CREATINE AS A SUBSTANCE FOR BETTER PERFORMANCE

A systematic collection of published scientific literature on “creatine and exercise in sports” 2000-2015

Åke Andrén-Sandberg

From the Department of Surgery, Karolinska Institutet at Karolinska University Hospital, Huddinge, S-141 86 Stockholm, Sweden
SUMMARY

INTRODUCTION
History of doping with creatine
A theoretically and practically working supplement
  - Oxygen transportation in muscles
  - Physiological aspects
  - A wide spectrum of indications
  - A safe substance
  - Hypertrophic response to resistance training
  - Short-term creatine supplementation
  - A commercial success

ON RESEARCH ON CREATINE
Inconsistent results
Few high-quality studies
Selected groups investigated

BIOCHEMISTRY OF CREATINE
Amounts and anatomy of creatine
  - Endogene production
  - Synthesis
  - Dietary intake
  - Anatomical localization
Exogenous and endogenous creatine
Glycine, arginine and methionine for synthesis of creatine
Uptake of oral creatine
  - Absorption of creatine supplied as a drink, in meat or in solid form
  - Pre-exercise oral creatine ingestion
Metabolism of creatine
  - Creatine kinases
  - Creatine degrades to creatinine
Creatine kinetics in healthy men and women
Urinary excretion
  - Excretion in urine of creatine

PHYSIOLOGY OF CREATINE
Possible multiple mechanisms for effect of creatine
Decreased levels of myostatin
Effect on myogenic satellite cells
  - Creatine monohydrate supplementation increases satellite cell mitotic activity
  - Enhanced satellite cell activity
  - Influence on myonuclei concentration
Effect on myosin heavy chain
  - Oral creatine and resistance training on myosin heavy chain expression
Increased protein synthesis
  - No effect on protein synthesis
  - Effect on human muscle protein turnover at rest
Influence of creatine on glucose metabolism
Muscle glycogen accumulation
  - Muscle glycogen supercompensation is enhanced by prior creatine supplementation
Effect on muscle ATP
ATP resynthesis

A buffering effect
Creatine reduces human muscle PCr and pH decrements and Pi accumulation

Relative importance of phosphocreatine (PCr) during exercise
Maintenance of a phosphorylcreatin reservoir for energy
Phosphocreatine (PCr) action on cell membrane structures
An antioxidant effect

MEDICAL USE OF CREATINE
Creatine as nutritional supplementation or medicinal product
In neurological, aging and psychiatric diseases
Protective effects on the brain
No effect on blood pressure

NON-MEDICAL USE OF CREATIN: SUPPLEMENTATION IN SPORTS
Use in young athletes
Creatine supplementation in high school football players
Physiologic basis for creatine use in children and adolescents
Creatine effects in select division I collegiate athletes
Use of creatine in high schools

Use in women
Use by military
Use of creatine by members of civilian and military health clubs

DOSAGE OF CREATINE
Creatine supplementation increases total body water
Increased body weight after creatine loading

Different preparations
Commercially available forms of creatine
Creatine monohydrate
Nothing better than the monohydrate
Creatine ethyl ester
Polyethylene glycosylated creatine
Hyperhydrating supplements containing creatine
Creatine as powder

Rudimentary legislations
Creatine purity

Safety
Upper storage limit
Time schedule of response
Effects of creatine supplementation before or after physical performance
Pre-season training
Effect of low-dose, short-duration creatine supplement
Typical dosage of creatine by athletes
Effects of creatine in males versus females
Creatine monohydrate enhances high-intensity exercise performance

EXOGENIC FACTORS’ INFLUENCE ON MUSCLE AND SERUM CREATINE LEVELS
Oral creatine supplementation
Strategies of creatine supplementation

Individual responses
Creatine supplementation affects muscle creatine during energy restriction
Plasma levels
Plasma levels after exercise
Endurance running
VEGETARIANS
Different responses
Lacto-ovo-vegetarian diet
Results also without meat

EFFECT OF CREATINE SUPPLEMENTATION ON EXERCISE PERFORMANCE
Improved training capacity
Acute (short time) resistance exercise
   - A meta-analysis
   - Contradicting results up to 2007
   - Hormonal effects
   - Effects on repeated bouts of supramaximal exercise
   - Effect of creatine on myostatin during resistance training
   - Effect of creatine loading on long-term sprint exercise performance and metabolism
   - Creatine loading, resistance exercise performance, and muscle mechanics
   - Creatine effects on periodized, off-season resistance-training program
   - Effects on isometric bench-press performance in resistance-trained humans
   - Short-term resistance training
   - In older males

Effects of creatine on anaerobic exercise
   - Creatine supplementation and upper extremity anaerobic response in females

Endurance
   - Effects on jumping, sprinting or cycling
   - Creatine reduces muscle inosine monophosphate depletion
   - Endurance followed by sprint
   - Effect of creatine loading on oxygen uptake during a 1-km cycling time trial
   - Creatine for endurance in female soccer players

Cardiorespiratory responses
   - Creatine supplementation alters the response to a graded cycle ergometer test
   - Increased enhances oxygen uptake during alternating intensity exercise

Effect on strength and power
   - Little effect on maximal strength
   - Improvement of strength? A meta-analysis
   - Effects of oral creatine on muscular strength and body composition
   - Strength loss after eccentric contractions is unaffected by creatine supplementation

Local effects on muscles
   - Effects of creatine supplementation on isometric force-time curve characteristics
   - Creatine supplementation and muscular adaptation to resistive overload
   - Creatine supplementation and lower limb strength performance
   - Effect of oral creatine supplementation on isokinetic torque production
   - Minimal effect on electromyographic fatigue threshold
   - Effects of creatine supplementation on skeletal muscle hypertrophy
   - Effect on skeletal muscle metabolism in physical exercise
   - Effect of exogenous creatine supplementation on muscle PCr metabolism
   - Effect of creatine loading on neuromuscular fatigue threshold
   - Contractile properties, fatigue and recovery are not influenced by short-term creatine

Effect of low doses
   - Effect of continuous low dose creatine on force, power, and total work
   - Effects of low doses of creatine on strength and urinary creatinine concentration
EFFECTS OF CREATINE IN SPECIFIED SPORTS

Running

In sprinting
Creatine supplementation improves sprint performance in male sprinters
Multiple sprint running
Sprint performance enhancement after one-week creatine supplementation
No acute effects of short-term creatine muscle properties and sprint performance
Similar ergogenic effect in sprinters and long-distance runners?

Biking

Effect of creatine loading on oxygen uptake during cycling
Effect of recovery interval on multiple-bout sprint cycling

Rowing

Track and field
Usage and education of track and field throwers in American universities

Football

Handball

Ice-hockey

Rugby

Swimming

Well trained swimmers
Female swimmers
Junior swimmers

Weightlifting

Taekwondo

Wrestling

No impact on upper-body anaerobic power in trained wrestlers

Tennis

Squash

POSSIBLE SIDE EFFECTS

Overview
Statement of the International Society of Sports Nutrition
Few side effects overall
Effects on the liver
Effects on the brain
Effects on the heart
Atrial fibrillation
Water retention
Increased body weight
Renal effects
Long-term effects in rats
Elevating of serum creatinine
Safety recommendations
Diarrhoea
Muscle cramping
Long-term use
Increased production of formaldehyde
Cytotoxicity
Cancer?
Children
Chronic exposition to individuals with chronic disease
Safety levels
Contaminants
EFFECT OF CREATINE IN COMBINATION WITH OTHER ORAL SUBSTANCES

Combination of creatine with glucose
Combination of creatine with carbohydrate and protein
Combination of creatine and carbohydrate or cinnamon
Combination of creatine-dextrose versus protein-dextrose
Combination of creatine and whey protein
Combination of creatine and beta-alanine
Combination of creatine and bicarbonate
Combination of creatine with ribose and glutamine
Combination of creatine with magnesium
Combination of creatine with ribose and glutamine
Combination of creatine with betaine
Combination of creatine and D-pinitol
Combination of creatine and conjugated linoleic acid
Combination of creatine and Russian tarragon

MISCELLANEOUS

Cognitive effects of creatine
Effect of creatine after sleep deprivation
Effects on inflammatory markers
Effect of creatine on plasma levels of pro-inflammatory cytokines
Effects of creatine on oxidative stress and inflammation markers after sprint
Experimentally
Neuroprotective effect of creatine
Acute creatine loading increases fat-free mass
Acute creatine loading enhances human growth hormone secretion
Creatine decreases plasma markers of adenine nucleotide degradation
Acute creatine supplementation in older men
Effect of pre-exercise creatine ingestion on performance in healthy aging males
Positive effects in hot environments
Physiological responses to short-term exercise in the heat after creatine loading
No effect on thermoregulation and isokinetic muscular performance
Role of creatine supplementation in exercise-induced muscle damage
Potential mechanisms of creatine on exercise-induced muscle damage
Protein- and carbohydrate-induced augmentation of creatine retention
No effect of resistance training and creatine supplementation on blood lipids
Decreased range of motion
Effect on musculoskeletal stiffness and performance
No positive effect of creatine on muscle wasting in cortisone treatment
Creatine deficiency syndromes
Experimental
Energetic driving forces are maintained in resting rat skeletal muscle after creatine
Effects of dietary creatine supplements on the contractile properties in the rat
Creatine loading and depletion on rat skeletal muscle contraction
Effect of creatine supplementation on cardiac muscle in rats
Decreased plasma lipid peroxidation and enhanced anaerobic performance in rats

Guanidinoacetic acid (GAA), a precursor of creatine

REFERENCES
SUMMARY

Creatine (alpha-methyl guandino-acetic acid) is an amino acid derivative synthesized from arginine, glycine, and methionine in the kidneys, liver, and pancreas.

History of doping with creatine

A French scientist named Chevreul is credited with first discovering creatine (Cr) in 1832, however, it was not until 1926 that the scientists Chanutin quantified Cr storage and retention in the body. The first reports that phosphocreatine content in human muscle can increase up to 50 percent following daily creatine supplement (5 g Cr monohydrate 4-6 × day for ≥ 2 days) was written by Harris et al in 1992.

Early work by Olexander Palladin established the role of creatine in muscle function. In the 1970s, Soviet scientists showed that oral creatine supplements improved athletic performance in short, intense activities such as sprints. Subsequent studies in the West substantiated these investigations and have led to the widespread acceptance and use of creatine supplements to enhance muscle function and athletic performance.

In 1992 a watershed paper was published by Roger Harris and co-workers on the capacity of the muscle to increase its phosphocreatine concentration following supplementation with a creatine product. This led to an explosion of interest in this unique ergogenic aid. In the space of two decades, the annual production and use of creatine supplements has grown exponentially, and the publication of creatine supplementation trials in the peer-reviewed literature can be measured in the hundreds.

A theoretically and practically working supplement

Creatine, the most extensively researched ergogenic aid, has been shown to increase strength and improve body composition in most individuals when combined with exercise. Creatine’s ergogenic abilities are derivative of its ability to rapidly replenish ATP stores, allowing for quicker recovery and potential increased training volume. To properly load creatine stores in the muscle, it is recommended that an individual consume roughly 0.3 g/kg/day for three days followed by a maintenance dose of 3-5g/day after the first three days. Alternately, a lower dose of 2-3 g/day may also be utilized to increase stores slowly. Supplementation of creatine is also beneficial for improving lean body mass when combined with exercise.

A wide spectrum of indications

Athletes, body builders, and military personnel have been used to use dietary creatine as an ergogenic aid to boost physical performance in short bursts of high-intensity muscle activity [12414]. A broad spectrum of beneficial effects has been ascribed to creatine (Cr), phosphocreatine (PCr) and their cyclic analogues cyclo-(cCr) and phospho-cyclocreatine (PcCr). Cr is widely used as nutritional supplement in sports and increasingly also as adjuvant treatment for pathologies such as myopathies and a plethora of neurodegenerative diseases. Additionally, Cr and its cyclic analogues have been proposed for anti-cancer treatment. The mechanisms involved in these pleiotropic effects are still controversial and far from being understood. However, some protective effects of Cr and analogues cannot be satisfactorily explained solely by effects on the cellular energy state.

On research on creatine

Creatine has been one of the more extensively studied dietary supplements. There had in
2013 been upward of 300 studies evaluating the effects of creatine on resistance training, with 70 percent reporting increases in strength in 2013. Several forms of creatine exist; however creatine monohydrate has been the most extensively studied, and its formulation has shown benefits in short-duration, high-intensity weightlifting and cycling.

Creatine supplementation is not banned by the International Olympic Committee and, with the exception of a small increase in body mass (approximately 1 kg) over the initial 36 d, does not appear to have any adverse side effects, at least with short-term use. Few scientific data are available for more prolonged use (months or years) but considering the large numbers of athletes using Cr over the past 6+ years and the absence of reported problems, it may be that the often discussed somewhat nebulous long term adverse effects are presently being overestimated. Intakes of 285-300 mg Cr/kg body mass 1 over 36 d or 3050 mg/kg body mass 1 over approximately 4 weeks are sufficient to produce benefits (muscle mass and high intensity power gains); however, not all study results are consistent.

A large number of studies have examined the effects of CrS on muscle metabolism and/or high-intensity exercise performance. Studies that have measured muscle total creatine (TCr) (phosphocreatine + creatine) have reported an elevation in TCr after CrS involving loading phases of 20-30 g/day for 3-6 days. Some studies found that both resting TCr and PCR content increased, whereas others reported significant increases in only TCr. However, on the whole, experimental evidence supporting an ergogenic effect for CrS is somewhat mixed. Several studies have demonstrated improved high-intensity exercise performance after CrS, whereas others have reported no beneficial effects. A possible explanation for the conflicting findings may relate to the experimental design used to examine the effects of CrS on exercise performance. Most studies have employed a cross-sectional experimental design or an ordered treatment allocation. Also, few studies have utilised a crossover experimental design, possibly due to the time required for muscle TCr to return to basal levels after CrS was unknown. It was indicated that a variety of factors including, but not limited to, sample size, exercise modality, rest and recovery intervals, residual effects of cessation of CrS, non-responders, gender and age effects and methodology used, make any interpretation of existing Cr literature extremely difficult.

Most of the evidence has been obtained from healthy young adult male subjects with mixed athletic ability and training status. Less research information is available related to the alterations due to age and gender.

**Biochemistry of creatine**

Creatine crystallizes from water as monoclinic prisms holding one molecule of water of crystallization per molecule of creatine. Continued drying of CM results in a loss of the water of crystallization at around 100°C, yielding anhydrous creatine. Creatine is a weak base with a pkb value of 11.02 at 25°C. As a result, creatine can only form salts with strong acids, having a pka value of less than 3.98. Creatine forms salts by the protonation of its guanidine moiety. In addition to salt formation, creatine is able to act as a complexing agent.

Almost all the Cr in the body is located in skeletal muscle in either the free (Cr: approximately 40 %) or phosphorylated (PCr: approximately 60 %) form and represents an average Cr pool of about 120-140 g for an average 70 kg person.

Creatine is distributed at approximately 95 percent in skeletal muscle mass; the remainder is located in the brain, the testes and the kidneys. Its synthesis starts mainly in the kidneys from glycine and arginine, forming alpha-methylguanidoacetic acid, which is conducted through the blood to the liver where it reacts with S-adenosylmethionine to synthesise creatine. Approximately 1-2 g of creatine is produced over 24 h and released mainly to the
skeletal muscle system. It may be assumed that there is a total creatine pool of approximately 120 g in a man of 70 kg body weight.

**Exogenous and endogenous creatine**

The exogenous sources of creatine are animal products such as red meat and fish. The normal dietary intake of creatine in an omnivorous diet is around 1 g per day. The liver, kidney, and pancreas form endogenous stores of creatine. The endogenous production of creatine is down-regulated during exogenous creatine supplementation; however the endogenous production returns to baseline after supplementation is discontinued. The first step in endogenous synthesis of creatine occurs in the kidney and starts with the amino acids glycine and arginine. The product is then transferred to the liver where a methyl group from methionine is added forming creatine. Circulating creatine is brought into skeletal muscle via transporters in the cell membrane. The rate of creatine uptake has been shown to be influenced by exercise, catecholamines, and insulin-like growth factor. Once within the cell, creatine can be phosphorylated to form phosphocreatine in a reversible enzymatic reaction facilitated by creatine kinase. The phosphate group comes from ATP forming adenosine diphosphate (ADP). The reverse reaction occurs when ATP is being used by the cell, and phosphocreatine can shuttle a phosphate group to ADP.

**Glycine, arginine and methionine for synthesis of creatine**

Three amino acids (glycine, arginine and methionine) and three enzymes (L-arginine:glycine amidinotransferase, guanidinoacetate methyltransferase and methionine adenosyltransferase) are required for creatine synthesis. The impact creatine synthesis has on glycine metabolism in adults is low, however the demand is more appreciable on the metabolism of arginine and methionine. Creatine ingested through supplementation is transported into the cells exclusively by CreaT1. However, there is another creatine transporter Crea T2, which is primarily active and present in the testes. Creatine uptake is regulated by various mechanisms, namely phosphorylation and glycosylation as well as extracellular and intracellular levels of creatine. Crea T1 has shown to be highly sensitive to the extracellular and intracellular levels being specifically activated when total creatine content inside the cell decreases. It has also been observed that in addition to cytosolic creatine, the existence of a mitochondrial isoform of Crea T1 allows creatine to be transported into the mitochondria. Indicating another intra-mitochondrial pool of creatine, which seems to play an essential role in the phosphate-transport system from the mitochondria to the cytosol.

**Uptake of oral creatine**

The uptake of creatine is simplified in a two-step approach: first, uptake into the bloodstream; second, uptake into the target tissue. The term “bioavailability” refers to both the intestinal absorption and the use of a substance by the body’s cells and tissues. First indications of a potential change of creatine bioavailability can be gathered from the amount of creatine taken up into the blood plasma after oral administration. However, a change in the total amount of creatine in the blood plasma cannot be directly extrapolated to a potential increase in desired performance. An increased amount of creatine in the plasma could be the result of decreased uptake into the target tissue resulting in an actual decrease in overall bioavailability. On the other hand, an initial rise in plasma creatine levels, followed by a reduction in plasma levels, is an indication of increased uptake into the target tissue. Dietary creatine is presumed to have high bioavailability since intestinal absorption of creatine monophosphate (CM) is already close to 100 percent. However, the response to creatine supplementation is heterogeneous, due in part to some non-responders, which might be overcome by alternative forms of creatine. There is some evidence that co-ingestion of CM with various nutrients (e.g. carbohydrate, protein, d-pinitol) may enhance creatine uptake to a
greater degree. However, there is no evidence that effervescent creatine, liquid creatine, and/or CEE promotes greater uptake of creatine to the muscle. Rather, there is some evidence that some of these forms of creatine may be less effective and/or be of greater clinical concern in terms of safety.

**Pre-exercise oral creatine ingestion**

Data suggest that although the pre-exercise ingestion of a large Cr dose was shown to have some impact on blood borne metabolites, it does not improve maximal prolonged intermittent sprint exercise performance, possibly due to an insufficient time allowed for uptake of serum Cr by skeletal muscle to occur. Therefore, this form of loading does not provide an alternative method of Cr supplementation to the traditional five-day supplementation regimes established by previous research.

**Metabolism of creatine**

Studies have indicated that creatine monophosphate (CM) is not degraded during normal digestion and that nearly 99 percent of orally ingested CM is either taken up by muscle or excreted in urine.

Creatine monohydrate powder is very stable showing no signs of degradation over years, even at elevated temperatures. To detect a potential degradation of creatine, one must measure the content of its degradation product, creatinine. The degradation of creatine can be reduced or even halted by either lowering the pH under 2.5 or increasing the pH. A very high pH results in the deprotonation of the acid group, thereby slowing down the degradation process by making it more difficult for the intramolecular cyclization. A very low pH results in the protonation of the amide function of the creatine molecule, thereby preventing the intramolecular.

**Creatine kinases**

Total creatine kinase (CK) levels depend on age, gender, race, muscle mass, physical activity and climatic condition. High levels of serum CK in apparently healthy subjects may be correlated with physical training status, as they depend on sarcomeric damage: strenuous exercise that damages skeletal muscle cells results in increased total serum CK. The highest post-exercise serum enzyme activities are found after prolonged exercise such as ultradistance marathon running or weight-bearing exercises and downhill running, which include eccentric muscular contractions. Total serum CK activity is markedly elevated for 24 h after the exercise bout and, when patients rest, it gradually returns to basal levels. Persistently increased serum CK levels are occasionally encountered in healthy individuals and are also markedly increased in the pre-clinical stages of muscle diseases. High CK serum levels in athletes following absolute rest and without any further predisposing factors should prompt a full diagnostic workup with special regards to signs of muscle weakness or other simple signs that, in both athletes and sedentary subjects, are not always promptly evident.

**Excretion in urine of creatine**

Following ingestion, the maximum of creatine excretion in urine is after about 2-3 h and a return to basal values is noted after 13 to 14 h. In physiological conditions, reference values of creatine excretion are scarce. However, creatine excretion can rise to 200 mmol/mol creatinine (26.2 g/mole creatinine). After a single oral supplementation of 2.1 g to three subjects, a kinetic investigation reveals a maximum concentration of 20 mmol/L (2.62 g/L), observed between 1 and 6 h after ingestion.
Physiological aspects

Creatine is synthesized at a rate of about 1-2 g/d. Creatine can also be obtained through the diet, mainly from meat and fish. The average person consumes about 1 g creatine each day from a regular diet. Creatine is degraded into creatinine and excreted in the urine at a rate of about 2 g/d. About 90-95 percent of the body’s creatine is found in skeletal muscle. Of this, approximately one-third is free creatine, whereas two-thirds exist as phosphocreatine (PCr). The uptake from circulation is an active process facilitated by a Na⁺-dependent transporter against a concentration gradient. PCr serves a major role in energy metabolism. When energy demands increase, PCr donates its phosphate to ADP to produce ATP. The ATP-PCr system can provide energy at high rates, but only for a few (10-15) seconds before the PCr store is emptied. Thus, creatine is involved in temporal energy buffering, and also in spatial energy buffering, proton buffering, and glycolysis regulation. Because PCr is a limiting factor in maintaining ATP resynthesis during maximal short-term exercise, an increased PCr concentration should theoretically increase the energy reserve for such exercise. Therefore, it has been suggested that creatine loading will improve performance during short-term maximal exercise, analogous to the effect of glycogen loading before endurance exercise. The normal concentration of total creatine in skeletal muscle is about 120 mmol/kg (dry mass), whereas the upper limit appears to be about 150–160 mmol/kg. It has repeatedly been shown that the muscle concentration of total creatine can indeed be increased by oral creatine supplementation. The supplementation protocols typically involve a loading phase of about 20 g creatine monohydrate for 4-6 days followed by a maintenance dose of about 5 g daily for 2–3 week. This regimen will theoretically increase the blood concentration of creatine to a level optimal for uptake in the muscle. Ingestion above this amount will likely lead to excess creatine wasted in the urine. Lower dose supplementation of 2-3 g/d will also elevate muscle creatine, but the increase occurs gradually over several weeks, rather than days.

Creatine is thus involved in the regulation of cellular energy demand. Under resting conditions, ATP is mainly formed in mitochondria through oxidative phosphorylation with ADP. Transported in sarcoplasm, some ATP molecules react with creatine, via the enzyme phosphorylcreatine kinase, to form phosphorylcreatine and ADP until equilibrium is reached. When ATP is needed for cellular energy, such as for muscle contraction, the phosphorylcreatine kinase reverse reaction replenishes the ATP content. Creatine thus acts indirectly to maintain a phosphorylcreatine reservoir for energy needs, more specifically to supply the muscle system with ATP.

To explain the elevated hypertrophic response with combined resistance training and creatine supplementation, an increased production of myogenic growth factors seems to occur in the muscle tissue itself. Thus, increased mRNA and protein levels of various myogenic regulatory factors (MyoD, myogenin, MRF4) have been observed following combined training and creatine intake. Although resistance training per se results in increased mRNA and protein content of MyoD, myogenin and MRF4, this increase is substantially accelerated when training is combined with creatine intake. A rise in myogenic regulatory factors with creatine supplementation may not per se elicit an enhanced hypertrophic response, rather it increases the sensitivity of the muscle cell to the resistance training stimulus, which in turn contributes to the accelerated hypertrophy.

The elevated muscle creatine content moderately improves contractile performance in sports with repeated high-intensity exercise bouts. More chronic ergogenic effects of creatine are to be expected when combined with several weeks of training. A more pronounced muscle hypertrophy and a faster recovery from atrophy have been demonstrated in humans involved in resistance training. The mechanism behind this anabolic effect of creatine may relate to satellite cell proliferation, myogenic transcription factors and insulin-like growth factor-1 signaling. An additional effect of creatine supplementation, mostly when combined with
training, is enhanced muscle glycogen accumulation and glucose transporter (GLUT4) expression. Thus, creatine may also be beneficial in sport competition and training characterized by daily glycogen depletion, as well as provide therapeutic value in the insulin-resistant state.

The mechanisms through which creatine supplementation improves exercise performance and body composition may also include metabolic enhancements (increased pre-exercise phosphocreatine, increased pre-exercise muscle glycogen), molecular adaptations (increased gene expression of growth factors) and reduced muscle damage. However, creatine supplementation does not increase skeletal muscle protein synthesis.

Research using muscle biopsy techniques has also shown increases in muscle cell diameter associated with 12 weeks of creatine supplementation and resistance training, which may be associated with the potential for creatine supplementation to amplify the resistance training-induced increase in satellite cell number and myonuclei concentration in skeletal muscle.

Animal experiments have shown that creatine supplementation may result in enhanced satellite cell activity. Given the fact that locally produced IGF-1 exerts similar effects, it is possible that combined creatine intake and resistance training leads to elevated satellite cell activation compared to resistance training alone, which amplifies the hypertrophic response.

Another possibility is that part of the increased body weight is caused by increased protein synthesis, conceivably stimulated by the cell swelling. Cell swelling has been shown to act as an anabolic signal stimulating protein synthesis and net protein deposition. In agreement with this, a stimulating effect of creatine on protein synthesis in animal cardiac and skeletal muscle in vitro has been found, although there are also studies that do not confirm this.

Creatine has no effect on turnover in the postabsorptive or fed states. Thus any increase in muscle mass accompanying creatine supplementation must be associated with increased physical activity.

There are results suggesting that creatine supplementation, combined with aerobic training, can improve glucose tolerance but does not affect insulin sensitivity, and may warrant further investigation with diabetic subjects.

An additional effect of creatine supplementation, mostly when combined with training, is enhanced muscle glycogen accumulation and glucose transporter (GLUT4) expression. Thus, creatine may also be beneficial in sport competition and training characterized by daily glycogen depletion, as well as provide therapeutic value in the insulin-resistant state.

It has been suggested that the improvements in performance associated with creatine supplementation are due to parallel improvements in ATP resynthesis during exercise as a consequence of increased PCr availability, particularly within the type II muscle fibers.

A buffering substance

During sustained high intensity exercise (HIE), PCr contributes about 26 percent of the anaerobic capacity and the 10 percent increase in PCr would correspond to an increased anaerobic capacity of about 3 percent (10% × 0.26). The reduced catabolism of adenine nucleotides after Cr loading during HIE gives experimental support for the role of Cr in improving energetic status. PCr-Cr acts as a temporal buffer of ATP, attenuating decreases in cellular ATP during high rates of energy demand. Cr kinase, the enzyme catalyzing bidirectional conversion of Cr-PCr, is localized to structural components close to sites of ATP utilization (myofibrils, sarcoplasmatic reticulum, and sarcolemma) and ATP formation.
(mitochondria). This provides the basis for PCr-Cr acting as a spatial buffer of ATP, by which intracellular gradients of ATP-ADP are diminished. Spatial buffering of ATP will reduce increases in ADP at the sites of energy consumption and may be an important factor to prevent contractile failure and fatigue. Increasing PCr by Cr loading will improve both the temporal and the spatial ATP-ADP buffering and explain part of the ergogenic effect. Another mechanism by which Cr loading can increase performance is related to muscle buffering. PCR-Pi accounts for more than 50 percent of the total muscle buffer capacity. It can be calculated that the improved muscle buffering after Cr loading, together with the knowledge that glycolysis accounts for 74 percent of anaerobic ATP production (sustained exercise at VO2max), can increase anaerobic capacity by about 4 percent \((10 \times 0.5 \times 0.74)\). The combined effect of Cr loading on ATP buffering and proton buffering may thus increase anaerobic capacity during sustained exercise by about 6 percent. However, due to the heterogenic response between subjects to Cr loading, one would expect that some subjects would benefit more, whereas others would not have any effect at all (non-responders).

**An antioxidant effect**

The beneficial effects can be barely explained on the basis of the sole ergogenic role of the Cr/CrP system. Indeed, a wide number of research articles indicate that Cr is capable of exerting multiple, non-energy related, effects on diverse and relevant cellular targets. Among these effects, the antioxidant activity of Cr emerges as an additional mechanism which is likely to play a supportive role in the Cr-cytoprotection paradigm.

**Medical use of creatine**

Most studies of the response to creatine supplementation have assessed exercise performance in healthy subjects. However, there are some indications that supplementation may be useful in the treatment of certain diseases, such as muscle fatigue secondary to impaired energy production and diseases resulting in muscle atrophy. The mechanisms underlying the effect of creatine in these circumstances are largely unknown, but may be due to the increased energy in the form of PCr, increased muscle accretion, and stabilization of membranes. Inborn errors of energy metabolism have been identified in 3 of the main steps in creatine metabolism: arginine:glycine aminidotransferase (AGAT), S-adenosyl-L-methionine:N-guanidino-acetate methyltransferase (GAMT) and the creatine transporter. Oral creatine has been shown to improve the clinical symptoms in both AGAT and GAMT deficiency, but not in the creatine transporter deficiency. Supplementation has also been shown to have neuroprotective effects in several animal models of neurological diseases, e.g. Huntington's disease, Parkinson's disease, and amyotrophic lateral sclerosis. However, this has to be confirmed in clinical studies in humans.

It is well accepted that creatine has a function in extremity muscles, but lesser known is the essential role creatine, a natural regulator of energy homeostasis, plays in brain function and development. Creatine supplementation has shown promise as a safe, effective, and tolerable adjunct to medication for the treatment of brain-related disorders linked with dysfunctional energy metabolism, such as Huntington's disease and Parkinson's disease. Impairments in creatine metabolism have also been implicated in the pathogenesis of psychiatric disorders, leaving clinicians, researchers and patients alike wondering if dietary creatine has therapeutic value for treating mental illness such as psychological stress, schizophrenia, mood and anxiety disorders. While present knowledge of the role of creatine in cognitive and emotional processing is in its infancy, further research on this endogenous metabolite has the potential to advance the understanding of the biological bases of psychopathology and improve current therapeutic strategies.
Dosage of creatine

Creatine supplementation appears to increase total body and lean body mass; however, short-term gains in total body mass may be primarily water, but long-term gains associated with resistance training appear to be lean muscle mass.

Creatine loading is associated with an increase in body weight of approximately 2 percent. Although it has been suggested that Cr loading might stimulate protein synthesis and muscle growth, the evidence for this is limited. It is more likely that the increased body weight after Cr loading is related to increased tissue water content due to the osmotic effect of increased intracellular concentrations of PCR and Cr or to increased glycogen storage. In subjects with otherwise stable body weight, the increased body weight may be used as a rough marker of the effect of Cr loading. The increased body weight will negatively affect performance in running and other sports where body weight influences energy demand, and may therefore reduce the potential ergogenic effects of Cr loading.

There are several different available forms of creatine: creatine anhydrous which is creatine with the water molecule removed in order to increase the concentration of creatine to a greater amount than that found in CM. Creatine has been manufactured in salt form: creatine pyruvate, creatine citrate, creatine malate, creatine phosphate, magnesium creatine, creatine orotate, Kre Alkalyn (creatine with baking soda). Creatine can also be manufactured in an ester form. Creatine ethyl ester (hydrochloride) is an example of this, as is creatine gluconate which is creatine bound to glucose. Another form is creatine effervescent which is creatine citrate or CM with citric acid and bicarbonate. The citric acid and bicarbonate react to produce an effervescent effect. When mixed with water the creatine separates from its carrier leaving a neutrally charged creatine, allowing it to dissolve to a higher degree in water. Manufacturers claim that creatine effervescent has a longer and more stable life in solution. When di-creatine citrate effervescent was studied for stability in solution it was found that the di-creatine citrate dissociates to citric acid and creatine in aqueous solutions which in turn forms CM and eventually crystallises out of the solution due to its low solubility. Some of the creatine may also convert to creatinine. In summary, creatine salts have been show to be less stable than CM. However the addition of carbohydrates could increase their stability. The potential advantages of creatine salts over CM include enhanced aqueous solubility and bioavailability which would reduce their possible gastrointestinal adverse effects. The possibility for new additional formulation such as tablets or capsules is interesting for its therapeutic application due to its attributed better dissolution kinetics and oral absorption compared to CM. However more complete in vivo pharmaceutical analysis of creatine salts are required to fully elucidate their potential advantages/disadvantages over the currently available supplement formulations.

Creatine monohydrate (CM), first marketed in the early 1990s, is the form most commonly found in dietary supplement/food products and most frequently cited in scientific literature.

Supplement manufacturers have continually introduced newer forms of creatine into the marketplace. These newer forms have been purported to have better physical and chemical properties, bioavailability, efficacy, and/or safety profiles than creatine monophosphate (CM). However, there is little to no evidence that any of the newer forms of creatine are more effective and/or safer than CM whether ingested alone and/or in combination with other nutrients.

The daily oral ingestion of supplementary creatine monohydrate can substantially elevate the creatine content of human skeletal muscle. One paper aimed to summarize the current knowledge regarding the impact muscle creatine loading can have on exercise performance and rehabilitation. The major part of the elevation of muscle creatine content is already
obtained after one week of supplementation, and the response can be further enhanced by a concomitant exercise or insulin stimulus.

Chronic supplementation with creatine monohydrate has been shown to promote increases in total intramuscular creatine, phosphocreatine, skeletal muscle mass, lean body mass and muscle fiber size. Furthermore, there is robust evidence that muscular strength and power will also increase after supplementing with creatine. Based on the magnitude inferences it appears that consuming creatine immediately post-workout is superior to pre-workout vis a vis body composition and strength.

As demonstrated by several laboratories, creatine supplementation is able to increase repetitions to failure. Therefore, many athletes have adopted creatine use during pre-season and/or off-season training for the cumulative effects of increased training volume, and they are not using creatine exclusively for acute performance benefits.

A typical creatine supplementation protocol consists of a loading phase of 20 g creatine monophosphate (CM)/d or 0.3 g CM/kg/d split into 4 daily intakes of 5 g each, followed by a maintenance phase of 3-5 g CM/d or 0.03 g CM/kg/d for the duration of the supplementation period. Other supplementation protocols are also used such as a daily single dose of around 3-6 g or between 0.03 to 0.1 g/kg/d. However, this method takes longer (between 21 to 28 days) to produce ergogenic effects. It was also found that a moderate protocol consisting of 20 g CM taken in 1g doses (evenly ingested at 30-min intervals) for 5 days resulted in reduced urinary creatine and methylamine excretion, leading to an estimated increase in whole body retention of creatine (+13 %) when compared with a typical loading supplementation protocol of 4 x 5 g/d during 5 days (evenly ingested at 3 hour intervals). This enhancement in creatine retention would lead to a significantly higher weight gain when people follow a moderate protocol ingestion of several doses of small amounts of CM evenly spread along the day.

However there is an upper limit of creatine stores that are possible in human muscle, which has been reported as high as 160 g in the human body. Therefore athletes with full stores of creatine in their muscles will not receive benefit from supplementation. People with lower creatine stores in their muscles receive the greatest effect on intramuscular creatine stores when supplemented with oral creatine.

Still the initial creatine content cannot fully explain the large intersubject variability in response to supplementation, suggesting that there are “responders” and “nonresponders.” An increased inward creatine transport has been found when creatine is ingested together with carbohydrates or a carbohydrate/protein mixture. This seems to be caused by an insulin effect on the uptake. Ingestion of creatine and 1 g glucose/kg body mass twice per day increased total muscle creatine by 9 percent versus creatine intake alone.

Strategies of creatine supplementation

A loading regime used in many studies is ingestion of 20 g of Cr monohydrate each day over a period of 5-7 days. The substance should be dissolved in water and distributed in 5 g doses in order to attain sufficient increases in plasma Cr concentration. The loading phase is followed by a period with a lower dose (2-3 g of Cr per day), which is sufficient to maintain the elevated muscle Cr level. The muscle uptake of Cr is largest during the initial loading phase, when about 30 percent of the dose administered is stored in muscle tissue and the remaining part excreted as creatinine in the urine. High doses of Cr after a loading phase, where the ceiling level of 150–160 mmol/kg dm has been reached, will be of no use since the surplus of Cr will be excreted in the urine. Muscle uptake of Cr can be enhanced by exercise, and the likely mechanism is increased muscle blood flow and thus exposure of the exercising
muscle to elevated plasma Cr. However, muscle Cr uptake is also stimulated by insulin, and the stimulating effect of exercise may thus, at least in part, relate to an exercise-induced increase in insulin sensitivity and/or increased exposure of the muscle to insulin. The practical recommendation is that Cr intake should be combined with a substantial amount of carbohydrate and exercise.

**Vegetarians**

Although well-controlled long-term studies assessing the effects of vegetarian diets on athletes have not been conducted, the following observations can be made: 1) well-planned, appropriately supplemented vegetarian diets appear to effectively support athletic performance; 2) provided protein intakes are adequate to meet needs for total nitrogen and the essential amino acids, plant and animal protein sources appear to provide equivalent support to athletic training and performance; 3) vegetarians (particularly women) are at increased risk for non-anemic iron deficiency, which may limit endurance performance; and 4) as a group, vegetarians have lower mean muscle creatine concentrations than do omnivores, and this may affect supramaximal exercise performance. Because their initial muscle creatine concentrations are lower, vegetarians are likely to experience greater performance increments after creatine loading in activities that rely on the adenosine triphosphate/phosphocreatine system.

**Improved training capacity**

Most studies document increases in muscle mass when creatine is supplemented during resistance training. The increase in muscle mass may be associated with a creatine supplementation-induced ability to do more repetitions during training, which may induce favorable genetic adaptations in the muscle.

Some measures of muscular performance and body composition are enhanced to a greater extent following the rebound phase of short-term resistance training overreaching with creatine supplementation and these changes are not related to changes in circulating hormone concentrations obtained in the resting, postabsorptive state. In addition, creatine supplementation appears to be effective for maintaining muscular performance during the initial phase of high-volume resistance training overreaching that otherwise results in small performance decrements.

**Effect on resistance training**

Creatine monohydrate’s effect on resistance training exercises has been extensively researched. There are numerous controlled studies that have reported increases in performance and muscle strength in short-duration, maximum-intensity exercises. Resistance training has been measured in many ways in the literature, including exercises such as bench press, leg press, biceps curls, leg extensions, jump squats, and bicycle ergometry. The method of measurement of strength and performance in creatine studies includes one repetition maximum, mean power, total force, and number of repetitions. The results regarding creatine supplementation’s ergogenic effect are not unanimous. However, there is a significant body of evidence that creatine increases performance in short-duration, maximum-intensity resistance training. Conflicting evidence exists regarding studies of the effect of creatine supplementation on anaerobic performance. Currently, studies consistently have observed no effect on aerobic performance with creatine supplementation.

A search of MEDLINE and SPORTDiscus using the phrase "creatine supplementation" revealed 96 English-language, peer-reviewed papers (100 studies), which included randomized group formation, a placebo control, and human subjects who were blinded to
treatments. ES was calculated for each body composition and performance variable. Small, but significant ES were reported for BC (n=163), ATP-PCr (n=17), G (n=135), and O (n=69). Effect size was greater for change in body composition following a loading-only creatine supplementation regimen compared to a maintenance regimen for repetitive-bout compared to single-bout exercise, and for upper-body exercise compared to lower and total body exercise. Effect size for laboratory-based tasks (e.g. isometric/isotonic/isokinetic exercise) were greater than those observed for field-based tasks (e.g. running, swimming). There were no differences in body composition or performance effects between males and females or between trained and untrained subjects. It was concluded that effect size was greater for changes in lean body mass following short-term CS, repetitive-bout laboratory-based exercise tasks ≤ 30 s (e.g. isometric, isokinetic, and isotonic resistance exercise), and upper-body exercise. Creatine supplementation does not appear to be effective in improving running and swimming performance. There is no evidence in the literature of an effect of gender or training status on effect following creatine supplementation.

Effects of creatine on anaerobic exercise

Creatine has demonstrated neuromuscular performance enhancing properties on short duration, predominantly anaerobic, intermittent exercises. It has been observed enhanced neuromuscular function of the elbow flexors in both electrically induced and voluntary contractions but not on endurance performance after 4 loading doses of 5 g creatine plus 15 g maltodextrin for 5/d in young, moderately trained men. Creatine supplementation may facilitate the reuptake of Ca2+ into the sacroplasmic reticulum by the action of the Ca2+ adenosine triphosphatase pump, which could enable force to be produced more rapidly through the faster detachment of the actomyosin bridges. A previous meta-analysis reported an overall creatine supplementation effect size of 0.24 ± 0.02 for activities lasting ≤30 s. (primarily using the ATP- phosphocreatine energy system). For this short high-intensity exercise, creatine supplementation resulted in a 7.5 ± 0.7 percent increase from baseline which was greater than the 4.3 ± 0.6 percent improvement observed for placebo groups. When looking at the individual selected measures for anaerobic performance the greatest effect of creatine supplementation was observed on the number of repetitions.

Effect on strength and power

Critical reviews of the scientific literature, including a meta-analysis and a monograph generally indicate that creatine supplementation may increase muscular strength and endurance as documented by enhanced performance in 1-repetition maximum strength tests, increased number of repetitions in various isotonic and isokinetic resistance exercise tasks, and increased work output during maximal short term (6-30 seconds) cycle ergometer tasks. In general, activities that involve sprinting, jumping or cycling performance show improved performance following creatine supplementation, but the beneficial effects appear to be less consistent. For example, using a standard creatine loading protocol with well-trained male sprinters as subjects, it was reported significant improvements in 100-meter sprint velocity and time to complete 6 intermittent 60-meter sprints. It was also reported significant increases in peak power and total work production in 10 sets of multiple 6-second bike sprints with varying periods of recovery in an 80-minute time frame following creatine supplementation. Conversely, it was reported no significant improvement in a 70-meter shuttle run sprint power test by well-trained tennis players and no beneficial effects on repeated 10-second skating sprints in ice-hockey players. Some research findings have a direct application to sports competition, such as an increased 1-RM performance in weight lifting and faster 100-meter sprint run times. The laboratory findings for other types of exercise performance are also rather strong, and do support a possible application to actual field competitions. For example, findings of increased muscle power output during intermittent sprint exercises may be applicable to football (soccer) and other sports
associated with high-intensity intermittent sprinting. In one such study, it was studied the effects of creatine supplementation on an exercise test protocol designed to simulate match play in soccer (football). The test involved 5 blocks of 11-minute exercise involving sprint running, agility runs, and a precision ball-kicking drill interspersed with recovery walks, jogs and runs. Creatine supplementation improved performance in some repeated sprint and agility tasks even though the subjects increased body mass, but the creatine had no effect on ball kicking accuracy. Thus, creatine supplementation might improve speed in repetitive sprints, important for many sports, but may not necessarily enhance sports skills. In support of this latter point, several studies reported no significant effects of creatine loading on tennis skill performance as measured by power and precision of their serves.

When maximal force or strength (dynamic or isotonic contractions) is the outcome measure following Cr ingestion, it generally appears that Cr does significantly impact force production regardless of sport, sex or age. The evidence is much more equivocal when investigating isokinetic force production and little evidence exists to support the use of Cr for isometric muscular performance.

The purpose of one study was to investigate whether creatine supplementation increases maximal strength and power in healthy adults through a meta-analysis of existing literature. It was searched MEDLINE (1966-2000) and the Cochrane Controlled Trials Register (through June 2001) to locate relevant articles. Sixteen studies were identified for inclusion. The summary difference in maximum weight lifted was 6.85 kg (95 % confidence interval 5.24 to 8.47) greater after creatine than placebo for bench press and 9.76 kg (95 % confidence interval 3.37 to 16.15) greater for squats; there was no difference for arm curls. In 7 of 10 studies evaluating maximal weight lifted, subjects were young men (younger than 36 years) engaged in resistance training. There was no difference in cycle ergometer or isokinetic dynamometer performance. It was concluded that oral creatine supplementation combined with resistance training increases maximal weight lifted in young men. There is no evidence for improved performance in older individuals or women or for other types of strength and power exercises.

Possible side effects

Usually, consumers do not report any adverse effects, but body mass increases. Gastrointestinal disturbances and muscle cramps have been reported occasionally in healthy individuals, but the effects are anecdotal. Liver and kidney dysfunction have also been suggested on the basis of small changes in markers of organ function and of occasional case reports, but well controlled studies on the adverse effects of exogenous creatine supplementation are almost nonexistent. It was investigated liver changes during medium term (4 weeks) creatine supplementation in young athletes. None showed any evidence of dysfunction on the basis of serum enzymes and urea production. Short term (5 days), medium term (9 weeks) and long term (up to 5 years) oral creatine supplementation has been studied in small cohorts of athletes whose kidney function was monitored by clearance methods and urine protein excretion rate. It was not ouind any adverse effects on renal function. Nevertheless, idiosyncratic effects may occur when large amounts of an exogenous substance containing an amino group are consumed, with the consequent increased load on the liver and kidneys. Regular monitoring is advised to avoid any abnormal reactions during oral creatine supplementation.

Cognitive effects of creatine

The effect of creatine supplementation and sleep deprivation, with intermittent moderate-intensity exercise, on cognitive and psychomotor performance, mood state, effort and salivary concentrations of cortisol and melatonin were examined. It was concluded that,
during sleep deprivation with moderate-intensity exercise, creatine supplementation only affects performance of complex central executive tasks.

**Creatine deficiency syndromes**

The cerebral creatine deficiency syndromes (CCDS), inborn errors of creatine metabolism, include the two creatine biosynthesis disorders, guanidinoacetate methyltransferase (GAMT) deficiency and L-arginine:glycine amidinotransferase (AGAT) deficiency, and the creatine transporter (CRTR) deficiency. Intellectual disability and seizures are common to all three CCDS. The majority of individuals with GAMT deficiency have a behavior disorder that can include autistic behaviors and self-mutilation; about 40 percent have movement disorder. Onset is between ages three months and three years. Only 14 individuals with AGAT deficiency have been reported.
INTRODUCTION

In regards to ergogenic supplementation, creatine’s effects have been researched in humans since the early 1990’s. Earnest and colleagues examined supplemental creatine’s effects on both body composition and muscular performance. After supplementing with creatine for only 2 weeks, subjects were tested with three 30s Wingate sprints, 1 repetition maximum in the bench press, and repetitions to failure at 70 percent 1RM. They discovered that creatine supplementation improved repeated sprint performance, bench press strength, fatigue resistance, body weight, and relative lifting volume. They also observed an insignificant trend for fat free mass. Creatine has since been verified as a potent supplement for each of these and more variables on numerous occasions [001].

History of doping with creatine

A French scientist named Chevreul is credited with first discovering creatine (Cr) in 1832, however, it was not until 1926 that the scientists Chanutin quantified Cr storage and retention in the body. The first reports that phosphocreatine content in human muscle can increase up to 50 percent following daily creatine supplement (5 g Cr monohydrate 4-6 × day for ≥ 2 days) was written by Harris et al in 1992 [002].

Early work by Olexander Palladin established the role of creatine in muscle function. In the 1970s, Soviet scientists showed that oral creatine supplements improved athletic performance in short, intense activities such as sprints. Subsequent studies in the West substantiated these investigations and have led to the widespread acceptance and use of creatine supplements to enhance muscle function and athletic performance [003].

In 1992 a watershed paper was published by Roger Harris and co-workers on the capacity of the muscle to increase its phosphocreatine concentration following supplementation with a creatine product. This led to an explosion of interest in this unique ergogenic aid – a product of apparently genuine value to biochemists, sports scientists, athletes, coaches, clinicians and the supplement industry. Clearly, the story of creatine is a remarkable one. In the space of two decades, the annual production and use of creatine supplements has grown exponentially, and the publication of creatine supplementation trials in the peer-reviewed literature can be measured in the hundreds. Creatine supplements owe their popularity both to the internet age and the evidence base of beneficial uses. As is the case for any supplement, however, it can be expected that the knowledge to continue to evolve, while the caution about individual products will need to be maintained in view of the frailties in the regulation of supplement manufacture and marketing [004].

A theoretically and practically working supplement

Creatine, the most extensively researched ergogenic aid, has been shown to increase strength and improve body composition in most individuals when combined with exercise. Creatine’s ergogenic abilities are derivative of its ability to rapidly replenish ATP stores, allowing for quicker recovery and potential increased training volume. To properly load creatine stores in the muscle, it is recommended that an individual consume roughly 0.3 g/kg/day for three days followed by a maintenance dose of 3-5g/day after the first three days. Alternately, a lower dose of 2-3 g/day may also be utilized to increase stores slowly. Supplementation of creatine is also beneficial for improving lean body mass when combined with exercise. According to the International Society of Sport Nutrition Position Stand on Creatine in 2014, creatine monohydrate was the most effective supplement for increasing anaerobic capacity and lean body mass [005].
Oxygen transportation in muscles

Anaerobic processes dominate during all-out exercise of a duration less than 1-2 min, but also have an essential role at submaximal work rates (70-90 % of VO2max), where muscle lactate may reach critically high levels. Improvements in anaerobic capacity with training or ergogenic supplements will improve performance primarily during short-term (<7 min) maximal exercise, when anaerobic energy release is an essential factor. Many sports include explosive activities where HIE occurs during a sustained period (sprint and middle distance) or during short repeated bursts with intervening periods of low-intensity exercise (e.g. team-sports such as soccer, handball, and basketball). Aerobic energy release is mainly limited by the rate at which ATP can be produced (i.e. aerobic power or VO2max). In contrast, anaerobic processes have a high power and are instead limited by the amount of ATP that can be produced (capacity). The capacity of the anaerobic processes is determined by the muscle store of high-energy phosphates and the maximal amount of lactate/protons that can be produced. By measurements of the maximal accumulated oxygen deficit, anaerobic capacity has been estimated to 52–90 ml O2, which corresponds to the energy demand of 1–2 min exercise at VO2max. The high-energy demand during high-intensity exercise (HIE) necessitates that anaerobic processes cover an extensive part of the adenosine triphosphate (ATP) requirement. Anaerobic energy release results in depletion of phosphocreatine (PCr) and accumulation of lactic acid, which set an upper limit of anaerobic ATP production and thus HIE performance. This report focuses on the effects of training and ergogenic supplements on muscle energetics and HIE performance. Anaerobic capacity (i.e. the amount of ATP that can be produced) is determined by the muscle content of PCr, the buffer capacity and the volume of the contracting muscle mass. HIE training can increase buffer capacity and the contracting muscle mass but has no effect on the concentration of PCr. Dietary supplementation with creatine (Cr), bicarbonate, or beta-alanine has a documented ergogenic effect. Dietary supplementation with Cr increases muscle Cr and PCr and enhances performance, especially during repeated short periods of HIE. The ergogenic effect of Cr is related to an increase in temporal and spatial buffering of ATP and to increased muscle buffer capacity. Bicarbonate loading increases extracellular buffering and can improve performance during HIE by facilitating lactic acid removal from the contracting muscle. Supplementation with beta-alanine increases the content of muscle carnosine, which is an endogenous intracellular buffer. It is clear that performance during HIE can be improved by interventions that increase the capacity of anaerobic ATP production, suggesting that energetic constraints set a limit for performance during HIE [006].

Physiological aspects

Creatine (alpha-methyl guandino-acetic acid) is an amino acid derivative synthesized from arginine, glycine, and methionine in the kidneys, liver, and pancreas. The synthesis rate is about 1-2 g/d. Creatine can also be obtained through the diet, mainly from meat and fish. The average person consumes about 1 g creatine each day from a regular diet. Creatine is degraded into creatinine and excreted in the urine at a rate of about 2 g/d. About 90-95 percent of the body’s creatine is found in skeletal muscle. Of this, approximately one-third is free creatine, whereas two-thirds exist as phosphocreatine (PCr). The uptake from circulation is an active process facilitated by a Na+-dependent transporter against a concentration gradient. PCr serves a major role in energy metabolism. When energy demands increase, PCr donates its phosphate to ADP to produce ATP. The ATP-PCr system can provide energy at high rates, but only for a few (10-15) seconds before the PCr store is emptied. Thus, creatine is involved in temporal energy buffering, and also in spatial energy buffering, proton buffering, and glycolysis regulation. Because PCr is a limiting factor in maintaining ATP resynthesis during maximal short-term exercise, an increased PCr concentration should theoretically increase the energy reserve for such exercise. Therefore, it has been suggested
that creatine loading will improve performance during short-term maximal exercise, analogous to the effect of glycogen loading before endurance exercise. The normal concentration of total creatine in skeletal muscle is about 120 mmol/kg (dry mass), whereas the upper limit appears to be about 150–160 mmol/kg. It has repeatedly been shown that the muscle concentration of total creatine can indeed be increased by oral creatine supplementation. The supplementation protocols typically involve a loading phase of about 20 g creatine monohydrate for 4-6 days followed by a maintenance dose of about 5 g daily for 2–3 week. This regimen will theoretically increase the blood concentration of creatine to a level optimal for uptake in the muscle. Ingestion above this amount will likely lead to excess creatine wasted in the urine. Lower dose supplementation of 2-3 g/d will also elevate muscle creatine, but the increase occurs gradually over several weeks, rather than days [007].

A wide spectrum of indications

Athletes, body builders, and military personnel have been used to use dietary creatine as an ergogenic aid to boost physical performance in short bursts of high-intensity muscle activity [12414]. A broad spectrum of beneficial effects has been ascribed to creatine (Cr), phosphocreatine (PCr) and their cyclic analogues cyclo-(cCr) and phospho-cyclocreatine (PcCr). Cr is widely used as nutritional supplement in sports and increasingly also as adjuvant treatment for pathologies such as myopathies and a plethora of neurodegenerative diseases. Additionally, Cr and its cyclic analogues have been proposed for anti-cancer treatment. The mechanisms involved in these pleiotropic effects are still controversial and far from being understood. The reversible conversion of Cr and ATP into PCr and ADP by creatine kinase, generating highly diffusible PCr energy reserves, is certainly an important element. However, some protective effects of Cr and analogues cannot be satisfactorily explained solely by effects on the cellular energy state [008].

A safe substance

There is an extensive and still growing body of the literature supporting the efficacy of creatine (Cr) supplementation. In sports, creatine has been recognized as the most effective nutritional supplement in enhancing exercise tolerance, muscle strength and lean body mass. From a clinical perspective, the application of Cr supplementation is indeed exciting. Evidences of benefits from this supplement have been reported in a broad range of diseases, including myopathies, neurodegenerative disorders, cancer, rheumatic diseases, and type 2 diabetes. In addition, after hundreds of published studies and millions of exposures creatine supplementation maintains an excellent safety profile. Thus, it is contended that the widespread application of this supplement may benefit athletes, elderly people and various patient populations [009].

Hypertrophic response to resistance training

Maximal muscle strength is strongly influenced by resistive-types of exercise, which induce adaptive changes in both neuromuscular function and muscle morphology. Further, timed intake of protein in conjunction with resistance training elicit greater strength and muscle size gains than resistance training alone. Creatine supplementation amplifies the hypertrophic response to resistance training, although some individuals may not respond positively. Locally produced muscle growth factors are upregulated during creatine supplementation, which contributes to increase the responsiveness of muscle cells to intensive training stimuli. Usage of anabolic steroids boosts muscle hypertrophy beyond inherent genetical limits, not only by increasing the DNA transcription rate for myofibrillar proteins but also by increasing the nucleus-to-cytoplasm ratio due to accelerated activation of myogenic satellite cells. However, severe tissue damaging effects exist with anabolic steroids, some of which are irreversible [010].
Short-term creatine supplementation

Creatine has become a popular nutritional supplement among athletes. Recent research has also suggested that there may be a number of potential therapeutic uses of creatine. This paper reviews the available research that has examined the potential ergogenic value of creatine supplementation on exercise performance and training adaptations. Review of the literature indicates that over 500 research studies have evaluated the effects of creatine supplementation on muscle physiology and/or exercise capacity in healthy, trained, and various diseased populations. Short-term creatine supplementation (e.g. 20 g/day for 5-7 days) has typically been reported to increase total creatine content by 10-30 percent and phosphocreatine stores by 10-40 percent. Of the approximately 300 studies that have evaluated the potential ergogenic value of creatine supplementation, about 70 percent of these studies report statistically significant results while remaining studies generally report non-significant gains in performance. No study reports a statistically significant ergolytic effect. For example, short-term creatine supplementation has been reported to improve maximal power/strength (5-15 %), work performed during sets of maximal effort muscle contractions (5-15 %), single-effort sprint performance (1-5 %), and work performed during repetitive sprint performance (5-15 %). Moreover, creatine supplementation during training has been reported to promote significantly greater gains in strength, fat free mass, and performance primarily of high intensity exercise tasks. Although not all studies report significant results, the preponderance of scientific evidence indicates that creatine supplementation appears to be a generally effective nutritional ergogenic aid for a variety of exercise tasks in a number of athletic and clinical populations [011].

A commercial success

During the past decade (1990-2000), the nutritional supplement creatine monohydrate has been gaining popularity exponentially, with reported annual sales in the US alone climbing from USD 50 million in 1996 to over USD 400 million during 2001. Creatine supplementation (CrS) first gained popular attention in the early 1990s, after high profile Olympic athletes competing in sprint and power events at the Barcelona Olympic Games believed their performance had benefited from CrS. Since this time creatine (Cr) has become one of the most widely used nutritional supplements with an estimated worldwide consumption of 2.7 million kilograms. Recently, many athletes and teams have implemented oral CrS in an effort to enhance sports performance, as CrS is not on the banned substance list by the International Olympic Committee (2003). Thus, using this supplement would not constitute anything illegal or unethical on behalf of the athlete or coach. Consequently, Cr has risen to the top of the modern athletes shopping list [002].
ON RESEARCH ON CREATINE

Creatine is one of the most popular athletic supplements with sales in the US surpassing 400 million dollars in 2004. Due to the popularity and efficacy of creatine supplementation over 200 studies have examined the effects of creatine on athletic performance. Despite the abundance of research suggesting the effectiveness and safety of creatine a fallacy appears to exist in the general public driven by media claims and anecdotal reports that creatine supplementation can result in muscle cramps and dehydration [012].

Due to the popularity and efficacy of creatine supplementation over 200 studies had in 2008 examined the effects of creatine on athletic performance. Despite the abundance of research suggesting the effectiveness and safety of creatine a fallacy appears to exist in the general public driven by media claims and anecdotal reports that creatine supplementation can result in muscle cramps and dehydration. Although, a number of published studies have refuted these claims, a position statement by the American College of Sports Medicine (ACSM) in 2001 advised individuals who are managing their weight and exercising intensely or in hot environments to avoid creatine supplementation [013].

Creatine has been one of the more extensively studied dietary supplements. There had in 2013 been upward of 300 studies evaluating the effects of creatine on resistance training, with 70 percent reporting increases in strength in 2013. Several forms of creatine exist; however creatine monohydrate has been the most extensively studied, and its formulation has shown benefits in short-duration, high-intensity weightlifting and cycling [014].

Inconsistent results

Short-term creatine (CrS) has been reported to improve maximal power/strength, work performed during sets of maximal effort muscle contractions, single-effort sprint performance, and work performed during repetitive sprint performance. During training CrS has been reported to promote significantly greater gains in strength, fat free mass, and exercise performance primarily of high intensity tasks. However, not all studies demonstrate a beneficial effect on exercise performance, as CrS does not appear to be effective in improving running and swimming performance [002].

Over the past few years there has been considerable interest in both the use of creatine (Cr) supplementation by athletes and the documentation of its effects by scientists. Some believe that this nitrogen-containing compound found in meat and fish has a performance-enhancing capability as important for brief intense exercise efforts as dietary carbohydrate is for activities where glycogen supplies limit performance. The mechanisms thought to be responsible for any ergogenic effect of acute (few d) Cr supplementation include: increased stores of muscle phosphocreatine (PCr), faster regeneration of PCr during exercise recovery, enhanced adenosine triphosphate (ATP) production from glycolysis secondary to increased hydrogen ion buffering, and/or possible shortened post contraction muscle relaxation time. With chronic (weeks to months) supplementation when combined with strength training, Cr may alter muscle protein metabolism directly (via decreasing protein breakdown or increasing synthesis) and/or indirectly as a result of a greater training load made possible by its acute ergogenic effects on strength and power. Cr supplementation is not banned by the International Olympic Committee and, with the exception of a small increase in body mass (approximately 1 kg) over the initial 36 d, does not appear to have any adverse side effects, at least with short-term use. Few scientific data are available for more prolonged use (mo or y) but considering the large numbers of athletes using Cr over the past 6+ years and the absence of reported problems, it may be that the often discussed somewhat nebulous long term adverse effects are presently being overestimated. Intakes of 285-300 mg Cr/kg body
mass 1 over 36 d or 3050 mg/kg body mass 1 over approximately 4 wk are sufficient to produce benefits (muscle mass and high intensity power gains); however, not all study results are consistent. The focus of this review is to outline some possible explanations for the inconsistent observations reported in the literature. Clearly, if proven to be consistent the benefits of Cr supplementation could extend far beyond the athletic arena to include individuals who experience muscle weakness for a variety of other reasons (e.g. age/disuse, muscle disease, exposure to microgravity, etc) [015].

It was stated in 2013 that multiple studies have investigated creatine supplementation effect on sprinting, swimming, and agility training and have failed to show an effect [014].

**Few high-quality studies**

Following the first reports in 1992 that PCr content in human muscle can increase up to 50 percent following daily CrS (5 g Cr monohydrate 4-6 × day for ≥ 2 days), a number of studies have examined the effects of CrS on muscle metabolism and/or high-intensity exercise performance. Studies that have measured muscle total creatine (TCr) (phosphocreatine + creatine) have reported an elevation in TCr after CrS involving loading phases of 20-30 g/day for 3-6 days. Some studies found that both resting TCr and PCr content increased, whereas others reported significant increases in only TCr. Theoretically, an increase in TCr stores may provide an ergogenic effect during high intensity exercise by enhancing the rate of ATP synthesis during contraction and by improving the rate of PCr resynthesis during recovery, which may be beneficial for repeated sprint activity. An investigation reported in 2000 supports such a contention, concluding that acute CrS favourably affected repeated sprint performance and limited the decay in jumping ability in highly trained soccer players. However, on the whole, experimental evidence supporting an ergogenic effect for CrS is somewhat mixed. Several studies have demonstrated improved high-intensity exercise performance after CrS, whereas others have reported no beneficial effects. A possible explanation for the conflicting findings may relate to the experimental design used to examine the effects of CrS on exercise performance. Most studies have employed a cross-sectional experimental design or an ordered treatment allocation. However, few studies have utilised a crossover experimental design, possibly due to the time required for muscle TCr to return to basal levels after CrS was unknown. It was indicated that a variety of factors including, but not limited to, sample size, exercise modality, rest and recovery intervals, residual effects of cessation of CrS, non-responders, gender and age effects and methodology used, make any interpretation of existing Cr literature extremely difficult [002].

**Selected groups investigated**

Most of the evidence has been obtained from healthy young adult male subjects with mixed athletic ability and training status. Less research information is available related to the alterations due to age and gender [016].
BIOCHEMISTRY OF CREATINE

Creatine (N-(aminoiminomethyl)-N-methyl glycine) is an ingredient commonly found in food, mainly in fish and meat, and is sold as a dietary supplement in markets around the world. Creatine crystallizes from water as monoclinic prisms holding one molecule of water of crystallization per molecule of creatine. Continued drying of CM results in a loss of the water of crystallization at around 100°C, yielding anhydrous creatine. Creatine is a weak base with a pkb value of 11.02 at 25°C. As a result, creatine can only form salts with strong acids, having a pka value of less than 3.98. Creatine forms salts by the protonation of its guanidine moiety. In addition to salt formation, creatine is able to act as a complexing agent [017].

Amounts and anatomy of creatine

Endogene production

Creatine monohydrate (Cr) is produced endogenously by the liver or ingested from exogenous sources such as meat and fish. Almost all the Cr in the body is located in skeletal muscle in either the free (Cr: approximately 40 %) or phosphorylated (PCr: approximately 60 %) form and represents an average Cr pool of about 120-140 g for an average 70 kg person [018].

Synthesis

Creatine, a derivative from three amino acids, is distributed at approximately 95 percent in skeletal muscle mass; the remainder is located in the brain, the testes and the kidneys. Its synthesis starts mainly in the kidneys from glycine and arginine, forming alpha-methylguanidoacetic acid, which is conducted through the blood to the liver where it reacts with S-adenosylmethionine to synthesise creatine. Approximately 1-2 g of creatine is produced over 24 h and released mainly to the skeletal muscle system [004].

Dietary intake

Some creatine is also added to the pool by adequate dietary intake, predominantly from meat and fish, with a typical diet supplying approximately 1-2 g of creatine daily. It may be assumed that there is a total creatine pool of approximately 120 g in a man of 70 kg body weight. In skeletal muscle, creatine is slowly degraded to creatinine (approximately 2 g/day), a reaction without any enzyme intervention, and is released to the blood and the kidney to be expelled through the urine [004].

Anatomical localization

Creatine, a derivative from three amino acids, is distributed at approximately 95 percent in skeletal muscle mass; the remainder is located in the brain, the testes and the kidneys [004].

Exogenous and endogenous creatine

Creatine is a nitrogenous amine that was discovered in 1832. It is found primarily in skeletal muscle, with 95 percent of the body's creatine stores found within skeletal muscle. The total amount of creatine in the body is equal to the free creatine plus the phosphocreatine, which equals approximately 120 g in a 70-kg person. The exogenous sources of creatine are animal products such as red meat and fish. The normal dietary intake of creatine in an
omnivorous diet is around 1 g per day. The liver, kidney, and pancreas form endogenous stores of creatine. The endogenous production of creatine is down-regulated during exogenous creatine supplementation; however the endogenous production returns to baseline after supplementation is discontinued. The first step in endogenous synthesis of creatine occurs in the kidney and starts with the amino acids glycine and arginine. The product is then transferred to the liver where a methyl group from methionine is added forming creatine. Circulating creatine is brought into skeletal muscle via transporters in the cell membrane. The rate of creatine uptake has been shown to be influenced by exercise, catecholamines, and insulin-like growth factor. Once within the cell, creatine can be phosphorylated to form phosphocreatine in a reversible enzymatic reaction facilitated by creatine kinase. The phosphate group comes from ATP forming adenosine diphosphate (ADP). The reverse reaction occurs when ATP is being used by the cell, and phosphocreatine can shuttle a phosphate group to ADP [014].

**Glycine, arginine and methionine for synthesis of creatine**

Creatine is produced endogenously at an amount of about 1 g/d. Synthesis predominately occurs in the liver, kidneys, and to a lesser extent in the pancreas. The remainder of the creatine available to the body is obtained through the diet at about 1 g/d for an omnivorous diet. Ninety-five percent of the bodies' creatine stores are found in the skeletal muscle and the remaining 5 percent is distributed in the brain, liver, kidney, and testes. As creatine is predominately present in the diet from meats, vegetarians have lower resting creatine concentrations. The majority of creatine in the human body is in two forms, either the phosphorylated form making up 60 percent of the stores or in the free form which makes up 40 percent of the stores. The average 70 kg young male has a creatine pool of around 120-140 g which varies between individuals depending on the skeletal muscle fiber type and quantity of muscle mass. The endogenous production and dietary intake matches the rate of creatinine production from the degradation of phosphocreatine and creatine at 2.6 percent and 1.1 percent/day respectively. In general, oral creatine supplementation leads to an increase of creatine levels within the body. Creatine can be cleared from the blood by saturation into various organs and cells or by renal filtration. Three amino acids (glycine, arginine and methionine) and three enzymes (L-arginine:glycine amidinotransferase, guanidinoacetate methyltransferase and methionine adenosyltransferase) are required for creatine synthesis. The impact creatine synthesis has on glycine metabolism in adults is low, however the demand is more appreciable on the metabolism of arginine and methionine. Creatine ingested through supplementation is transported into the cells exclusively by CreaT1. However, there is another creatine transporter Crea T2, which is primarily active and present in the testes. Creatine uptake is regulated by various mechanisms, namely phosphorylation and glycosylation as well as extracellular and intracellular levels of creatine. Crea T1 has shown to be highly sensitive to the extracellular and intracellular levels being specifically activated when total creatine content inside the cell decreases. It has also been observed that in addition to cytosolic creatine, the existence of a mitochondrial isoform of Crea T1 allows creatine to be transported into the mitochondria. Indicating another intra-mitochondrial pool of creatine, which seems to play an essential role in the phosphate-transport system from the mitochondria to the cytosol. Myopathy patients have demonstrated reduced levels of total creatine and phosphocreatine as well as lower levels of CreaT1 protein, which is thought to be a major contributor to these decreased levels [019].

**Uptake of oral creatine**

The uptake of creatine is simplified in a two-step approach: first, uptake into the blood stream; second, uptake into the target tissue. The term “bioavailability” refers to both the
intestinal absorption and the use of a substance by the body's cells and tissues. First indications of a potential change of creatine bioavailability can be gathered from the amount of creatine taken up into the blood plasma after oral administration. However, a change in the total amount of creatine in the blood plasma cannot be directly extrapolated to a potential increase in desired performance. An increased amount of creatine in the plasma could be the result of decreased uptake into the target tissue resulting in an actual decrease in overall bioavailability. On the other hand, an initial rise in plasma creatine levels, followed by a reduction in plasma levels, is an indication of increased uptake into the target tissue. This has been demonstrated in vivo by combining creatine with insulin-stimulating ingredients such as high amounts of glucose or protein. Conclusive proof of an increase in relevant bioavailability can only be gained by assessing the amount of creatine reaching the target tissue, the muscle, measured by muscle biopsy and/or whole body creatine retention assessed by measuring the difference between creatine intake and urinary excretion. Dietary creatine is presumed to have high bioavailability since intestinal absorption of creatine monophosphate (CM) is already close to 100 percent. However, the response to creatine supplementation is heterogeneous, due in part to some non-responders, which might be overcome by alternative forms of creatine. Over the years, there has been significant commercial interest in determining whether creatine could be delivered in a liquid form. The thought has been since CM is relatively insoluble that development of a liquid or suspended form of creatine may be more convenient to consume, be more readily absorbed into the blood stream, and promote a greater efficiency in transport of creatine to the muscle. Some companies have even claimed that minimal amounts of liquid creatine would need to be ingested because of enhanced efficiency in transport through the blood and into the muscle. A limitation with these theories is that CM is not stable for any substantial length of time in liquid. Consequently, while researchers have been working on ways to suspend creatine within gels and fluids, it has been generally considered to be impractical to develop into a product due to limitations in shelf-life. In addition, while people may prefer the taste of liquid or gel versions of creatine, there is no evidence that these delivery forms provide a superior performance benefit. In analysis of this literature, it is clear that CM supplementation promotes significant increases in muscle creatine levels in most individuals. There is some evidence that co-ingestion of CM with various nutrients (e.g. carbohydrate, protein, d-pinitol) may enhance creatine uptake to a greater degree. However, there is no evidence that effervescent creatine, liquid creatine, and/or CEE promotes greater uptake of creatine to the muscle. Rather, there is some evidence that some of these forms of creatine may be less effective and/or be of greater clinical concern in terms of safety [017].

Absorption of creatine supplied as a drink, in meat or in solid form

It was examined the plasma concentration curve obtained over 6 h after the ingestion of 2 g of creatine (Cr) (equivalent to 2.3 g Cr hydrated) contained in meat or in solution in five non-users of creatine supplements. Peak plasma creatine concentration was lower after the ingestion of meat but was maintained close to this for a longer period. Measurements of the area under the plasma concentration curve indicated approximate bioequivalence of creatine contained in meat with the same dose supplied in a solution. In a separate study, it was examined the plasma concentration time curve after ingestion of solid Cr x H2O. Creatine ingested as a lozenge (crushed in the mouth and swallowed) or as a crystalline suspension in ice cold water resulted in a 20 percent lower peak concentration and 30-35 percent smaller area under the plasma creatine concentration curve than the same dose administered in solution. Despite a possibly lower bioavailability, hydrated 2.3 g Cr supplied in either solid form was nonetheless sufficient to raise the plasma concentration five- to six-fold in individuals with a mean body mass of 76 kg. It was conclude that creatine administered as meat or in solid form is readily absorbed but may result in slightly lower peak concentrations than when the same dose is ingested as a solution [020].
**Pre-exercise oral creatine ingestion**

One investigation determined whether pre-exercise oral Cr ingestion could enhance prolonged intermittent sprint exercise performance in a randomised, double-blind crossover design was employed. Testing was performed at the Western Australian Institute of Sport and participants were monitored and treated by both scientific and medical personnel. Eight active, but not well-trained males with a background in multiple-sprint based sports acted as subjects for this investigation. The subjects ingested either 15 g Cr or placebo 120 min and 60 min prior to the start of an 80-min maximal sprint cycling task (10 sets of multiple 6-sec sprints with varying active recoveries). Subjects were retested 14 days later, being required to ingest the alternate supplement and repeat the exercise test. Performance variables (work done and peak power) were obtained throughout the exercise challenge. Muscle biopsies (vastus lateralis) were raised to a peak of 2348 ± 223 micromol/L prior to the commencement of exercise after Cr ingestion. There were no significant changes in any cycling performance parameters following Cr ingestion, although blood La- was significantly lower than placebo at all time points during were taken preexercise as well as immediately and 3 min post-exercise in order to determine concentrations of ATP, PCr, Cr, La- and glycogen. Venous blood was drawn prior to and on four occasions during the exercise test, and analysed for Cr, NH3+, La- and pH. Serum Cr concentrations exercise, and plasma NH3+ accumulation was also significantly reduced in the Cr condition, but only in the second half of the 80-min exercise test. Muscle ATP and TCr levels as well as postexercise PCr replenishment were unaffected following Cr administration. The data suggest that although the pre-exercise ingestion of a large Cr dose was shown to have some impact on blood borne metabolites, it does not improve maximal prolonged intermittent sprint exercise performance, possibly due to an insufficient time allowed for uptake of serum Cr by skeletal muscle to occur. Therefore, this form of loading does not provide an alternative method of Cr supplementation to the traditional five-day supplementation regimes established by previous research [021].

**Metabolism of creatine**

Studies have indicated that creatine monophosphate (CM) is not degraded during normal digestion and that nearly 99 percent of orally ingested CM is either taken up by muscle or excreted in urine [017].

Creatine monohydrate powder is very stable showing no signs of degradation over years, even at elevated temperatures. To detect a potential degradation of creatine, one must measure the content of its degradation product, creatinine. The degradation of creatine can be reduced or even halted by either lowering the pH under 2.5 or increasing the pH. A very high pH results in the deprotonation of the acid group, thereby slowing down the degradation process by making it more difficult for the intramolecular cyclization. A very low pH results in the protonation of the amide function of the creatine molecule, thereby preventing the intramolecular [017].

**Creatine kinas**

Total creatine kinase (CK) levels depend on age, gender, race, muscle mass, physical activity and climatic condition. High levels of serum CK in apparently healthy subjects may be correlated with physical training status, as they depend on sarcomeric damage: strenuous exercise that damages skeletal muscle cells results in increased total serum CK. The highest post-exercise serum enzyme activities are found after prolonged exercise such as ultradistance marathon running or weight-bearing exercises and downhill running, which include eccentric muscular contractions. Total serum CK activity is markedly elevated for 24 h after the exercise bout and, when patients rest, it gradually returns to basal levels.
Persistently increased serum CK levels are occasionally encountered in healthy individuals and are also markedly increased in the pre-clinical stages of muscle diseases. Some authors, studying subjects with high levels of CK at rest, observed that, years later, subjects developed muscle weakness and suggested that early myopathy may be asymptomatic. Others demonstrated that, in most of these patients, hyperCKemia probably does not imply disease. In many instances, the diagnosis is not formulated following routine examination with the patients at rest, as symptoms become manifest only after exercise. Some authors think that strength training seems to be safe for patients with myopathy, even though the evidence for routine exercise prescription is still insufficient. Others believe that, in these conditions, intense prolonged exercise may produce negative effects, as it does not induce the physiological muscle adaptations to physical training given the continuous loss of muscle proteins. High CK serum levels in athletes following absolute rest and without any further predisposing factors should prompt a full diagnostic workup with special regards to signs of muscle weakness or other simple signs that, in both athletes and sedentary subjects, are not always promptly evident. These signs may indicate subclinical muscle disease, which training loads may evidence through the onset of profound fatigue. It is probably safe to counsel athletes with suspected myopathy to continue to undertake physical activity at a lower intensity, so as to prevent muscle damage from high intensity exercise and allow ample recovery to favour adequate recovery. CK values show great variability among individuals. Some athletes are low responders to physical training, with chronically low CK serum levels. Some athletes are high responders, with higher values of enzyme: the relationship among level of training, muscle size, fibre type and CK release after exercise should be investigated further. In addition, more details about hyperCKemia could come from the evaluation of the kinetics of CK after stress in healthy athletes with high levels of CK due to exercise, comparing the results with the ones obtained from athletes with persistent hyperCKemia at rest. Finally, it would be important to quantify the type of exercise more suited to athletes with myopathy and the intensity of exercise not dangerous for the progression of the pathology [022].

Under such conditions the catalytic concentration of CK in the serum displays a far greater increase than the serum concentration of other muscle proteins. The serum concentration of CK peaks 1-4 days after exercise and remains elevated for several days. Thus, athletes participating in daily training have higher resting values than non-athletes, although this response to training is mitigated by the so-called repeated-bout effect. The serum concentration of creatine kinase (CK) is used widely as an index of skeletal muscle fibre damage in sport and exercise. Since athletes have higher CK values than non-athletes, comparing the values of athletes to the normal values established in non-athletes is pointless. The purpose of this study was to introduce reference intervals for CK in athletes. CK was assayed in serum samples from 483 male athletes and 245 female athletes, aged 7-44. Samples had been obtained throughout the training and competition period. For comparison, CK was also assayed in a smaller number of non-athletes. The reference intervals were 82-1083 U/L (37 degrees C) in male and 47-513 U/L in female athletes. The upper reference limits were twice the limits reported for moderately active non-athletes in the literature or calculated in the non-athletes in this study. The upper limits were up to six times higher than the limits reported for inactive individuals in the literature. When reference intervals were calculated specifically in male football (soccer) players and swimmers, a threefold difference in the upper reference limit was found (1492 vs 523 U/L, respectively), probably resulting from the different training and competition demands of the two sports. It was concluded that sport training and competition have profound effects on the reference intervals for serum CK. Introducing sport-specific reference intervals may help to avoid misinterpretation of high values and to optimise training [023].
Creatine degrades to creatinine

Creatine is one of the main compounds in muscular energetic metabolism leading to phosphocreatine to maintain high ATP levels. Creatine is found in blood and excreted in small amounts in urine. Approximately 95 percent of total creatine is located in skeletal muscles in free and phosphorylated forms, the latter being essentially used in intense short-term activities. The phosphorylation step is reversible and phosphocreatine maintains the adenosine triphosphate/adenosine diphosphate ratio in muscle tissues. Creatine liberated from phosphocreatine undergoes nonenzymatic cyclization to form creatinine, which is excreted in urine in high amounts and at a constant rate. Therefore, creatine may be used as a dietary supplement, and a positive effect of pure creatine ingestion has sometimes been noted on physical performances [024].

Creatine kinetics in healthy men and women

Creatine kinetics were measured in young healthy subjects, eight males and seven females, age 20-30 years, after an overnight fast on creatine-free diet. Whole body turnover of glycine and its appearance in creatine was quantified using [1-13C] glycine and the rate of protein turnover was quantified using L-ring [2H5] phenylalanine. The creatine pool size was estimated by the dilution of a bolus [C2H3] creatine. Studies were repeated following a five days supplement creatine 21 g/day and following supplement amino acids 14.3 g/day. Creatine caused a ten-fold increase in the plasma concentration of creatine and a 50 percent decrease in the concentration of guanidinoacetic acid. Plasma amino acids profile showed a significant decrease in glycine, glutamine, and taurine and a significant increase in citrulline, valine, lysine, and cysteine. There was a significant decrease in the rate of appearance of glycine, suggesting a decrease in de-novo synthesis. The fractional and absolute rate of synthesis of creatine was significantly decreased by supplemental creatine. Amino acid supplement had no impact on any of the parameters. This is the first detailed analysis of creatine kinetics and the effects of creatine supplement in healthy young men and women. These methods can be applied for the analysis of creatine kinetics in different physiological states [025].

Urinary excretion

The aim of one study is to investigate urinary creatine (URCR) and urinary creatinine (URCRN) response to CR supplementation in conjunction with exercise performance. Twenty-one sprint trained males were randomly divided into 3 groups. Each group followed a different CR dosage (10 g, 25 g and 35 g/day for 4 days) and placebo (Pl) in the 1st and 2nd week, respectively. A double-blind design was used. Subjects’ urine was collected every 24 hours during the entire period of supplementation (SP). All groups, at the end of each SP performed 3 times the Anaerobic Wingate Test (AWT) with 6 min active recovery (60 rpm) on a cycle ergometer. Significantly higher peak and mean power values were produced during the CR compared to Pl condition. A significant correlation was also observed between peak power improvement (PPI) and URCR for the 3 groups. No such relationship was found between URCRN. Across all groups, URCR and URCRN increased significantly following ingestion compared with Pl. URCR post- supplementation presented a 7.4 fold, 36 fold and 21 fold increase for 10 g, 25 g and 35 g dose respectively, whilst URCRN presented a mean 2.4 fold increase for all different doses, which clearly shows the magnitude of sensitivity of these indices to CR supplementation. A strong correlation observed between dose of CR ingestion and mean URCR (MRUCR) with prediction formula: CR = -0.936 + (5.613 x MRUCR) (SEE=3.5). URCR was an effective measure of each CR dosage administered as well as of the excretion pattern that each group followed throughout the SP. Furthermore the
strong relationship of URCR and PPI could be particularly useful for monitoring and optimising CR loading in athletic populations [026].

**Excretion in urine of creatine**

Creatine is now commercially available over-the-counter in the United States and is widely used by athletes. The daily ingested dose is generally 5 g, which is equivalent to about 1 kg of uncooked meat. Most published analytical techniques for creatine analysis involve high-performance liquid chromatography. With liquid chromatography coupled to mass spectrometry in the chemical ionization mode, it was determined creatine, creatinine, and glycoctamine in serum and urine. It was also separated and quantitated creatine and creatinine by capillary electrophoresis with ultraviolet (UV) detection. The authors followed the excretion of creatine in urine as a function of time, after creatine ingestion by two athletes (100 mg/kg body mass). Following ingestion, the maximum of creatine excretion in urine is after about 2-3 h and a return to basal values is noted after 13 to 14 h. In physiological conditions, reference values of creatine excretion are scarce. However, creatine excretion can rise to 200 mmol/mol creatinine (26.2 g/mole creatinine). In one paper its mean excretion is 0.19 + 0.03 mmol/L (24.9 mg/L) in humans. In another study, creatine excretion in adults ranges from 150 to 1200 pmol/24 h (20 to 157 rag/24 h). On the contrary, the sale of creatine is prohibited in France; it is considered a doping substance. Creatine supplementation and athletic performances are supposed to be correlated, particularly in intensive and intermittent efforts. After oral creatine supplementation, a 1H nuclear magnetic resonance (NMR) spectroscopy method was developed for its direct analysis, without any pretreatment of urine samples. This method can be used to detect any supplementation of creatine, a substance prohibited in France. The detection limit is 10 micromol/L (1.31 mg/L) and analysis is performed in 10 min. After a single oral supplementation of 2.1 g to three subjects, a kinetic investigation reveals a maximum concentration of 20 mmol/L (2.62 g/L), observed between 1 and 6 h after ingestion. This procedure was used to test 13 urine specimens obtained from bodybuilders. From the concentrations measured (range: 0.41 to 10.30 mmol/L, 54 to 1350 mg/L), the doping practices of at least nine athletes could be observed. Creatine is not often analyzed in hospital laboratories. Nuclear magnetic resonance (NMR) analysis can be used to determine a wide range of small molecules in biological fluids and offers several advantages: it is rapid, nondestructive, and the determination of many different chemical species is possible on the same analysis. This technique has already contributed to clinical diagnosis and thus has a promising use in urine analysis. Among compounds which can be detected, creatinine determination by 1H NMR spectroscopy can be used. Considering the chemical analogy between creatine and creatinine and their metabolic link, it is suitable to determine both compounds simultaneously. Their detection and quantitation can be obtained by 1H NMR spectroscopy using their respective singlet peaks at characteristic chemical shifts. One paper documents how easily creatine can be determined and quantitated by 1H NMR spectroscopy [024].
Creatine is involved in the regulation of cellular energy demand. Under resting conditions, ATP is mainly formed in mitochondria through oxidative phosphorylation with ADP. Transported in sarcoplasm, some ATP molecules react with creatine, via the enzyme phosphorylcreatine kinase, to form phosphorylcreatine and ADP until equilibrium is reached. When ATP is needed for cellular energy, such as for muscle contraction, the phosphorylcreatine kinase reverse reaction replenishes the ATP content. Creatine thus acts indirectly to maintain a phosphorylcreatine reservoir for energy needs, more specifically to supply the muscle system with ATP [004].

Creatine supplementation’s ergogenic effects are thus largely explained by increasing intramuscular phosphocreatine (PCr) stores. The increase in PCr allows adenosine diphosphate (ADP) to be readily rephosphorylated to adenosine triphosphate (ATP), which is depleted rapidly during strenuous exercise. Thus, increasing the amount of PCr through creatine supplementation increases the capacity to rephosphorylate ADP to ATP, and this allows the athlete to resist fatigue and maintain a higher level of performance. This increase in fatigue resistance created by creatine supplementation allows for increased training volume [001].

To explain the elevated hypertrophic response with combined resistance training and creatine supplementation, an increased production of myogenic growth factors seems to occur in the muscle tissue itself. Thus, increased mRNA and protein levels of various myogenic regulatory factors (MyoD, myogenin, MRF4) have been observed following combined training and creatine intake. Although resistance training per se results in increased mRNA and protein content of MyoD, myogenin and MRF4, this increase is substantially accelerated when training is combined with creatine intake. A rise in myogenic regulatory factors with creatine supplementation may not per se elicit an enhanced hypertrophic response, rather it increases the sensitivity of the muscle cell to the resistance training stimulus, which in turn contributes to the accelerated hypertrophy [010].

**Possible multiple mechanisms for effect of creatine**

The elevated muscle creatine content moderately improves contractile performance in sports with repeated high-intensity exercise bouts. More chronic ergogenic effects of creatine are to be expected when combined with several weeks of training. A more pronounced muscle hypertrophy and a faster recovery from atrophy have been demonstrated in humans involved in resistance training. The mechanism behind this anabolic effect of creatine may relate to satellite cell proliferation, myogenic transcription factors and insulin-like growth factor-1 signaling. An additional effect of creatine supplementation, mostly when combined with training, is enhanced muscle glycogen accumulation and glucose transporter (GLUT4) expression. Thus, creatine may also be beneficial in sport competition and training characterized by daily glycogen depletion, as well as provide therapeutic value in the insulin-resistant state [027].

The mechanisms through which creatine supplementation improves exercise performance and body composition may also include metabolic enhancements (increased pre-exercise phosphorylcreatine, increased pre-exercise muscle glycogen), molecular adaptations (increased gene expression of growth factors) and reduced muscle damage. However, creatine supplementation does not increase skeletal muscle protein synthesis [004].
Decreased levels of myostatin

In addition to chronic performance benefits, creatine aids those seeking to increase muscle mass. Muscle mass increases can be explained in part by creatine increasing training volume, but it is also partially explained by other factors. It was confirmed creatine’s effects on increasing lean body mass after chronic resistance training, and in addition, they observed decreased levels of myostatin in the creatine supplemented group and demonstrated increased myonuclei and muscle fiber area after 16 weeks of concurrent resistance training and creatine supplementation. Additionally, 12 weeks creatine supplementation has been demonstrated to increase myogenic regulatory factor expression and myogenin, both of which initiate transcription and regulate gene expression, which likely contribute to the anabolic effects of creatine [001].

Effect on myogenic satellite cells

A series of studies were conducted in which compounds commonly shown to be ergogenic aids for strength athletes if taken orally were evaluated for their ability to directly induce postnatal muscle stem cell proliferation or differentiation/fusion in vitro. Compounds tested were creatine monohydrate, creatine pyruvate, L-glutamine, dehydroepiandrosterone (DHEA), androstenedione, Ma Huang (Ephedra sinensis) extract, and Zhi Shi (Citrus aurantium) extract. Dulbecco’s modified eagle medium, supplemented with minimal levels of serum and antibiotics, was used as the initial vehicle for the test compounds. Subsequently, a defined treatment medium termed ITTC was used. Satellite cells were exposed to the test compounds for the indicated times and then evaluated by counting mononucleated and multinucleated (fused) cells. In serum-containing media, none of the treatment groups displayed increased proliferation over that of the control. However, in the differentiation cultures, 0.10 percent creatine monohydrate increased differentiation over that of the control cultures. When 0.10 percent creatine monohydrate was added to defined media formulations, all treatments but one demonstrated increased differentiation over the 0.5 percent serum control. Time course experiments, which followed the effect of 0.10 percent creatine monohydrate contained in ITTC defined media over 120 h, suggested that cells exposed to this treatment differentiated earlier and to a greater level than cells exposed to ITTC alone. It was concluded that creatine in the monohydrate form induced differentiation of myogenic satellite cells. Other agents examined did not increase satellite cell proliferation or differentiation. These results provide initial evidence for a mechanistic understanding of observed effects in vivo of increased muscular size and strength from creatine supplementation [028].

Creatine monohydrate supplementation increases satellite cell mitotic activity

Nutritional status influences muscle growth and athletic performance, but little is known about the effect of nutritional supplements, such as creatine, on satellite cell mitotic activity. The purpose of this study was to examine the effect of oral creatine supplementation on muscle growth, compensatory hypertrophy, and satellite cell mitotic activity. Compensatory hypertrophy was induced in the rat plantaris muscle by removing the soleus and gastrocnemius muscles. Immediately following surgery, a group of six rats was provided with elevated levels of creatine monohydrate in their diet. Another group of six rats was maintained as a non-supplemented control group. Twelve days following surgery, all rats were implanted with mini-osmotic pumps containing the thymidine analog 5-bromo-2'-deoxyuridine (BrdU) to label mitotically active satellite cells. Four weeks after the initial surgery the rats were killed, plantaris muscles were removed and weighed. Subsequently, BrdU-labeled and non-BrdU-labeled nuclei were identified on enzymatically isolated myofiber segments. Muscle mass and myofiber diameters were larger in the muscles that underwent
compensatory hypertrophy compared to the control muscles, but there were no differences between muscles from creatine-supplemented and non-creatine-supplemented rats. Similarly, compensatory hypertrophy resulted in an increased number of BrdU-labeled myofiber nuclei, but creatine supplementation in combination with compensatory hypertrophy resulted in a higher number of BrdU-labeled myofiber nuclei compared to compensatory hypertrophy without creatine supplementation. Thus, creatine supplementation in combination with an increased functional load results in increased satellite cell mitotic activity [029].

Enhanced satellite cell activity

Animal experiments have shown that creatine supplementation may result in enhanced satellite cell activity. Given the fact that locally produced IGF-1 exerts similar effects, it is possible that combined creatine intake and resistance training leads to elevated satellite cell activation compared to resistance training alone, which amplifies the hypertrophic response [010].

Influence on myonuclei concentration

Research using muscle biopsy techniques has also shown increases in muscle cell diameter associated with 12 weeks of creatine supplementation and resistance training, which may be associated with the potential for creatine supplementation to amplify the resistance training-induced increase in satellite cell number and myonuclei concentration in skeletal muscle [030].

Effect on myosin heavy chain

It was studied the effect of 12 weeks of creatine supplementation and resistance training on muscular strength and myosin heavy chain (MHC) mRNA and protein expression. Compared to both a control and placebo group, the creatine group significantly increased fat-free mass and strength. Additionally, it was found that in general the MHC mRNA and protein expression were significantly higher in the creatine group compared to the other groups, and suggested that the increased strength and muscle size associated with creatine supplementation may be attributed to increase MHC synthesis [030].

Oral creatine and resistance training on myosin heavy chain expression

One study examined 12 weeks of creatine (Cr) supplementation and heavy resistance training on muscle strength and myosin heavy chain (MHC) isoform mRNA and protein expression. Twenty-two untrained male subjects were randomly assigned to either a control (CON), placebo (PLC), or Cr (CRT) group in a double-blind fashion. Muscle biopsies were obtained before and after 12 wk of heavy resistance training. PLC and CRT trained thrice weekly using three sets of 6-8 repetitions at 85-90 percent 1-RM on the leg press, knee extension, and knee curl exercises. CRT ingested 6 g/d of Cr for 12 weeks, whereas PLC consumed the equal concentration of placebo. There were no significant differences for percent body fat. However, for total body mass, fat-free mass, thigh volume, muscle strength, and myofibrillar protein, CRT and PLC exhibited significant increases after training when compared to CON, whereas CRT was also significantly greater than PLC. For Type I, IIa, and IIx MHC mRNA expression, CRT was significantly greater than CON and PLC, whereas PLC was greater than CON. For MHC protein expression, CRT was significantly greater than CON and PLC for Type I and IIx but was equal to PLC for IIa. It was concluded that long-term Cr supplementation increases muscle strength and size, possibly as a result of increased MHC synthesis [031].
**Increased protein synthesis**

Another possibility is that part of the increased body weight is caused by increased protein synthesis, conceivably stimulated by the cell swelling. Cell swelling has been shown to act as an anabolic signal stimulating protein synthesis and net protein deposition. In agreement with this, a stimulating effect of creatine on protein synthesis in animal cardiac and skeletal muscle in vitro has been found, although there are also studies that do not confirm this. The effect of creatine on protein synthesis in human skeletal muscle has been studied more directly. It was determined the effect of creatine intake (20 g/d for 5 d followed by 5 g/d for 3–4 d) on indices of protein metabolism. 13C-leucine was infused before and after the supplementation period. Creatine had no effect on mixed muscle protein fractional synthetic rate (m. vastus lateralis). In men, but not in women, reduced leucine oxidation and rate of appearance of plasma leucine (estimate of whole body protein breakdown) were observed. Total body mass or fat free mass was not affected. The author concluded that short-term supplementation may have some anticatabolic action in some protein in men, but does not increase whole-body or mixed-muscle protein synthesis. It was tested the effect of creatine supplementation on myofibrillar protein synthesis (MPS; measured as incorporation of 1-13C-leucine in the quadriceps muscle) and muscle protein breakdown (MPB; measured as dilution of 13C-leucine or 2H5-phenylalanine across the forearm). In this study, 6 men were tested twice, before and after creatine ingestion (21 g/d for 5 d). In each study the subjects were tested both before (fasted) and after intake of maltodextrin and protein (postabsorptive). Feeding led to a doubling of MPS, and a 40 percent depression of MPB, but no effect of creatine was found on these parameters either in the fed or fasted states [007].

**No effect on protein synthesis**

In yet another report it was stated that addition to performance measurements creatine supported increases in fat-free mass and type II muscle fiber area. However, there is no evidence that creatine supplementation affects protein synthesis [014].

**Effect on human muscle protein turnover at rest**

Dietary creatine supplementation is associated with increases in muscle mass, but the mechanism is unknown. It was tested the hypothesis that creatine supplementation enhanced myofibrillar protein synthesis (MPS) and diminished muscle protein breakdown (MPB) in the fed state. Six healthy men (26 years) were studied twice, 2–4 week apart, before and after ingestion of creatine (21 g/day, 5 days). It was carried out two sets of measurements within 5.5 h of both MPS (by incorporation of [1-13C]leucine in quadriceps muscle) and MPB (as dilution of [1-13C]leucine or [2H5]phenylalanine across the forearm); for the first 3 h, the subjects were postabsorptive but thereafter were fed orally (0.3 g maltodextrin and 0.083 g protein/kg body wt/h). Creatine supplementation increased muscle total creatine by approximately 30 percent. Feeding had significant effects, doubling MPS and depressing MPB by approximately 40 percent, but creatine had no effect on turnover in the postabsorptive or fed states. Thus any increase in muscle mass accompanying creatine supplementation must be associated with increased physical activity [032].

**Influence of creatine on glucose metabolism**

Findings have indicated that creatine supplementation may affect glucose metabolism. One study aimed to examine the effects of creatine supplementation, combined with aerobic training, on glucose tolerance in sedentary healthy male. Subjects (n=22) were randomly divided in two groups and were allocated to receive treatment with either creatine (CT) (approximately 10 g/day over three months) or placebo (PT) (dextrose). Administration of
treatments was double blind. Both groups underwent moderate aerobic training. An oral glucose tolerance test (OGTT) was performed and both fasting plasma insulin and the homeostasis model assessment (HOMA) index were assessed at the start, and after four, eight and twelve weeks. CT demonstrated significant decrease in OGTT area under the curve compared to PT. There were no differences between groups or over time in fasting insulin or HOMA. The results suggest that creatine supplementation, combined with aerobic training, can improve glucose tolerance but does not affect insulin sensitivity, and may warrant further investigation with diabetic subjects [033].

**Muscle glycogen accumulation**

An additional effect of creatine supplementation, mostly when combined with training, is enhanced muscle glycogen accumulation and glucose transporter (GLUT4) expression. Thus, creatine may also be beneficial in sport competition and training characterized by daily glycogen depletion, as well as provide therapeutic value in the insulin-resistant state [034].

Muscle glycogen levels may also be affected by creatine supplementation, likely as a result of increased cellular water content. Increases in body mass with creatine supplementation have been reported as far back as 1928. However current evidence suggests that the increase in body mass observed with creatine is due to the decreased urine output and water retention during the initial stages of creatine loading [014].

It is suggested that another mechanism for the effect of creatine could be enhanced muscle glycogen accumulation and GLUT4 expression, when creatine supplementation is combined with a glycogen depleting exercise. Whereas it has been observed that creatine supplementation alone does not enhance muscle glycogen storage it was observed positive effects of creatine supplementation for enhancing initial and maintaining a higher level of muscle glycogen during 2 hours of cycling. In general, it is accepted that glycogen depleting exercises, such as high intensity or long duration exercise should combine high carbohydrate diets with creatine supplementation to achieve heightened muscle glycogen stores [035].

**Muscle glycogen supercompensation is enhanced by prior creatine supplementation**

Recently, it was shown that glycogen supercompensation tended to be greater if creatine and glycogen were loaded simultaneously. Because the authors suggested that creatine loading increased cell volumes and, therefore, enhanced glycogen supercompensation, we decided to determine whether an enhanced glycogen supercompensation could be realized if the glycogen loading protocol was preceded by a 5-d creatine load. Twelve men (19-28 years) performed two standard glycogen loading protocols interspersed with a standard creatine load of 20 g/d for 5 d. The vastus lateralis muscle was biopsied before and after each loading protocol. The initial glycogen loading protocol showed a significant 4 percent increase in muscle glycogen (Delta upward arrow 164 ± 87 mmol/kg d.m.), and no change in total muscle creatine. Biopsies pre- and post-creatine loading showed significant increases in total muscle creatine levels in both the left leg and the right leg, with no change in either leg's muscle glycogen content. After the final glycogen loading, a significant 53% increase in muscle glycogen was detected. Finally, the postcreatine load total glycogen content (694 ± 156 mmol/kg d.m.) was significantly greater than the precreatine load total glycogen content (597 ± 142 mmol/kg d.m.). It is suggested that a muscle’s glycogen loading capacity is influenced by its initial levels of creatine and the accompanying alterations in cell volume [036].
Effect on muscle ATP

During short-duration, high-intensity exercises, ATP needs are met by both anaerobic glycolysis and phosphocreatine shuttle. Anaerobic glycolysis is the dominant form of ATP production between 10 and 30 s when at maximal effort, while the phosphocreatine shuttle predominates as an ATP source during maximal effort exercises less than 10 s. By increasing stores of phosphocreatine with creatine supplementation, the belief is one can decrease muscle fatigue and increase performance by prolonging the phosphocreatine shuttle. In addition to increasing phosphocreatine stores, there are other proposed mechanisms by which creatine supplementation can improve performance during these exercises. One proposed mechanism is faster resynthesis of phosphocreatine during rest and recovery between bouts of maximal exercises; more creatine in the muscles would equate to more potential phosphocreatine. Conflicting data exist regarding creatine supplementation improving phosphocreatine resynthesis. Other mechanisms include aiding ATP production via glycolysis by increasing phosphofructokinase activity or by buffering hydrogen ions [014].

Cr is a compound that is both made within the body from amino acids and obtained through diet. Most of the body's Cr is stored within skeletal muscle where it plays a role in metabolism, with the daily turnover of Cr for the average sized person of about 2 g. It has been suggested that the adenosine triphosphate-phosphocreatine (ATP-PCr) energy system has the greatest power potential. Muscle stores of PCr may split and release energy for rapid resynthesis of ATP, although the supply of PCr is limited, with the combined total ATP and PCr capable of sustaining all out maximal effort exercise lasting up to 5 to 10 seconds. Therefore, fatigue may be attributed to the rapid decrease in PCr. Generation of peak anaerobic power and anaerobic capacity in short-term, high-intensity exercise may be dependent upon endogenous levels of ATP and PCr, particularly, PCr as a means to rapidly regenerate the limited intramuscular supply of ATP for anaerobic capacity. Thus, an increase in muscle total creatine (TCr) through exogenous CrS may provide an ergogenic effect by enhancing the rate of ATP synthesis during intermittent, high-intensity, short-duration exercise and by improving the rate of PCr resynthesis during recovery. This contention is supported by the findings of the rate of ATP synthesis through PCr hydrolysis and glycolysis and mean power output during a 10 second maximal dynamic handgrip exercise (Ex10) using 31-phosphorus magnetic resonance spectroscopy before and after CrS (30 g/day for 14 days). ATP synthesis rate through PCr hydrolysis positively correlated with mean power output during Ex10 in all subjects after CrS. The authors concluded that a daily dose of 30 g CrS for 14 days improved ATP synthesis through PCr hydrolysis and mean power output during short-term, maximal exercise. Moreover, it is strongly indicated that an improvement in performance during Ex10 was associated with the increased PCr availability for the synthesis of ATP. The body has several different ways in which it restores ATP. As previously stated, energy is released when one of the phosphates in ATP is cleaved off. When this happens, ATP becomes adenosine diphosphate (ADP). Returning ADP to its high-energy state of ATP by adding another phosphate group to it can then recycle ADP. One such ATP producing system is glycolysis, which is achieved anaerobically. Another system that the body extracts energy from is oxidative phosphorylation, which incorporates oxygen to yield ATP [002].

**ATP resynthesis**

It has been suggested that the improvements in performance associated with creatine supplementation are due to parallel improvements in ATP resynthesis during exercise as a consequence of increased PCr availability, particularly within the type II muscle fibers [030].
A buffering effect

During sustained high intensity exercise (HIE), PCr contributes about 26 percent of the anaerobic capacity and the 10 percent increase in PCr would correspond to an increased anaerobic capacity of about 3 percent (10% × 0.26). The reduced catabolism of adenine nucleotides after Cr loading during HIE gives experimental support for the role of Cr in improving energetic status. PCr-Cr acts as a temporal buffer of ATP, attenuating decreases in cellular ATP during high rates of energy demand. Cr kinase, the enzyme catalyzing bidirectional conversion of Cr-PCr, is localized to structural components close to sites of ATP utilization (myofibrils, sarcoplasmatic reticulum, and sarcolemma) and ATP formation (mitochondria). This provides the basis for PCr-Cr acting as a spatial buffer of ATP, by which intracellular gradients of ATP-ADP are diminished. Spatial buffering of ATP will reduce increases in ADP at the sites of energy consumption and may be an important factor to prevent contractile failure and fatigue. Increasing PCr by Cr loading will improve both the temporal and the spatial ATP-ADP buffering and explain part of the ergogenic effect. Another mechanism by which Cr loading can increase performance is related to muscle buffering. PCR-Pi accounts for more than 50 percent of the total muscle buffer capacity. It can be calculated that the improved muscle buffering after Cr loading, together with the knowledge that glycolysis accounts for 74 percent of anaerobic ATP production (sustained exercise at VO_{2\text{max}}), can increase anaerobic capacity by about 4 percent (10 × 0.5 × 0.74). The combined effect of Cr loading on ATP buffering and proton buffering may thus increase anaerobic capacity during sustained exercise by about 6 percent. However, due to the heterogenic response between subjects to Cr loading, one would expect that some subjects would benefit more, whereas others would not have any effect at all (non-responders). An additional function of the PCr–Cr system is in the control of oxidative phosphorylation. Cr kinase is localized to the inner mitochondrial membrane and forms a functional unit with adenine nucleotide translocase. Increases in Cr and ADP stimulate oxidative phosphorylation, whereas PCr has an inhibitory role. Although it is clear that the PCr–Cr couple have a role in controlling aerobic ATP production, at least in oxidative muscle fibers, there is limited evidence that Cr supplementation can improve performance during endurance exercise. Cr loading has also been shown to increase the rate of force relaxation in human muscle. A faster relaxation would certainly be an advantage during sprints, where the rapid movements require a fast adjustment of muscles between their contraction and relaxation phases [006].

The degree to which skeletal muscle will use PCr may be intensity and duration dependant. When intensity exceeds the power of the aerobic system the muscle begins to rely on the anaerobic system, which includes the use of PCr and muscle glycogen as fuels. Consequently, during the most intense periods of exercise or sport, the muscle will tax the PCr store most highly. Therefore, some have argued that CrS may benefit certain athletes in particular sports. CrS has been suggested as a means to "load" the muscle with Cr and increase storage of PCr. Theoretically, this would serve to improve the ability to produce energy during explosive, high-intensity exercise bouts and/or enhance the ability to recover from intense exercise. In support of the contention research has shown that CrS increases intramuscular PCr concentrations. Furthermore, the CrS-related increase in PCr concentration may allow a “mopping up” the acid producing hydrogen ions produced during the breakdown of ATP and other anaerobic processes. Therefore, PCr may contribute to the maintenance of optimal pH levels within the muscle and allows continued performance with minimal fatigue [002].

Creatine reduces human muscle PCr and pH decrements and P_i accumulation

The purpose of one study was to examine with ^{31}\text{P}-magnetic resonance spectroscopy energy metabolism during repeated plantar flexion isometric exercise (Ex-1-Ex-4) at 32 ± 1 and 79 ±
4 percent of maximal voluntary contraction (MVC) before and during a creatine (Cr) feeding period of 5 g/day for 11 days. Eight trained male subjects participated in the study. ATP was unchanged with Cr supplementation at rest and during exercise at both intensities. Resting muscle phosphocreatine (PCr) increased from 18.3 ± 0.9 (before) to 19.6 ± 1.0 mmol/kg wet wt after 9 days. At 79 percent MVC, PCr used, Pi accumulated, and pH at the end of Ex-1-Ex-4 were similar after 4 and 11 days of Cr supplementation. In contrast, PCr utilization and P(i) accumulation were lower and pH was higher for exercise at 32 percent MVC with Cr supplementation, suggesting aerobic resynthesis of PCr was more rapid during exercise. These results suggest that elevating muscle Cr enhances oxidative phosphorylation during mild isometric exercise, where it is expected that oxygen delivery matches demands and predominantly slow-twitch motor units are recruited [037].

Relative importance of phosphocreatine (PCr) during exercise

The muscle store of phosphocreatine (PCr) can be depleted almost completely during exhaustive exercise, providing an equimolar amount of ATP (about 70 mmol per kg dry muscle [dm]) in humans. Anaerobic glycolysis (i.e. glycogen to lactate) gives 1.5 mmol ATP per mmol of lactate. During exhaustive cycling at VO\textsubscript{2max}, muscle lactate can increase from 5 to 113 mmol/kg dm corresponding to 162 mmol ATP/kg dm. Some of the produced lactate will be co-transported with H\textsuperscript{+} to the extracellular fluid, thus decreasing the intracellular acid load and enabling further glycolysis. Lactate efflux is dependent on muscle blood flow (capillary density and degree of restricting muscle contraction), the amount of lactate transport proteins, the extracellular buffer capacity, and the extracellular lactate concentration. Increasing the efflux of lactate will enhance glycolytic ATP production. The amount of lactate transported to blood has been estimated to be about 17 percent of total lactate production during cycling at VO\textsubscript{2max} but will be lower during exercise of shorter durations. The contribution of anaerobic glycolysis (i.e. lactate formation) to anaerobic ATP production during cycling at VO\textsubscript{2max} is thus 195 mmol/kg dm (162 + 33 mmol/kg dm) or 74 percent of total anaerobic ATP production, with the remaining part covered by PCr depletion. Although there is extensive anaerobic energy utilization during this type of exercise (cycling at VO\textsubscript{2max}), with concomitant depletion of PCr stores and high lactate levels in muscle and blood, the major part (84 %) of the energy demand is covered by aerobic processes. The proportion of ATP derived from PCr utilization and anaerobic glycolysis is dependent on the duration and intensity of exercise. The maximal rate of ATP supply from PCr is higher than that from glycolysis and, during the first 3 s of contraction, PCr breakdown contributes to 70 % of the ATP formation. The rate of PCr breakdown declines after a few seconds of maximal contraction, after which glycolysis increases in importance. There is a complex metabolic interaction between both aerobic and anaerobic processes and between PCr utilization and glycolysis. PCr utilization is coupled to increased concentration of Pi, which is a limited substrate for the flux-generating step of glycolysis, i.e. glycogen phosphorylase. The increase in Pi will promote glycolysis and could explain the shift in ATP provision from PCr to glycolysis. Alternately, the reduced rate of PCr utilization may be a consequence of reduced muscle content of PCr and kinetic constraints of the creatine kinase reaction, where the reduced PCr/Cr ratio will reduce the maximal rate of PCr breakdown. A reduction in PCr may therefore reduce anaerobic power and contribute to fatigue even before complete depletion of the PCr store. The proportion of anaerobic ATP production covered by PCr will be high at the onset of exercise, whereas that of glycolysis will dominate after about 6 s of exercise. PCr is rapidly resynthesized during recovery (half of the depletion restored after ~30 s), whereas removal of muscle lactate is slower (half of the accumulated lactate removed after ~10 min). Therefore, during interval exercise with short recovery periods, PCr utilization will be the dominating process of anaerobic ATP production. To sum up, the relative importance of PCr utilization and anaerobic glycolysis for anaerobic ATP production will depend on the duration, intensity, and type of exercise. During sustained HIE with a duration exceeding 6 s, anaerobic glycolysis will dominate, whereas at shorter durations and
especially during interval exercise with short recovery periods, PCr will be the main source of anaerobic ATP [006].

*Maintenance of a phosphorylcreatine reservoir for energy*

Creatine monohydrate is a dietary supplement that increases muscle performance in short-duration, high-intensity resistance exercises, which rely on the phosphocreatine shuttle for adenosine triphosphate. Many situations within athletics and during training for sport require fast and intense muscle contractions. Intense sport activities less than 10 s in duration are dependent on intramuscular stores of adenosine triphosphate (ATP) and phosphocreatine [014].

Creatine is involved in the regulation of cellular energy demand. Under resting conditions, ATP is mainly formed in mitochondria through oxidative phosphorylation with ADP. Transported in sarcoplasm, some ATP molecules react with creatine, via the enzyme phosphorylcreatine kinase, to form phosphorylcreatine and ADP until equilibrium is reached. When ATP is needed for cellular energy, such as for muscle contraction, the phosphorylcreatine kinase reverse reaction replenishes the ATP content. Creatine thus acts indirectly to maintain a phosphorylcreatine reservoir for energy needs, more specifically to supply the muscle system with ATP [004].

The classical role of creatine-phosphate (PCr) is seen as a reservoir of high-energy phosphates defending cellular ATP levels under anaerobic conditions, high rates of energy transfer or rapid fluctuations in energy requirement. Although the high concentration of PCr in glycolytic fast-twitch fibers supports the role of PCr as a buffer of ATP, the primary importance of the creatine kinase (CK) reaction may in fact be to counteract large increases in ADP, which could otherwise inhibit cellular ATPase-mediated systems. A primary role for CK in the maintenance of ADP homeostasis may explain why, in many conditions, there is an inverse relationship between PCr and muscle contractility but not between ATP and muscle contractility. The high rate of ATP hydrolysis during muscle contraction combined with restricted diffusion of ADP suggests that ADP concentration increases transiently during the contraction phase (ADP spikes) and that these are synchronized with the contraction. The presence of CK, structurally bound in close vicinity to the sites of ATP utilization, will reduce the amplitude and duration of the ADP spikes through PCr-mediated phosphotransfer. When PCr is reduced, the efficiency of CK as an ATP buffer will be reduced and the changes in ADP will become more prominent. The presence of ADP spikes is supported by the finding that other processes known to be activated by ADP (i.e. AMP deamination and glycolysis) are stimulated during exercise but not during anoxia, despite the same low global energy state. Breakdown of PCr is driven by increases in ADP above that depicted by the CK equilibrium and the current method to calculate ADPfree from the CK reaction in a contracting muscle is therefore questionable [037].

*Phosphocreatine (PCr) action on cell membrane structures*

At the beginning, the survival of humans was strictly related to their physical capacity. There was the need to resist predators and to provide food and water for life. Achieving these goals required a prompt and efficient energy system capable of sustaining either high intensity or maintaining prolonged physical activity. Energy for skeletal muscle contraction is supplied by anaerobic and aerobic metabolic pathways. The former can allow short bursts of intense physical activity (60-90 sec) and utilizes as energetic source the phosphocreatine shuttle and anaerobic glycolysis. The aerobic system is the most efficient ATP source for skeletal muscle. The oxidative phosphorylation of carbohydrates, fats and, to a minor extent, proteins, can sustain physical activity for many hours. Carbohydrates are the most efficient fuel for working muscle and their contribution to total fuel oxidation is positively related to the
intensity of exercise. The first metabolic pathways of carbohydrate metabolism to be involved are skeletal muscle glycogenolysis and glycolysis. Later circulating glucose, formed through activated gluconeogenesis, becomes an important energetic source. Among glucose metabolites, lactate plays a primary role as either direct or indirect (gluconeogenesis) energy source for contracting skeletal muscle. Fat oxidation plays a primary role during either low-moderate intensity exercise or protracted physical activity (over 90-120 min). Severe muscle glycogen depletion results in increased rates of muscle proteolysis and branched chain amino acid oxidation. Endurance training ameliorates physical performance by improving cardiopulmonary efficiency and optimizing skeletal muscle supply and oxidation of substrates [038].

Here it was used mainly liposome model systems to provide evidence for interaction of phosphocreatine (PCr) and PcCr with different zwitterionic phospholipids by applying four independent, complementary biochemical and biophysical assays: chemical binding assay, surface plasmon resonance spectroscopy (SPR), (solid-state 31P-NMR, and (differential scanning calorimetry (DSC). SPR revealed low affinity PCr/phospholipid interaction that additionally induced changes in liposome shape as indicated by NMR and SPR. Additionally, DSC revealed evidence for membrane packing effects by PCr, as seen by altered lipid phase transition. Finally, PCr efficiently protected against membrane permeabilization in two different model systems: liposome-permeabilization by the membrane-active peptide melittin, and erythrocyte hemolysis by the oxidative drug doxorubicin, hypoosmotic stress or the mild detergent saponin. These findings suggest a new molecular basis for non-energy related functions of PCr and its cyclic analogue. PCr/phospholipid interaction and alteration of membrane structure may not only protect cellular membranes against various insults, but could have more general implications for many physiological membrane-related functions that are relevant for health and disease [039].

An antioxidant effect

The beneficial effects can be barely explained on the basis of the sole ergogenic role of the Cr/CrP system. Indeed, a wide number of research articles indicate that Cr is capable of exerting multiple, non-energy related, effects on diverse and relevant cellular targets. Among these effects, the antioxidant activity of Cr emerges as an additional mechanism which is likely to play a supportive role in the Cr-cytoprotection paradigm [040].
MEDICAL USE OF CREATINE

Most studies of the response to creatine supplementation have assessed exercise performance in healthy subjects. However, there are some indications that supplementation may be useful in the treatment of certain diseases, such as muscle fatigue secondary to impaired energy production and diseases resulting in muscle atrophy (90). The mechanisms underlying the effect of creatine in these circumstances are largely unknown, but may be due to the increased energy in the form of PCr, increased muscle accretion, and stabilization of membranes. Inborn errors of energy metabolism have been identified in 3 of the main steps in creatine metabolism: arginine:glycine aminotransferase (AGAT), S-adenosyl-L-methionine:N-guanidino-acetate methyltransferase (GAMT) and the creatine transporter. Oral creatine has been shown to improve the clinical symptoms in both AGAT and GAMT deficiency, but not in the creatine transporter deficiency. Supplementation has also been shown to have neuroprotective effects in several animal models of neurological diseases, e.g. Huntington's disease, Parkinson's disease, and amyotrophic lateral sclerosis. However, this has to be confirmed in clinical studies.

Creatine as nutritional supplementation or medicinal product

Because of assumed ergogenic effects, the creatine administration has become popular practice among subjects participating in different sports. Appropriate creatine monohydrate dosage may be considered a medicinal product since, in accordance with the Council Directive 65/65/EEC, any substance which may be administered with a view to restoring, correcting or modifying physiological functions in humans beings is considered a medicinal product. Thus, quality, efficacy and safety must characterise the substance. In addition, the European Court of Justice has held that a product which is recommended or described as having preventive or curative properties is a medicinal product even if it is generally considered as a foodstuff and even if it has no known therapeutic effect in the present state of scientific knowledge. In biochemical terms, creatine administration increases creatine and phosphocreatine muscle concentration, allowing for an accelerated rate of ATP synthesis. In thermodynamics terms, creatine stimulates the creatine-creatine kinase-phosphocreatine circuit, which is related to the mitochondrial function as a highly organised system for the control of the subcellular adenylate pool. In pharmacokinetics terms, creatine entry into skeletal muscle is initially dependent on the extracellular concentration, but the creatine transport is subsequently downregulated. In pharmacodynamics terms, the creatine enhances the possibility to maintain power output during brief periods of high-intensity exercises. In spite of uncontrolled daily dosage and long-term administration, no researches on creatine monohydrate safety in humans were set up by standardised protocols of clinical pharmacology and toxicology, as currently occurs in phases I and II for products for human use. More or less documented side effects induced by creatine monohydrate are weight gain; influence on insulin production; feedback inhibition of endogenous creatine synthesis; long-term damages on renal function. A major point that related to the quality of creatine monohydrate products is the amount of creatine ingested in relation to the amount of contaminants present. During the industrial production of creatine monohydrate from sarcosine and cyanamide, variable amounts of contaminants (dicyandiamide, dihydrotriazines, creatinine, ions) are generated and, thus, their tolerable concentrations (ppm) must be defined and made consumers known. Furthermore, because sarcosine could originate from bovine tissues, the risk of contamination with prion of bovine spongiform encephalopathy (BSE or mad-cow disease) can t be excluded. Thus, French authorities forbade the sale of products containing creatine. Creatine, as other nutritional factors, can be used either at supplementary or therapeutic levels as a function of the dose. Supplementary doses of nutritional factors usually are of the order of the daily turnover, while therapeutic ones are three or more times higher. In a subject of 70 kg with a total creatine pool of 120 g,
the daily turnover is approximately of 2 g. Thus, in healthy subjects nourished with fat-rich, carbohydrate, protein-poor diet and participating in a daily recreational sport, the oral creatine monohydrate supplementation should be of the order of the daily turnover, i.e., less than 2.5-3 g per day, bringing the gastrointestinal absorption to account. In healthy athletes submitted daily to high-intensity strength or sprint training, the maximal oral creatine monohydrate supplementation should be of the order of two times the daily turnover, i.e., less than 5-6 g per day for less than two weeks, and the creatine monohydrate supplementation should be taken under appropriate medical supervision. The oral administration of more that 6 g per day of creatine monohydrate should be considered as a therapeutic intervention and should be prescribed by physicians only in the cases of suspected or proven deficiency, or in conditions of severe stress and/or injury. The incorporation of creatine into the medicinal product class is supported also by the use in pathological conditions, e.g., some mitochondrial cytopathies, the guanidinoacetate methyltransferase deficiency, etc [041].

In neurological, aging and psychiatric diseases

It is well accepted that creatine has a function in extremity muscles, but lesser known is the essential role creatine, a natural regulator of energy homeostasis, plays in brain function and development. Creatine supplementation has shown promise as a safe, effective, and tolerable adjunct to medication for the treatment of brain-related disorders linked with dysfunctional energy metabolism, such as Huntington’s disease and Parkinson’s disease. Impairments in creatine metabolism have also been implicated in the pathogenesis of psychiatric disorders, leaving clinicians, researchers and patients alike wondering if dietary creatine has therapeutic value for treating mental illness such as psychological stress, schizophrenia, mood and anxiety disorders. While present knowledge of the role of creatine in cognitive and emotional processing is in its infancy, further research on this endogenous metabolite has the potential to advance the understanding of the biological bases of psychopathology and improve current therapeutic strategies [042].

It is worth commenting that creatine supplements have potentially greater and more mainstream value than as performance enhancers for athletes. Creatine supplementation can improve muscle mass and fatigue resistance in sarcopenic older adults in whom a better function means an enhanced ability to perform activities of daily living. The benefits of creatine ingestion have been extended to patient populations as well, and there are many reports of improved muscle function in patients with various muscle (e.g. muscular dystrophy) and degenerative central nervous system disorders (e.g. Parkinson’s and Huntington’s diseases). Promising new data have emerged demonstrating that creatine supplementation can improve cognitive processing in older adults [004].

Protective effects on the brain

In addition to its role in physical performance, creatine supplementation has protective effects on the brain in models of neuronal damage and also alters mood state and cognitive performance. Creatine is found in high protein foods, such as fish or meat, and is also produced endogenously from the biosynthesis of arginine, glycine, and methionine. Changes in brain creatine levels, as measured using magnetic resonance spectroscopy, are seen in individuals exposed to drugs of abuse and depressed individuals. These changes in brain creatine indicate that energy metabolism differs in these populations relative to healthy individuals. Recent work shows that creatine supplementation has the ability to function in a manner similar to antidepressant drugs and can offset negative consequences of stress. These observations are important in relation to addictive behaviors as addiction is influenced by psychological factors such as psychosocial stress and depression. The significance of
altered brain levels of creatine in drug-exposed individuals and the role of creatine supplementation in models of drug abuse have yet to be explored and represent gaps in the current understanding of brain energetics and addiction [043].

No effect on blood pressure

The effects of short-term oral creatine (Cr) supplementation on exercise performance and on blood pressure and renal function were assessed. Thirty-five healthy, active duty, U.S. Army volunteers (20 men and 15 women; age, 22-36 years) at Fort Sam Houston, Texas, supplemented their diet for 7 days with 20 g/day of either Cr or taurine (as placebo). There was no significant difference in 2-minute push-up counts between the Cr and taurine groups from before to after supplementation. The Cr group demonstrated a significant increase in serum creatinine levels, compared with the taurine group, and this increase could be misinterpreted as impairment of renal function. No adverse changes in blood pressure, body composition, weight, or serum Cr phosphokinase levels were observed. It was concluded that short-term creatine supplementation appears to be safe but does not enhance push-up performance [044].
NON-MEDICAL USE OF CREATIN: SUPPLEMENTATION IN SPORTS

For many years, the popularity of creatine rose dramatically, especially among athletes. In the USA alone, creatine-containing dietary supplements make up a large portion of the estimated USD 2.7 billion in annual sales of sports nutrition supplements [017].

A survey of first division athletes in 1999 found that 48 percent of male athletes reported current or prior use of creatine. Creatine also was found to be the most popular supplement used by a cohort of high school athletes in a survey completed in Iowa in 2001. It was found that usage increased in high school by grade, and in the 11th and 12th grade, the usage was about 12 percent in the US. One survey, however, showed in 2013 a decrease in popularity of creatine with whey protein being the more popular [014].

Creatine is a very popular sports supplement. Studies of various athletic groups report usage ranging from 17-75 percent. The most cited reasons for taking creatine are enhanced performance and appearance. Indeed, creatine continues to be one of the most popular sports supplements of all time [030].

Use in young athletes

Creatine is a nutritional supplement that is purported to be a safe ergogenic aid in adults. Although as many as 28 percent of collegiate athletes admit taking creatine, there is little information about creatine use or potential health risk in children and adolescents. Although the use of creatine is not recommended in people less than 18 years of age, numerous anecdotal reports indicate widespread use in young athletes. The purpose of this study was to determine the frequency, risk factors, and demographics of creatine use among middle and high school student athletes. Before their annual sports preparticipation physical examinations, middle and high school athletes aged 10 to 18 in Westchester County, a suburb north of New York City, were surveyed in a confidential manner. Information was collected regarding school grade, gender, specific sport participation, and creatine use. Overall, 62 of 1103 participants (6%) admitted taking creatine. Creatine use was reported in every grade, from 6 to 12. Forty-four percent of grade 12 athletes surveyed reported using creatine. Creatine use was significantly more common among boys (53/604, 9%) than girls (9/492, 2%). Although creatine was taken by participants in every sport, use was significantly more common among football players, wrestlers, hockey players, gymnasts, and lacrosse players. The most common reasons cited for taking creatine were enhanced performance (74% of users) and improved appearance (61%), and the most common reason cited for not taking creatine was safety (46% of nonusers). Despite current recommendations against use in adolescents less than 18 years old, creatine is being used by middle and high school athletes at all grade levels. The prevalence in grades 11 and 12 approaches levels reported among collegiate athletes. Until the safety of creatine can be established in adolescents, the use of this product should be discouraged [045].

Competitive athletes, including adolescents, seek ways to gain advantage over competitors. One ergogenic aid is creatine, a naturally occurring nitrogen compound found primarily in skeletal muscle. Increasing creatine levels may prolong skeletal muscle activity, enhancing work output. A questionnaire assessing awareness and use of creatine supplementation was completed by 674 athletes from 11 high schools. Data were statistically analyzed to determine variation among groups. Of those surveyed, 75 percent had knowledge of creatine supplements, and 16 percent used creatine to enhance athletic performance. Percentage of use increased with age and grade level. Awareness and use were greater among boys than girls. Adverse effects were reported by 26 percent. Most athletes consumed creatine using a method inconsistent with scientific recommendations. Use of creatine by adolescent athletes
is significant and inconsistent with optimal dosing. Physicians, athletic trainers, and coaches should disseminate proper information and advise these adolescent athletes [046].

**Creatine supplementation in high school football players**

To describe creatine supplementation patterns and behaviors associated with creatine supplementation in high school football players a cross-sectional, multisite, anonymous, descriptive survey was conducted between October 1999 and February 2000 in 37 public high schools in a total of 1,349 high school football players, grades 9-12. Self-reported prevalence of creatine use, as well as perceived benefits and risks. In addition, sources of information and influence regarding creatine supplementation were assessed. Thirty percent of the respondents reported using creatine. Creatine use was lowest in the 9th grade (10 %) and highest in the 12th grade (51 %). Forty-one percent of the players at small schools stated they used creatine compared with 29 percent of the players in large schools. Enhanced recovery following a workout was the most likely perceived benefit of creatine supplementation, while dehydration was cited most often as a risk of creatine use. Users were encouraged to take creatine most often by their friends while their parents discouraged creatine use. It was concluded that creatine use is widespread in high school football players. High school football players who use creatine may not be aware of the risks and benefits associated with creatine supplementation. Sports medicine professionals who work with this population need to educate athletes, coaches, and parents about the use of creatine as a performance-enhancing supplement [047].

**Physiologic basis for creatine use in children and adolescents**

The purported ergogenic benefits of creatine for the adult population have been well documented. In able-bodied children and adolescents, there is a paucity of data on creatine use and the purported ergogenic effects of creatine. Only 1 study to date has investigated the ergogenic properties of creatine in the adolescent population. The purpose of this review was to try to establish a rationale for creatine use in the child and adolescent population. The limited literature available in this area did not provide a strong enough rationale from either a physiologic or performance perspective for creatine supplementation in these populations. However, significantly more research is required before definitive conclusions can be made [048].

To determine the prevalence, frequency, and patterns of creatine use among a local US population of high school athletes. Male and female high school athletes completed an anonymous questionnaire on creatine use during the August 1999 preparticipation examinations at a single institutional sports medicine center. A total of 328 students (182 males and 146 females) aged 14 to 18 years (15.2 ± 1.3 years) completed the survey (100 % response rate), although not all athletes answered each question. Twenty-seven athletes (8.2 % of total group), 1 of whom was female, reported creatine use. Of these 27 athletes, 14 (52 %) were taking creatine at the time of the survey. The frequency of creatine use among past and current users was equally distributed among rarely (30 %), weekly (35 %), and daily (35 %). Creatine users were older than nonusers (mean 16.5 ± 1.2 vs 15.0 ± 1.3 years). Of creatine users, 21 (78 %) were male football players. Nineteen of 24 respondents (79 %) believed creatine improved their performance. Overall, 78 percent of users either did not know how much creatine they were taking (12/22 respondents) or were taking greater than the recommended doses (5/22 respondents). Minor gastrointestinal side effects or muscle cramps were reported by 5 (20 %) of 25 respondents. Creatine users were more likely than nonusers to know other creatine users and to use other supplements (67 % vs 9 %). Creatine users obtained creatine information primarily from friends (74 %) and purchased creatine predominantly from health food stores (86 %). It was concluded that high school male and female athletes as young as 14 years use creatine. Of high school athletes
participating in our study, 8.2 percent reported creatine use. Relatively minor side effects, diarrhea, cramps, and loss of appetite, were reported. Creatine users seem to believe that creatine improves their performance, but they may lack sufficient information to make informed decisions regarding creatine use [049].

Creatine effects in select division I collegiate athletes

To describe patterns of creatine use in select Division I collegiate athletes based on recommended dosages according to body weight. Further, to report the perceived effects noted with creatine supplementation an anonymous open-ended self-report descriptive questionnaire was used. Two-hundred and nineteen male and female collegiate athletes representing eight varsity sports participated. In addition, perceived positive, negative, and no effects associated with creatine usage patterns were determined from athlete responses on this self-report measure. Considering this select group of collegiate athletes, highly variable patterns of creatine supplementation were noted for loading/maintenance phases based on recommended dosages/days and body weight. Of the 219 athletes surveyed, 90 (41 %) reported using creatine, while creatine supplementation was more prevalent among men than women. Creatine users (80 athletes, 89 %) reporting perceived positive effects were primarily at or below recommended dosages for the loading phase but above recommended dosages in the maintenance phase. Creatine users (34 athletes, 38 %) reporting perceived negative effects were primarily at or below recommended dosages in the loading phase but noticeably above recommended dosages in the maintenance phase. Ironically, all creatine users who reported negative side effects also reported positive effects. Creatine users (10 athletes, 11 %) reporting no effects were below recommended loading dosages but above recommended maintenance dosages. The perceived positive effects noted support current research (strength/weight gains), while the perceived negative effects (cramping/gastrointestinal distress) were consistent with anecdotal reports surrounding creatine supplementation. Apparently, collegiate athletes in this study are in need of education regarding the proper use of creatine supplementation. Additional studies are needed to ascertain creatine supplementation patterns of collegiate athletes in various settings [050].

Use of creatine in high schools

Creatine is a nutritional supplement used to enhance athletic performance in collegiate and professional athletes. There is increasing evidence that high school athletes are using creatine as well. The objective of one study was to describe patterns of creatine supplementation as well as the behaviors and beliefs associated with creatine use in high school athletes. 4011 high school student-athletes from 37 public high schools in Wisconsin took part in a cross-sectional, multi-site, anonymous, descriptive survey. Measurements included self-reported patterns of creatine use. Seventeen percent of the athletes (25 % males, 4 % females) reported using creatine. Creatine use was lowest in the 9th grade (8 %) and highest in the 12th grade (25 %). The percentage of participants in each sport who used creatine varied considerably from 1.3 percent (female cross country) to 30 percent (football). Increased strength was the most likely perceived benefit of creatine supplementation, while dehydration was cited most often as a perceived risk of creatine use. Users were encouraged to take creatine most often by their friends while their parents discouraged its use. It was concluded that despite the lack of research regarding the efficacy or safety of creatine supplementation in high school athletes, creatine was used by 25 percent of males and 4 percent of female high school athletes in Wisconsin. High school athletes who use creatine may not be aware of the risks and benefits associated with creatine supplementation. Primary care providers and sports medicine professionals need to educate athletes, coaches and parents about the creatine use as a performance enhancing supplement [051].
Use in women

Many surveys have found creatine to be the most commonly used supplement. Creatine use is consistently higher in men compared with women. The 2001 NCAA study of substance use habits of college student-athletes showed that 26 percent of athletes had tried creatine at least once in the past year. The same survey repeated in 2005 found an increased prevalence of 40 percent athletes who had tried creatine. The study did not differentiate between women and men. A survey given to high preadolescent and adolescent boys and girls found that 0.4 percent of girls had tried creatine in the past year, compared with 4.3 percent of boys. Further, a study evaluating high school athletes found that significantly more boys than girls had used creatine (21 % vs 3 %) [052].

Use by military

Creatine is considered an effective nutritional ergogenic aid to enhance exercise performance. In spite of the publication of several reviews in the last decade on the topic of exercise performance/sports and creatine there is a need for an update related to the military given the lack of information in this area. The aim of one study was to critically assess original research addressing the use of creatine supplements in the military. A search of the electronic databases PubMed and SPORTDiscus, for the following key words: military personnel, trainees, recruit, soldier, physical fitness, physical conditioning, creatine supplementation, creatine ingestion, nutritional supplements to identify surveys and randomised clinical trials from journal articles and technical reports investigating the effect of creatine supplementation on military populations. Thirty-three out of 90 articles examined the use of creatine as a dietary supplement in military personnel. Twenty-one studies were finally selected on the basis of stated inclusion criteria for military surveys and randomised clinical trials. Most of the surveys (15/17) in the military indicate a high popularity of creatine (average 27 %) among supplement users. In contrast, in most of the exercise protocols used (6/9) during randomised clinical trials creatine has produced a non-significant performance-enhancing effect. Experimental research suggests that creatine supplementation does not enhance physical performance in the military. However, limitations in creatine dosage, military fitness testing and sample group selection might have underestimated the ergogenic properties of creatine. Recent studies also indicate positive effects on various aspects of total force fitness such as cognitive-psychomotor performance, bone health, musculoskeletal damage and neuromuscular function [053].

Use of creatine by members of civilian and military health clubs

A survey was used to collect anonymous cross-sectional data on demographics, exercise habits, and use of creatine and other supplements by exercisers in civilian (C) and military (M) health clubs. M (n=33) reported more aerobic training and less use of creatine and protein supplements than C (n=96). Supplement users (SU, n=194) and nonusers (SNU, n=35) engaged in similar frequency and duration of aerobic exercise, as well as number of resistance exercise repetitions, but SU completed more sets for each resistance exercise than SNU. Significant associations were observed between SU and resistance training goal of strength (as opposed to endurance), as well as greater frequency of resistance training. Male gender, resistance training goal of strength, lower frequency and duration of aerobic training, and use of protein, beta-hydroxy-beta-methyl butyrate, and androstenedione/dehydroepiandrosterone supplements were all associated with creatine use. For creatine users, the dose and length of creatine supplementation was 12.2 ± 2.7 g/day for 40 ± 5 weeks. Popular magazines were the primary source of information on creatine (69 %) compared to physicians (14 %) or dietitians (10 %). One study underscores two potential public health concerns: (a) reliance on popular media rather than allied-health professionals
for information on creatine, and (b) use of creatine, a popular supplement with unknown long-
term effects, in combination with other anabolic supplements of questionable efficacy and/or
safety [054].
DOSAGE OF CREATINE

Creatine supplementation increases the intracellular pool of phosphocreatine in skeletal muscle. Phosphocreatine provides a reserve of energy to rapidly regenerate ATP, which is consumed as a result of muscle contraction [055].

Creatine supplementation increases total body water

Creatine supplementation appears to increase total body and lean body mass; however, short-term gains in total body mass may be primarily water, but long-term gains associated with resistance training appear to be lean muscle mass [030].

One study aimed to evaluate changes in total body water (TBW) in soccer athletes using a deuterium oxide dilution method and bioelectrical impedance (BIA) formulas after 7 days of creatine supplementation. In a double-blind controlled manner, 13 healthy (under-20) soccer players were divided randomly in 2 supplementation groups: Placebo (Pla, n=6) and creatine supplementation (CR, n=7). Before and after the supplementation period (0.3 g/kg/d during 7 days), TBW was determined by deuterium oxide dilution and BIA methods. 7 days of creatine supplementation lead to a large increase in TBW (2.3 ± 1.0 L) determined by deuterium oxide dilution, and a small but significant increase in total body weight (1.0 ± 0.4 kg) in Cr group compared to Pla. The Pla group did not experience any significant changes in TBW or body weight. Although 5 of 6 BIA equations were sensitive to determine TBW changes induced by creatine supplementation, the Kushner et al. 16 method presented the best concordance levels when compared to deuterium dilution method. In conclusion, 7-days of creatine supplementation increased TBW determined by deuterium oxide dilution or BIA formulas. BIA can be useful to determine TBW changes promoted by creatine supplementation in soccer athletes, with special concern for formula choice [056].

Increased body weight after creatine loading

Cr loading is associated with an increase in body weight of approximately 2 percent. Although it has been suggested that Cr loading might stimulate protein synthesis and muscle growth, the evidence for this is limited. It is more likely that the increased body weight after Cr loading is related to increased tissue water content due to the osmotic effect of increased intracellular concentrations of PCr and Cr or to increased glycogen storage. In subjects with otherwise stable body weight, the increased body weight may be used as a rough marker of the effect of Cr loading. The increased body weight will negatively affect performance in running and other sports where body weight influences energy demand, and may therefore reduce the potential ergogenic effects of Cr loading [006].

Different preparations

Creatine is one of the most popular and widely researched natural supplements. The majority of studies have focused on the effects of creatine monohydrate on performance and health; however, many other forms of creatine exist and are commercially available in the sports nutrition/supplement market. Creatine is a nitrogen-containing substance found naturally in small amounts in animal foods. In recent years, creatine has been synthesized, mainly as creatine monohydrate, and has been marketed to athletes at all levels. Creatine supplements come in various forms (powder, pills, candy, chews, gels, serum, micronized) for both strength and endurance athletes, including products marketed specifically for males, females, and adolescents [030].
**Commercially available forms of creatine**

There are several different available forms of creatine: creatine anhydrous which is creatine with the water molecule removed in order to increase the concentration of creatine to a greater amount than that found in CM. Creatine has been manufactured in salt form: creatine pyruvate, creatine citrate, creatine malate, creatine phosphate, magnesium creatine, creatine orotate, Kre Alkalyn (creatine with baking soda). Creatine can also be manufactured in an ester form. Creatine ethyl ester (hydrochloride) is an example of this, as is creatine gluconate which is creatine bound to glucose. Another form is creatine effervescent which is creatine citrate or CM with citric acid and bicarbonate. The citric acid and bicarbonate react to produce an effervescent effect. When mixed with water the creatine separates from its carrier leaving a neutrally charged creatine, allowing it to dissolve to a higher degree in water. Manufacturers claim that creatine effervescent has a longer and more stable life in solution. When di-creatine citrate effervescent was studied for stability in solution it was found that the di-creatine citrate dissociates to citric acid and creatine in aqueous solutions which in turn forms CM and eventually crystallises out of the solution due to its low solubility. Some of the creatine may also convert to creatinine. In summary, creatine salts have been show to be less stable than CM. However the addition of carbohydrates could increase their stability. The potential advantages of creatine salts over CM include enhanced aqueous solubility and bioavailability which would reduce their possible gastrointestinal adverse effects. The possibility for new additional formulation such as tablets or capsules is interesting for its therapeutic application due to its attributed better dissolution kinetics and oral absorption compared to CM. However more complete in vivo pharmaceutical analysis of creatine salts are required to fully elucidate their potential advantages/disadvantages over the currently available supplement formulations [035].

**Creatine monohydrate**

Creatine monohydrate (CM), first marketed in the early 1990s, is the form most commonly found in dietary supplement/food products and most frequently cited in scientific literature [017].

Creatine monohydrate (CrM) has been consistently reported to increase muscle creatine content and improve high-intensity exercise capacity. However, a number of different forms of creatine have been purported to be more efficacious than CrM. The purpose of this study was to determine if a buffered creatine monohydrate (KA) that has been purported to promote greater creatine retention and training adaptations with fewer side effects at lower doses is more efficacious than CrM supplementation in resistance-trained individuals. In a double-blind manner, 36 resistance-trained participants (20 years, 181 cm, 82 kg, and 15 % body fat) were randomly assigned to supplement their diet with CrM (Creapure® AlzChem AG, Trostberg, Germany) at normal loading (4 x 5 g/d for 7-days) and maintenance (5 g/d for 21-days) doses; KA (Kre-Alkalyn®, All American Pharmaceutical, Billings, MT, USA) at manufacturer's recommended doses (KA-L, 1.5 g/d for 28-days); or, KA with equivalent loading (4 x 5 g/d for 7-days) and maintenance (5 g/d) doses of CrM (KA-H). Participants were asked to maintain their current training programs and record all workouts. Muscle biopsies from the vastus lateralis, fasting blood samples, body weight, DEXA determined body composition, and Wingate Anaerobic Capacity (WAC) tests were performed at 0, 7, and 28-days while 1RM strength tests were performed at 0 and 28-days. Data were analyzed by a repeated measures multivariate analysis of variance (MANOVA) and are presented as mean ± SD changes from baseline after 7 and 28-days, respectively. Muscle free creatine content obtained in a subgroup of 25 participants increased in all groups over time after 7 and 28-days, respectively, with no significant differences among groups. However, while no overall group differences were observed, pairwise comparison between the KA-L and CrM groups revealed that changes in muscle creatine content tended to be greater in the CrM
group. Although some significant time effects were observed, no significant group x time interactions were observed in changes in body mass, fat free mass, fat mass, percent body fat, or total body water; bench press and leg press 1RM strength; WAC mean power, peak power, or total work; serum blood lipids, markers of catabolism and bone status, and serum electrolyte status; or, whole blood makers of lymphocytes and red cells. Serum creatinine levels increased in all groups with higher doses of creatine promoting greater increases in serum creatinine but the increases observed (0.1-0.2 mg/dL) were well within normal values for active individuals (i.e. <1.28 ± 0.2 mg/dL). Serum LDL was decreased to a greater degree following ingesting loading doses in the CrM group but returned to baseline during the maintenance phase. No side effects were reported. It was concluded that neither manufacturers recommended doses of KA (1.5 g/d) or KA with equivalent loading (20 g/d for 7-days) and maintenance doses (5 g/d for 21-days) of CrM promoted greater changes in muscle creatine content, body composition, strength, or anaerobic capacity than CrM (20 g/d for 7-days, 5 g/d for 21-days). There was no evidence that supplementing the diet with a buffered form of creatine resulted in fewer side effects than CrM. These findings do not support claims that consuming a buffered form of creatine is a more efficacious and/or safer form of creatine to consume than creatine monohydrate [057].

Nothing better than the monohydrate

Supplement manufacturers have continually introduced newer forms of creatine into the marketplace. These newer forms have been purported to have better physical and chemical properties, bioavailability, efficacy, and/or safety profiles than creatine monophosphate (CM). However, there is little to no evidence that any of the newer forms of creatine are more effective and/or safer than CM whether ingested alone and/or in combination with other nutrients [017].

Claims include improved stability when combined with other ingredients or in liquids, improved solubility in water, improved bioavailability, and even an increase in performance. Creatine salts such as citrate, maleate, fumarate, tartrate, pyruvate, ascorbate, and orotate were first introduced to the marketplace as early as the late 1990s. Creatine and acids with multiple acid moieties such as citric acid can form salts as well as complexation products. In addition to creatine and its salts, derivatives of creatine such as creatine ester or even creatine alcohols are currently marketed as dietary supplements. Both ingredients do not contain creatine as such, since they have been chemically altered. While it is assumed that the human body will transfer those molecules into creatine upon intake, there are no published data available to base firm conclusions. The amount of creatine in different forms of creatine varies. Creatine monohydrate contains 88 percent of creatine, whereas the creatine content in other forms of creatine is lower with the exception of creatine anhydrous. Commercial creatine salts are formed in solution or by mechanical processes such as milling or grinding under the presence of residual water. Complexes are formed by the subsequent replacement of the solvating molecules by the new ligands [017].

Creatine ethyl ester

Creatine is a hydrophilic polar molecule that consists of a negatively charged carboxyl group and a positively charged functional group. The hydrophilic nature of creatine limits its bioavailability. In an attempt to increase creatines bioavailability creatine has been esterified to reduce the hydrophilicity; this product is known as creatine ethyl ester. Manufacturers of creatine ethyl ester promote their product as being able to by-pass the creatine transporter due to improved sarcolemmal permeability toward creatine. The results of at least one study showed that ethyl ester was not as effective as CM to enhance serum and muscle creatine stores. Furthermore creatine ethyl ester offered no additional benefit for improving body composition, muscle mass, strength, and power. This research did not support the claims of
the creatine ethyl ester manufacturers [019].

Creatine ethyl ester has received recent attention. In order to increase the intramuscular creatine levels, one of the latest creatine variations is creatine ethyl ester. Creatine ethyl ester is alleged to increase creatine bioavailability. Esterification of creatine decreases its hydrophilicity, and manufacturers of creatine ethyl ester claim that this allows it to bypass the creatine transporter due to enhanced sarcolemmal permeability toward creatine. Studies have shown that creatine ethyl ester is a substrate for creatine kinase. Yet recent studies show that creatine ethyl ester is converted to creatinine, not creatine. Increases in plasma creatinine were found with creatine ethyl ester. In addition, nonenzymatic cleavage of creatine ethyl ester was reported, leading them to report that creatine ethyl ester is a pronutrient for creatinine rather than creatine under all physiological conditions encountered during transit through the various tissues; thus no ergogenic effect is to be expected from supplementation. Other forms of creatine, such as a buffered form of creatine, to increase hydrophilic nature of the molecule is a more efficacious and/or safer form of creatine to consume than creatine monohydrate. Creatine ethyl ester has received recent attention. In order to increase the intramuscular creatine levels, one of the latest creatine variations is creatine ethyl ester. Creatine ethyl ester is alleged to increase creatine bioavailability. Esterification of creatine decreases its hydrophilicity, and manufacturers of creatine ethyl ester claim that this allows it to bypass the creatine transporter due to enhanced sarcolemmal permeability toward creatine. Studies have shown that creatine ethyl ester is a substrate for creatine kinase. Yet recent studies show that creatine ethyl ester is converted to creatinine, not creatine. Increases in plasma creatinine were found with creatine ethyl ester. In addition, nonenzymatic cleavage of creatine ethyl ester was reported, leading them to report that creatine ethyl ester is a pronutrient for creatinine rather than creatine under all physiological conditions encountered during transit through the various tissues; thus no ergogenic effect is to be expected from supplementation. Other forms of creatine, such as a buffered form of creatine, to increase hydrophilic nature of the molecule is a more efficacious and/or safer form of creatine to consume than creatine monohydrate [014].

Polyethylene glycosylated creatine

Polyethylene glycol is a non-toxic, water-soluble polymer that is capable of enhancing the absorption of creatine and various other substances. Polyethylene glycol can be bound with CM to form polyethylene glycosylated creatine. There are that results seem to indicate that the addition of polyethylene glycol could increase the absorption efficiency of creatine but further research is needed before a definitive recommendation can be reached [019].

The purpose of this study was to examine the effects of 28 days of polyethylene glycosylated creatine (PEG-creatine) supplementation (1.25 and 2.50 g/d) on anaerobic performance measures (vertical and broad jumps, 40-yard dash, 20-yard shuttle run, 3-cone drill), upper and lower body muscular strength and endurance (bench press and leg extension), and body composition. The study used a randomized, double-blind, placebo-controlled, parallel design. Seventy-seven adult males (mean age 22 years; body mass 82 kg) volunteered to participate and were randomly assigned to a placebo (n=23), 1.25 g/d of PEG-creatine (n=27) or 2.50 g/d of PEG-creatine (n=27) group. The subjects performed anaerobic performance measures, muscular strength (1RM) and endurance (80 % 1RM) tests for bench press and leg extension, and underwater weighing for the determination of body composition at Day 0 (baseline), Day 14, and Day 28. The results indicated there were significant improvements in vertical jump, 20-yard shuttle run, 3-cone drill, muscular endurance for bench press, and body mass for at least one of the PEG-creatine groups without changes for the placebo group. Thus, the present results demonstrated that PEG-creatine supplementation at 1.25 and/or 2.50 g/d had an ergogenic effect on lower body vertical power, agility, change-of-direction ability, upper body muscular endurance, and body mass [058].
Hyperhydrating supplements containing creatine

The addition of carbohydrate (CHO) in the form of simple sugars to creatine (Cr) supplements is central. One study aimed to determine whether ingestion of glucose (Glu) simultaneously with Cr and glycerol (Cr/Gly) supplement is detrimental to plasma lipids of endurance-trained individuals and find out whether modification arising can be attenuated by replacing part of the Glu with alpha lipoic acid (Ala). Twenty-two endurance-trained cyclists were randomized to receive Cr/Gly/Glu (11.4 g Cr·H₂O, 1 g Gly/kg BM, and 150 g Glu) or Cr/Gly/Glu/Ala (11.4 g Cr·H₂O, 1 g Gly/kg BM, 100 g Glu, and 1 g Ala) for 7 days. Fasting concentration of TAG increased significantly after supplementation with Cr/Gly/Glu (before: 0.9 ± 0.2 mmol/L; after: 1.3 ± 0.4 mmol/L) and Cr/Gly/Glu/Ala (before: 0.8 ± 0.2 mmol/L; after: 1.2 ± 0.5 mmol/L) but changes were not different between the groups. Supplementation significantly increased the TAG to HDL-cholesterol ratio but had no effect on fasting concentration of total, HDL-, and LDL-cholesterol and insulin resistance. Thus, addition of Glu to Cr containing supplements enhances plasma TAG concentration and the TAG to HDL-cholesterol ratio and this enhancement cannot be attenuated by partial replacement of Glu with Ala [059].

Creatine as powder

One study examined the effects of supplementation with either creatine monohydrate powder in solution (CP) or a widely available creatine serum (CS) on performance in a repeated maximal sprint cycling test (10 x 6 seconds, 24-second passive rest between sprints). Using a randomized, double-blind, crossover design, 11 competitive male athletes supplemented with creatine in 2 forms according to the manufacturer's recommendations on 2 separate occasions. The 2 supplementation protocols were (a) 20 g.day⁻¹ x 6 days of creatine powder in solution plus a placebo serum (CP) or (b) 5 ml.day⁻¹ x 6 days of creatine serum plus a placebo powder (CS). Subjects completed 2 familiarization trials before the 6-day supplementation period. A repeated maximal sprint cycling test was performed prior to and immediately postsupplementation. A 7-week washout period separated the 2 supplementation protocols. Subjects' total work (9.6 %) and peak power (3.4 %) in the cycle sprint improved significantly after loading with CP, but there was little change after loading with CS. The present data support previous research findings showing an ergogenic effect of CP supplementation but indicate that supplementation with CS does not affect sprint cycling performance. Although the levels of creatine in each formulation were not determined, a substantial conversion of creatine into creatinine has been reported in many formulations and may explain the present findings [060].

Rudimentary legislations

Whereas the safety, efficacy, and regulatory status of creatine monophosphate (CM) is clearly defined in almost all global markets; the safety, efficacy, and regulatory status of other forms of creatine present in today's marketplace as a dietary or food supplement is less clear. Accompanying the explosive growth in sales has been the introduction of different forms of creatine. Where the legal and regulatory status of the forms of creatine in the USA and other markets around the world is at best uncertain. To date, with the exception of Japan, CM is the only form of creatine to be officially approved or accepted in key markets.
such as the USA, European Union (EU), Canada and South Korea. The continued presence of other forms of creatine in the marketplace, especially in the US, may be due to a multitude of factors. These include, but may not be limited to, a lack of awareness or understanding on the part of marketers of applicable laws and regulations, intentional noncompliance with the law, and/or inadequate enforcement of the law. The public health implications of widespread distribution and use of these unauthorized forms of creatine is unknown and warrants careful monitoring. New forms of creatine are marketed with claims of improved physical, chemical, and physiological properties in comparison to creatine monohydrates [017].

Explanations as to why creatine forms are prevalent in the marketplace despite not having met the legal and regulatory requirements in the various markets are likely two-fold. Legal definitions of and regulatory requirements for “dietary supplements” (USA and Korea), “food supplements” (EU), “natural health products” (Canada), and “non-drug food additives” (Japan) are complex, differ between countries/regions, and can be confusing. Lack of awareness and/or understanding of the given country’s applicable requirements may be one explanation for the lack of compliance on the part of some marketers. To the extent that the laws and regulations are known and understood, inadequate enforcement by regulators can create an environment where noncompliance is perceived to be without consequences, resulting in the forgoing of required registration or notification requirements. In the USA, the increased prevalence of these alternate forms (CEE in particular) in dietary supplement products, with no enforcement action from FDA, has helped to support this misperception. The public health implications of having unsanctioned or unapproved forms of creatine on the market remain to be fully realized. While classical animal toxicity and short- and long-term clinical safety studies have been conducted in humans, the basic safety data on new forms of creatine is lacking. At present, there do not appear to be any imminent or specific safety concerns associated with any of these alternate forms. However, this must be monitored carefully through post-market surveillance and published case reports. As far as the marketplace is concerned, the presence of these newer and typically more expensive forms of creatine in a multitude of consumer products that are often marketed with misleading and/or unsubstantiated claims of greater bioavailability, efficacy, and safety sets a negative precedent. The reality that companies need not fulfill the necessary registration or notification requirements to satisfy regulatory authorities, but still feel free to market their ingredients and products without penalty establishes an “unlevel” playing field among competitors. This undermines any incentive to invest upfront resources to establish ingredients as safe and efficacious prior to reaching consumers. Inevitably, this will result in unintended and unforeseen consequences, which will serve to erode consumer confidence [017].

**Creatine purity**

Creatine is an unregulated dietary supplement that is readily available to consumers and legal for use in athletic training. The supplement is not banned by The National Collegiate Athletic Association (NCAA) or by the International Olympic Committee (IOC). The NCAA does prohibit individual institutions from distributing creatine supplements. The IOC ruled to allow creatine given the substance is readily found in animal proteins. However creatine is a dietary supplement that falls under the Dietary Supplement Health and Education Act, and is not directly regulated by the Food and Drug Administration. In general, those who participate in athletics under the supervision of institutions such as the NCAA and IOC should use caution when using any dietary supplement due to reported contamination of creatine [014].

**Safety**

The safety of creatine monohydrate has previously been confirmed. However with each novel form of creatine that emerges, its safety must be verified. Therefore, the purpose of
this study was to examine the safety of a novel form of creatine, creatine nitrate (CN), over a 28 day period. Fifty-eight young males and females (24 ± 4 years) participated in one study across two laboratories. Subjects were equally and randomly assigned to consume either 1 g (n=18) or 2 g (n=20) of CN or remained unsupplemented (n=20). Blood draws for full safety panels were conducted by a trained phlebotomist prior to and at the conclusion of the supplementation period. Pooled data from both laboratories revealed significant group x time interactions for absolute lymphocytes and absolute monocytes. Analysis of the 1 g treatment revealed lab x time differences for red blood cell distribution width, platelets, absolute monocytes, creatinine, blood urea nitrogen (BUN):creatinine, sodium, protein, and alanine aminotransferase (ALT). Analysis of the 2 g treatment revealed lab x time differences for BUN:creatinine and ALT. BUN and BUN:creatinine increased beyond the clinical reference range for the 2 g treatment of Lab 2, but BUN did not reach statistical significance. Overall, CN appears to be safe in both 1 g and 2 g servings daily for up to a 28 day period. While those with previously elevated BUN levels may see additional increases resulting in post-supplementation values slightly beyond normal physiological range, these results have minor clinical significance and are not cause for concern. Otherwise, all hematological safety markers remained within normal range, suggesting that CN supplementation has no adverse effects in daily doses up to 2 g over 28 days and may be an alternative to creatine monohydrate supplementation [061].

Young athletes, however, must be cautious about taking creatine because its effects on growth and development are unknown and long-term safety has not been established. Variability in research study designs and small sample sizes have left many questions unanswered regarding the safety and efficacy of chronic supplementation. This is an active area of clinical investigation and the results of ongoing and future research should guide the appropriate use of creatine to enhance athletic performance among athletes of all ages [062].

**Upper storage limit**

Athletes continue to search for the most effective supplement to aid performance. Athletes use a wide variety of commercial supplements because of the belief that the supplements possess beneficial effects. Some examples include l-carnitine, ginseng, chromium, glutamine, amino acids, protein powders, and creatine monohydrate, taken alone or in combination. Some supplements may cause long-term or short-term harmful effects, a possibility that has stimulated concern among health care professionals, exercise physiologists, and coaches. Because we do not know conclusively which supplements are ergogenic or ergolytic, we need to further examine specific supplements and their effects on exercise. Recently, creatine monohydrate has become the nutritional supplement of choice for athletes. This compound has accounted for most of the supplement sales during the past few years, and the market continues to grow as a result of endorsement by professional athletes. In addition, creatine does not appear on the banned list of substances of any sports federation. Professional athletes have a tremendous influence on many other athletes at various levels of competition. Currently, college, high school, and recreational athletes are questioning the effects of creatine. Recent studies have supported creatine as an effective, harmless ergogenic aid with no short-term contraindications. However, the effect of long-term use of creatine monohydrate on athletes is unclear; hence, the medical community has raised concerns, and possible health risks have been suggested. Creatine supplementation has resulted in positive physiologic effects on skeletal muscle creatine phosphate stores; short-duration, high-intensity anaerobic exercise; strength; and body composition in physically active subjects. These subjects have used a loading protocol of 20 to 30 g/d for up to 7 days, but most of these studies were not conducted in sport-specific settings. If creatine supplementation, specifically loading, causes a surplus of creatine in the muscle, then any excess can be converted and excreted in the form of creatinine. Creatinine is produced from
creatine as a byproduct of catabolism in skeletal muscle, and it appears in the urine. Therefore, creatine loading may not be beneficial to athletes if the muscle can only hold a predetermined concentration. It was shown that approximately 155 mmol/kg of dry muscle mass may represent the upper storage limit of creatine when a subject ingests 5 g 4 to 6 times per day. Thus, creatine loading may result in an excessive creatine concentration, which can be converted to creatinine and excreted. This finding suggests that creatine loading may not be necessary for those athletes (e.g. football players) engaged in long-term supplementation [063].

**Time schedule of response**

The daily oral ingestion of supplementary creatine monohydrate can substantially elevate the creatine content of human skeletal muscle. One paper aimed to summarize the current knowledge regarding the impact muscle creatine loading can have on exercise performance and rehabilitation. The major part of the elevation of muscle creatine content is already obtained after one week of supplementation, and the response can be further enhanced by a concomitant exercise or insulin stimulus [027].

The elevated muscle creatine content moderately improves contractile performance in sports with repeated high-intensity exercise bouts. More chronic ergogenic effects of creatine are to be expected when combined with several weeks of training [027].

**Effects of creatine supplementation before or after physical performance**

Chronic supplementation with creatine monohydrate has been shown to promote increases in total intramuscular creatine, phosphocreatine, skeletal muscle mass, lean body mass and muscle fiber size. Furthermore, there is robust evidence that muscular strength and power will also increase after supplementing with creatine. However, it is not known if the timing of creatine supplementation will affect the adaptive response to exercise. Thus, the purpose of one investigation was to determine the difference between pre versus post exercise supplementation of creatine on measures of body composition and strength. Nineteen healthy recreational male bodybuilders (mean; age 23; height 166 cm; weight: 80 kg) participated in this study. Subjects were randomly assigned to one of the following groups: PRE-SUPP or POST-SUPP workout supplementation of creatine (5 grams). The PRE-SUPP group consumed 5 grams immediately before exercise. On the other hand, the POST-SUPP group consumed 5 grams immediately after exercise. Subjects trained on average five days per week for four weeks. Subjects consumed the supplement on the two non-training days at their convenience. Subjects performed a periodized, split-routine, bodybuilding workout five days per week (Chest-shoulders-triceps; Back-biceps, Legs, etc.). Body composition (Bod Pod(R)) and 1-RM bench press (BP) were determined. Diet logs were collected and analyzed (one random day per week; four total days analyzed). There was a significant time effect for fat-free mass (FFM) and BP, however, fat mass (FM) and body weight did not reach significance. While there were trends, no significant interactions were found. However, using magnitude-based inference, supplementation with creatine post workout is possibly more beneficial in comparison to pre workout supplementation with regards to FFM, FM and 1-RM BP. Qualitative inference represents the likelihood that the true value will have the observed magnitude. Furthermore, there were no differences in caloric or macronutrient intake between the groups. It was concluded that creatine supplementation plus resistance exercise increases fat-free mass and strength. Based on the magnitude inferences it appears that consuming creatine immediately post-workout is superior to pre-workout vis a vis body composition and strength [064].
Pre-season training

As demonstrated by several laboratories, creatine supplementation is able to increase repetitions to failure. Therefore, many athletes have adopted creatine use during pre-season and/or off-season training for the cumulative effects of increased training volume, and they are not using creatine exclusively for acute performance benefits [001].

Effect of low-dose, short-duration creatine supplement

To examine the efficacy of a low-dose, short-duration creatine monohydrate supplement, 40 physically active men were randomly assigned to either a placebo or creatine supplementation group (6 g of creatine monohydrate per day). Testing occurred before and at the end of 6 days of supplementation. During each testing session, subjects performed three 15-second Wingate anaerobic power tests. No significant group or time differences were observed in body mass, peak power, mean power, or total work. In addition, no significant differences were observed in peak power, mean power, or total work. However, the change in the rate of fatigue of total work was significantly lower in the creatine supplementation group than in the placebo group, indicating a reduced fatigue rate in subjects supplementing with creatine compared with the placebo. Although the results of this study demonstrated reduced fatigue rates in patients during high-intensity sprint intervals, further research is necessary in examining the efficacy of low-dose, short-term creatine supplementation [065].

Typical dosage of creatine by athletes

Many studies have demonstrated that oral creatine supplementation can maximise muscle creatine levels by either a loading dose of 20 g/day for approximately 5 days followed by a maintenance dose of 2–3 g/day; or by the maintenance dose of 2–3 g/day for approximately 30 days [004].

A typical creatine supplementation protocol consists of a loading phase of 20 g creatine monophosphate (CM)/d or 0.3 g CM/kg/d split into 4 daily intakes of 5 g each, followed by a maintenance phase of 3-5 g CM/d or 0.03 g CM/kg/d for the duration of the supplementation period. Other supplementation protocols are also used such as a daily single dose of around 3-6 g or between 0.03 to 0.1 g/kg/d. However, this method takes longer (between 21 to 28 days) to produce ergogenic effects. It was also found that a moderate protocol consisting of 20 g CM taken in 1g doses (evenly ingested at 30-min intervals) for 5 days resulted in reduced urinary creatine and methylamine excretion, leading to an estimated increase in whole body retention of creatine (+13 %) when compared with a typical loading supplementation protocol of 4 x 5 g/d during 5 days (evenly ingested at 3 hour intervals). This enhancement in creatine retention would lead to a significantly higher weight gain when people follow a moderate protocol ingestion of several doses of small amounts of CM evenly spread along the day [035].

Studies have shown that intramuscular stores of total creatine and phosphocreatine can be increased by supplementing with oral creatine monohydrate for 5 to 7 d with a dose of 20 to 25 g/d. The greatest increase of creatine and phosphocreatine is reported to be in the first 2 days of supplementation. The typical dosing in studies that have shown increases in strength performance includes both a loading and maintenance phase. Depending on the study, the loading phase varies from 5 to 7 days at 0.3 g/kg/day. During the loading phase, the daily dose is divided into four equal doses throughout the day dissolved in a liquid. After the 5- to 7-d loading phase, the athlete continues with the maintenance phase at 0.03 g/kg/day. The
length of the maintenance phase varies in studies from 28 d to 10 week. When a carbohydrate or protein is added to creatine supplementation, there may be an increase in muscle retention of creatine, particularly in the first few days, resulting in a decreased need for loading. However alternative dosing methods also have been shown to effectively increase creatine stores and have effects on strength gains. Regimens without the creatine loading phase, 3 to 6 g/day for 28 days and 6 g/day for 12 weeks, also have been shown to be effective in increasing creatine stores. The increase in creatine stores occurs more slowly and therefore may take longer to see the strength training effects [014].

Oral creatine supplementation, usually as creatine monohydrate, has been reported to increase muscle supplies of free creatine and creatine phosphate (phosphocreatine; PCr), a high-energy phosphagen. A typical creatine loading protocol involves the ingestion of 20 grams of creatine monohydrate over the course of a day, usually in four separate doses of 5 grams each, for 4-6 consecutive days. Once loaded, a daily dose of 2–5 grams helps maintain elevated muscle creatine levels [030].

**Effects of creatine in males versus females**

*Creatine monohydrate enhances high-intensity exercise performance*

Creatine monohydrate supplementation has been shown to enhance high-intensity exercise performance in some but not all studies. Part of the controversy surrounding the ergogenic effect(s) of creatine monohydrate supplementation may relate to design issues that result in low statistical power. A further question that remains unresolved in the creatine literature is whether or not males and females respond in a similar manner to supplementation. It was studied the effect of creatine supplementation upon high intensity exercise performance in 24 subjects (n=12 males, n=12 females). Creatine monohydrate (Cr; 5g, 4x/d 3 4d) and placebo (Pl; glucose polymer 3 4d) were provided using a randomized, double-blind crossover design (7 week washout). Outcome measures included: 2 3 30-s anaerobic cycle test, with plasma lactate pre- and post-test; dorsi-flexor: maximal voluntary contraction (MVC), 2-min fatigue test, and electrically stimulated peak and tetanic torque; isokinetic knee extension torque and 1-min ischemic handgrip strength. Significant main effects of Cr treatment included: increased peak and relative peak anaerobic cycling power (3.7 %), dorsi-flexion MVC torque (6.6 %), and increased lactate (20.8 %) with no gender specific responses. It was concluded that short-term Cr supplementation can increase indices of high-intensity exercise performance for both males and females [066].
Oral creatine supplementation

Numerous studies have found that creatine monophosphate (CM) supplementation increases muscle phosphagen levels generally by 10 to 40 percent. Acute and chronic supplementation of CM has been reported to improve performance primarily during high intensity, intermittent activities. The impact on performance has been associated with the magnitude of change in baseline phosphagen levels. Numerous studies have shown that CM supplementation during training promotes greater gains in performance and/or fat-free mass [067].

Several studies have shown increases in intramuscular stores of creatine and phosphocreatine with creatine monohydrate supplementation, and the increases range from 10 to 40 percent. However there is an upper limit of creatine stores that are possible in human muscle, which has been reported as high as 160 g in the human body. Therefore athletes with full stores of creatine in their muscles will not receive benefit from supplementation. People with lower creatine stores in their muscles receive the greatest effect on intramuscular creatine stores when supplemented with oral creatine. Therefore the theory behind creatine supplementation is to increase stores in the muscle to facilitate ATP and phosphocreatine production, delaying the onset of muscle fatigue. The effective dosing for creatine supplementation includes loading with 0.3 g/kg/d for 5 to 7 days, followed by maintenance dosing at 0.03 g/kg/d most commonly for 4 to 6 weeks [014].

Creatine supplementation amplifies the adaptive response to resistance training both in female and male subjects, resulting in greater increases in maximal muscle strength and fat free mass in parallel with greater increases in muscle cross-sectional area compared to placebo intake. Notably, not all subjects respond with elevated muscle cell creatine content following creatine loading (about 10 %), particularly not individuals with initially high muscle creatine concentration. Creatine loading initially gives rise to an increased retention of water in the body, along with fluid shifts into the muscle fibers due to elevated osmotic gradients caused by the increase in intracellular creatine concentration. Data exist to suggest that this initial osmotic-induced increase in muscle fiber volume provide a stimulus per se for increased cellular protein synthesis [010].

The increase in muscle creatine content with supplementation is usually greatest in subjects with low initial concentration, whereas subjects with higher starting concentration may experience little or no increase. Hence, it has been shown that vegetarians typically are more responsive to creatine supplementation, since their initial levels are low from a diet that contains little creatine. Still the initial creatine content cannot fully explain the large intersubject variability in response to supplementation, suggesting that there are "responders" and "nonresponders." An increased inward creatine transport has been found when creatine is ingested together with carbohydrates or a carbohydrate/protein mixture. This seems to be caused by an insulin effect on the uptake. Ingestion of creatine and 1 g glucose/kg body mass twice per day increased total muscle creatine by 9 percent versus creatine intake alone [007].

**Strategies of creatine supplementation**

A loading regime used in many studies is ingestion of 20 g of Cr monohydrate each day over a period of 5-7 days. The substance should be dissolved in water and distributed in 5 g doses in order to attain sufficient increases in plasma Cr concentration. The loading phase is followed by a period with a lower dose (2-3 g of Cr per day), which is sufficient to maintain
the elevated muscle Cr level. The muscle uptake of Cr is largest during the initial loading phase, when about 30 percent of the dose administered is stored in muscle tissue and the remaining part excreted as creatinine in the urine. High doses of Cr after a loading phase, where the ceiling level of 150–160 mmol/kg dm has been reached, will be of no use since the surplus of Cr will be excreted in the urine. Muscle uptake of Cr can be enhanced by exercise, and the likely mechanism is increased muscle blood flow and thus exposure of the exercising muscle to elevated plasma Cr. However, muscle Cr uptake is also stimulated by insulin, and the stimulating effect of exercise may thus, at least in part, relate to an exercise-induced increase in insulin sensitivity and/or increased exposure of the muscle to insulin. The practical recommendation is that Cr intake should be combined with a substantial amount of carbohydrate and exercise [006].

**Individual responses**

Regardless of the form, supplementation with creatine has regularly shown to increase strength, fat free mass, and muscle morphology with concurrent heavy resistance training more than resistance training alone. Creatine may be of benefit in other modes of exercise such as high-intensity sprints or endurance training. However, it appears that the effects of creatine diminish as the length of time spent exercising increases. Even though not all individuals respond similarly to creatine supplementation, it is generally accepted that its supplementation increases creatine storage and promotes a faster regeneration of adenosine triphosphate between high intensity exercises. These improved outcomes will increase performance and promote greater training adaptations. More recent research suggests that creatine supplementation in amounts of 0.1 g/kg of body weight combined with resistance training improves training adaptations at a cellular and sub-cellular level. Finally, although presently ingesting creatine as an oral supplement is considered safe and ethical, the perception of safety cannot be guaranteed, especially when administered for long period of time to different populations (athletes, sedentary, patient, active, young or elderly) [019].

Creatine (Cr) supplementation is widely used to enhance high intensity performance. It is well known that the muscle content of Cr and PCr can be increased by dietary supply of Cr. Oral intake of Cr increases the concentration in blood and muscle, and part of the increased muscle Cr is transformed to PCr – a process catalyzed by Cr kinase. In an average person, Cr supplementation increases total Cr (TCr = Cr + PCr) by about 20 percent, with the PCr component accounting for 10 percent of the increase. However, there are large differences in the response between subjects, with a more pronounced effect in subjects with low initial muscle TCr content (e.g. vegetarians) and absent in subjects with already high initial contents of TCr, suggesting a ceiling of maximal TCr of 150-160 mmol/kg dm. It is well documented that Cr supplementation can increase performance during high intensity exercise, especially during interval exercise when PCr is the dominating anaerobic source of ATP. Cr loading prior to or during a training period can also, due to the ergogenic effect of Cr, increase the training load and thus enhance the training adaptation. This may explain the increased gain in muscle strength when resistance training is combined with Cr loading [006].

**Creatine supplementation affects muscle creatine during energy restriction**

Anaerobic performance and body protein may decrease with energy restriction practiced by some athletes for weight loss. One investigation examined the effects of creatine (Cr) supplementation during energy restriction on muscle Cr, exercise performance (10 sprints of 6 s, with 30-s rest), nitrogen balance, and body composition in male resistance trainers. Creatine supplemented (CrS, 20 g/d of Cr) and those given a placebo (P1) consumed a
formula diet of 75.3 kJ (18 kcal)/kg/d (54.7 % C, 21.3 % P, 24 % F) for 4 d. A control group was unsupplemented and continued their normal diet. There were no changes in body composition or performance of the control group. CrS and P1 demonstrated similar decreases in body weight and percent body fat. The percent change in fat-free mass was more for P1 (2.4 ± 0.3 % reduction) than CrS (1.4 ± 0.4 %), but urinary nitrogen losses were similar. Significant increases in muscle total Cr and CrP of 15-16 percent were demonstrated by CrS over the energy restriction period, whereas P1 had no changes in muscle Cr. Total work done during the sprints expressed per body weight tended to be 3.8 percent higher in CrS and 0.5 percent less in P1 after the energy restriction. It was concluded that Cr supplementation increased muscle Cr during short-term energy restriction but did not affect body fat or protein loss. The change in muscle creatine was reflected in a tendency for higher total sprint work for the Cr group [068].

**Plasma levels**

*Plasma levels after exercise*

One study examined whether plasma total glutathione levels could explain the intersubject variability in the creatine kinase (CK) response to eccentric exercise. It was hypothesized that the increase in plasma CK activity after eccentric exercise would be lower for individuals with low plasma total glutathione (<2.5 micromol/L) compared with individuals with high total glutathione (>3.8 micromol/L), but other indicators of muscle damage would be the same between groups. Resting blood samples were obtained over 2 d from 60 subjects and analyzed for plasma total glutathione. Eight subjects who had total glutathione values below 2.5 micromol/L (LG), and nine who had values above 3.8 micromol/L (HG) performed 50 maximal eccentric actions of the elbow flexors. Maximal voluntary isometric contraction (MVC), relaxed arm angle (RANG), and blood samples for CK, myoglobin (Mb), and total glutathione were obtained pre, post (except blood samples), 24, 48, 72, 96, and 120 h after exercise. There was a significant group-by-time interaction in analysis of MVC, RANG, total glutathione, CK, and Mb response to exercise. Although LG showed a smaller CK response to eccentric exercise compared with HG, LG also showed a smaller increase in plasma Mb, a faster recovery of MVC and RANG, and an increase in plasma total glutathione. It was concluded that subjects with low plasma total glutathione levels had a smaller plasma CK and Mb response and a faster recovery from eccentric exercise compared with subjects having high plasma total glutathione levels. It was suggested that a blunted inflammatory response in subjects with low plasma glutathione may be one explanation for these findings [069].

**Endurance running**

Very high blood creatine kinase (CK) concentrations have been observed among finishers of the 161-km Western States Endurance Run (WSER), and it has been suggested that there is a link between rhabdomyolysis and hyponatremia. Therefore, the purpose of one study was to compare CK concentrations of finishers in the 2010 WSER with past values, and to determine whether there was an association between blood CK and sodium concentrations. Consenting 2010 WSER finishers provided blood samples at the finish for determination of blood CK and sodium concentrations. Finish time, age, and gender were obtained from official race results, and running experience was determined from our database as number of prior 161-km ultramarathon finishes. From 216 (66 %) of the 328 finishers, median and mean CK concentrations were found to be 20 850 IU/L and 32 956 IU/L, respectively (range 1500-264 300 IU/L), and 13 (6%) had values greater than 100 000 IU/L. These values were statistically higher than those reported from the 1995 WSER. The CK concentration was not significantly associated with finish time, age, gender, or running experience. Blood sodium
concentrations were obtained from a subgroup of 159 runners, and the relationship between blood CK and sodium concentrations did not reach statistical significance. It was concluded that creatine kinase concentrations of 2010 WSER finishers are higher than values previously reported. More research should focus on explaining this observation and on whether there is a possible link between higher CK concentrations and hyponatremia [070].
To compare the change in muscle creatine, fiber morphology, body composition, hydration status, and exercise performance between vegetarians and nonvegetarians with 8 weeks of creatine supplementation and resistance training. Eighteen VG and 24 NV subjects (19-55 years) were randomly assigned (double blind) to four groups: VG + creatine (VGCr, n=10), VG + placebo (VGPl, n=8), NV + creatine (NVCr, n=12), and NV + placebo (NVPl, n=12). Before and at the end of the study, muscle biopsies were taken from the vastus lateralis m, body composition was assessed by DXA, and strength was assessed using 1-RM bench press and leg press. Subjects participated in the same 8-wk resistance-training program. Creatine dosage was based on lean tissue mass. Biopsy samples indicated that total creatine (TCr=free Cr + PCr) was significantly lower in VG compared with NV at baseline. For Cr subjects, there was a greater increase in PCr, TCr, bench-press strength, isokinetic work, Type II fiber area, and whole-body lean tissue compared with subjects on placebo. Vegetarians who took Cr had a greater increase in TCr, PCr, lean tissue, and total work performance than nonvegetarians who took Cr. The change in muscle TCr was significantly correlated with initial muscle TCr, and the change in lean tissue mass and exercise performance. These findings confirm an ergogenic effect of Cr during resistance training and suggest that subjects with initially low levels of intramuscular Cr (vegetarians) are more responsive to supplementation.

With the growing interest in the potential health benefits of plant-based diets, it is relevant to consider whether vegetarian dietary practices could influence athletic performance. Accordingly, this review examines whether nutrients that may differ between vegetarian and omnivorous diets could affect physical performance. It was also described studies that attempt to assess the effects of a vegetarian diet on performance and comment on other nutritional aspects of vegetarianism of relevance to athletes. Although well-controlled long-term studies assessing the effects of vegetarian diets on athletes have not been conducted, the following observations can be made: 1) well-planned, appropriately supplemented vegetarian diets appear to effectively support athletic performance; 2) provided protein intakes are adequate to meet needs for total nitrogen and the essential amino acids, plant and animal protein sources appear to provide equivalent support to athletic training and performance; 3) vegetarians (particularly women) are at increased risk for non-anemic iron deficiency, which may limit endurance performance; and 4) as a group, vegetarians have lower mean muscle creatine concentrations than do omnivores, and this may affect supramaximal exercise performance. Because their initial muscle creatine concentrations are lower, vegetarians are likely to experience greater performance increments after creatine loading in activities that rely on the adenosine triphosphate/phosphocreatine system. 5) Coaches and trainers should be aware that some athletes may adopt a vegetarian diet as a strategy for weight control. Accordingly, the possibility of a disordered eating pattern should be investigated if a vegetarian diet is accompanied by unwarranted weight loss.

The effect of creatine supplementation on exercise performance in vegetarians was examined. Creatine was ingested for 1 week by a group of vegetarians (VC) and meat-eaters (MC); a control group of meat-eaters was fed only glucose (MG). Exercise performance during three, 20-s maximal cycling tests (modified Wingate anaerobic test, WAnT) was determined before and after creatine supplementation. Blood samples were also drawn before and after exercise prior to and after supplementation. Basal plasma creatine (after an overnight fast) averaged 11 microM in VC, and 24 and 23 microM in MG and MC, respectively, a statistically significant effect. These findings were expected, since most of the body's exogenous creatine source is meat. There was no significant difference in any other parameter between groups prior to supplementation. Creatine feedings significantly increased body mass (approximately 1 kg) and mean power output during the WAnTs (approximately 5 %) to a similar extent in the VC and MC groups. These parameters were
not affected by supplementation in the MG group. Peak power output was also significantly increased by supplementation in MC (approximately 5 percent, but not in VC. It is concluded that vegetarians and meat-eaters respond to creatine feedings with similar increases in mean power output during short-term, maximal exercise [073].

**Different responses**

Some individuals, particularly vegetarians with initially low levels of intramuscular creatine, may respond more effectively to creatine supplementation, while individuals with higher intramuscular creatine and phosphocreatine levels and those with fewer type II muscle fibers may be less responsive to supplementation [030].

**Lacto-ovo-vegetarian diet**

The purpose of one investigation was to examine the effects of preceding oral creatine monohydrate with a lacto-ovo-vegetarian diet on muscle creatine concentration. Thirty-two healthy men, who regularly consumed an omnivorous diet, were randomly assigned to consume a weight maintaining, lacto-ovo-vegetarian (LOV; n=16) or omnivorous (Omni; n=16) diet for 26 days. In addition to their assigned diet, on day 22 of the study, subjects were assigned in a double-blind manner to receive either creatine monohydrate (CM: 0.3 g kg d 1 + 20 g Polycose) or an equivalent dose of placebo (PL) for 5 days. There were no significant differences between the LOV and Omni groups at baseline with respect to age, height, and weight. The results demonstrated that consuming a LOV diet for 21 days was an effective procedure to decrease muscle creatine concentration in individuals who normally consume meat and fish in their diet. However, muscle total creatine (TCr) following creatine supplementation did not differ statistically between LOV and Omni diet groups (149 vs 142 mmol/kg d.m.) [074].

**Results also without meat**

Creatine supplementation improves repetitive, short-term performance. However, it has not been shown that exclusion of meat from the diet would impair repetitive short-term performance. In contrast, reduction of protein intake and a concomitant increase of carbohydrate intake during a period of 3-5 days improves anaerobic (2-7 minutes) performance. The protein intake in a mixed or lacto-vegetarian diet is adequate even for elite athletes, providing that energy requirements are met. Many dietary supplements have been suggested to increase muscle mass and/or to decrease fat mass [075].
EFFECT OF CREATINE SUPPLEMENTATION ON EXERCISE PERFORMANCE

Improved training capacity

Most studies document increases in muscle mass when creatine is supplemented during resistance training. The increase in muscle mass may be associated with a creatine supplementation-induced ability to do more repetitions during training, which may induce favorable genetic adaptations in the muscle [030].

The possible ergogenic effect of creatine has led to a widespread use of supplementation in sport. It has been estimated that 80 percent of the athletes competing in the 1996 Olympic Games were probably using, or had been using, creatine and the 1998 sale was estimated at USD 100 million in the United States alone. Many athletes ingest creatine over long periods of time, and not only in connection with special events. Often it is used during training periods aimed to increase strength and body mass. The question then remains whether creatine has any ergogenic effects. A large number of studies have been performed to test the effect of creatine on exercise performance. These have been summarized in several previous reviews on this topic. From controlled laboratory tests, it can be concluded that creatine supplementation appears to improve performance on repeated sprints (<30 s), whereas the effect on single sprints and on muscle strength is less conclusive. Thus, the major advantage of creatine may derive from improved training capacity. Also, the effect on performance during longer exercise (>90 s) is unclear. This is not surprising, since the contribution of the PCr-ATP system decreases as the exercise duration increases. No effect has been found in longer-duration aerobic type exercise. Even though creatine supplementation did not increase the performance during the endurance part of prolonged cycling, it did, however, increase sprint performance within and after this phase. The results from more recent field studies are variable, probably caused by factors such as intake of meat in the placebo group, sample size, and type and duration of exercise. In meta-analyses of the effect of creatine on exercise performance, the conclusions are also somewhat variable. Nevertheless, taken together, it is suggested that creatine supplementation is of benefit to exercise lasting 30 s or less [007].

Many athletes use creatine in connection with resistance training as an aid to increase the training effect on muscle mass and strength. Meta-analyses have shown an effect of creatine supplementation on body composition and muscular strength in resistance training program. Twelve weeks of resistance training combined with creatine supplementation increased muscle fiber diameter by 35 percent in both Type 1 and 2 muscle fibers in men versus 6-15 percent in placebo-supplemented resistance trained subjects. The effect on body mass may result from supplemented subjects training on higher workloads than the placebo control subjects, since higher creatine and PCr stores in the muscle would theoretically improve work capacity during this kind of exercise. Thus, athletes should be able to perform more repetitions and recover faster between sets compared to nonsupplemented controls. In accordance with this notion, no difference in strength and weight lifting performance was found between creatine and placebo groups during an 8 weeks resistance training program, when the training volume was constant between groups (76). Thus, the beneficial effects of creatine on muscular strength and body composition probably occur by the following sequence: increased muscle creatine, increased training intensity, greater training stimulus, and enhanced physiological adaptations to training. Creatine supplementation is also associated with an immediate increase in body mass. Typically this amounts to about 1-2 kg in 4-7 days. The mechanism responsible for this appears to be an increase in total body water. It was reported that creatine markedly reduced urinary volume during the initial days of supplementation, suggesting that the increased body weight primarily was water retention. It must be pointed out that this increase in body weight makes a blinding of studies difficult [007].
Acute (short time) resistance exercise

It was also examined the possible stimulatory effect of creatine in conjunction with acute resistance exercise. Seven healthy men performed $20 \times 10$ repetitions of leg extension-flexion at 75 percent of 1-repetition maximum in 1 leg, before and after creatine intake (same dose as in previous study). The subjects were studied both in fasted and fed state, and again no effect of creatine was found on the synthetic rates of myofibrillar and sarcoplasmic proteins or MPB in any state, whereas exercise increased the synthetic rates of proteins by 2–3 fold. In fact, the rate of MPS in the exercised leg in the creatine supplemented subjects was about 60 percent lower than in the placebo group, but this failed to reach significance. The authors suggested that this may be an indication that creatine feeding acutely inhibits the postexercise increase in MPS, possibly by blunting any stimulus of MPS that depends on lowering of energy status during the exercise bout. Hence, any effect of creatine on translation of pre-existing messenger RNA (mRNA) seems unlikely. However, it cannot be ruled out that creatine (in combination with resistance exercise) can change transcription or activate satellite cells. Clearly, both exercise in itself and food are much stronger stimuli for protein synthesis than creatine intake in healthy individuals [007].

To determine the effects of creatine supplementation during short-term resistance training overreaching on performance, body composition, and resting hormone concentrations, 17 men were randomly assigned to supplement with 0.3 g/kg per day of creatine monohydrate (CrM: $n=9$) or placebo (P: $n=8$) while performing resistance exercise (5 days/week for 4 weeks) followed by a 2-week taper phase. Maximal squat and bench press and explosive power in the bench press were reduced during the initial weeks of training in P but not CrM. Explosive power in the bench press, body mass, and lean body mass (LBM) in the legs were augmented to a greater extent in CrM by the end of the 6-week period. A tendency for greater 1-RM squat improvement was also observed in CrM. Total testosterone (TT) and the free androgen index (TT/SHBG) decreased in CrM and P, reaching a nadir at week 3, whereas sex hormone binding globulin (SHBG) responded in an opposite direction. Cortisol significantly increased after week 1 in CrM (+29 %), and returned to baseline at week 2. Insulin was significantly depressed at week 1 (-24 %) and drifted back toward baseline during weeks 2-4. Growth hormone and IGF-I levels were not affected. Therefore, some measures of muscular performance and body composition are enhanced to a greater extent following the rebound phase of short-term resistance training overreaching with creatine supplementation and these changes are not related to changes in circulating hormone concentrations obtained in the resting, postabsorptive state. In addition, creatine supplementation appears to be effective for maintaining muscular performance during the initial phase of high-volume resistance training overreaching that otherwise results in small performance decrements [076].

The purpose of one study was to examine the influence of short-term creatine (Cr) supplementation on exercise-induced transverse relaxation time (T2) and sprint performance during maximum intermittent cycling exercise using the muscle functional magnetic resonance imaging (mfMRI) technique. Twelve men were divided into a Cr supplementation group [the Cr group, taking 4 x (5 g Cr monohydrate + 2.5 g maltodextrin)/day], or a placebo supplementation group (the P group, taking 4 x 7.5 g maltodextrin/day). The allocation to the groups was based on cycling tests and the subject's physical characteristics, and thus was not randomized. A double-blind research design was employed for a 5-day supplementation period. mfMR images of the right thigh were collected at rest and immediately after two, five, and ten 6-s sprint bouts of maximum intermittent cycling exercise with a 30-s recovery interval between sets. Before and after supplementation, blood was taken to calculate lactate accumulation, and the muscle volume of the thigh was determined by MRI. Following supplementation, there was significant body mass gain in the Cr group, whereas the P group did not change. The exercise-induced T2, blood lactate levels and sprint performance were not affected by Cr supplementation in any sprint bouts. These results suggest that short-term
Cr supplementation does not influence short duration repetitive sprint performance and muscle activation and/or metabolic state during sprint cycling evaluated by mfMRI of the skeletal muscle in humans [077].

Muscle hypertrophy during resistance training is reportedly increased by creatine supplementation. Having previously failed to find an anabolic effect on muscle protein turnover at rest, either fed or fasted, we have now examined the possibility of a stimulatory effect of creatine in conjunction with acute resistance exercise. Seven healthy men (21 years) performed 20 x 10 repetitions of leg extension-flexion at 75 percent one-repetition maximum in one leg, on two occasions, 4 wk apart, before and after ingesting 21 g/day creatine for 5 days. The subjects ate approximately 21 g maltodextrin + 6 g protein/h for 3 h postexercise. It was measured incorporation of [1-^{13}C]leucine into quadriceps muscle proteins in the rested and exercised legs. Leg protein breakdown (as dilution of [2H5]phenylalanine) was also assessed in the exercised and rested leg postexercise. Creatine supplementation increased muscle total creatine by approximately 21 percent. Exercise increased the synthetic rates of myofibrillar and sarcoplasmic proteins by two- to threefold, and leg phenylalanine balance became more positive, but creatine was without any anabolic effect [078].

Increased creatine levels in muscle lead to improved performance of repeated high-intensity exercise, increased strength and lean body mass and enhanced fatigue resistance for exercise tasks lasting 30 s or less, particularly when combined with progressive resistance training [004].

Creatine monohydrate’s effect on resistance training exercises has been extensively researched. There are numerous controlled studies that have reported increases in performance and muscle strength in short-duration, maximum-intensity exercises. Resistance training has been measured in many ways in the literature, including exercises such as bench press, leg press, biceps curls, leg extensions, jump squats, and bicycle ergometry. The method of measurement of strength and performance in creatine studies includes one repetition maximum, mean power, total force, and number of repetitions. The results regarding creatine supplementation’s ergogenic effect are not unanimous. However, there is a significant body of evidence that creatine increases performance in short-duration, maximum-intensity resistance training. Conflicting evidence exists regarding studies of the effect of creatine supplementation on anaerobic performance. Currently, studies consistently have observed no effect on aerobic performance with creatine supplementation [014].

It was examined the effects of creatine supplementation on the response to repeated bouts of resistance exercise. Young men (24 year) were divided into creatine (CM, n=9) and Placebo (PL, n=9) groups. On day (D) 1 and D15, subjects performed four sets of bicep curls at 75% 1-RM to concentric failure. On D8-D13, subjects consumed either 20 g/d creatine monohydrate or placebo. Muscle soreness and elbow joint range of motion (ROM) were assessed on D1-D5 and D15-D19. Serum creatine kinase activity (CK) was assessed on D1, D3, D5, D15, D17, and D19. The first exercise bout produced increases in muscle soreness and CK, and decreases in ROM in both groups. The second bout produced lesser rises in serum CK, muscle soreness, and a lesser decrease in ROM with greater attenuation of these damage markers in CM than PL. CK levels on D17 were lower (+110 % over D15 for CM vs +343 % for PL), muscle soreness from D15-19 was lower (-75 % for CM vs -56 % for PL compared with first bout), and elbow ROM was decreased in PL, but not CM on D16. It was concluded creatine supplementation provides an additive effect on blunting the rise of muscle damage markers following a repeated bout of resistance exercise. The mechanism by which creatine augments the repeated bout effect is unknown but is likely due to a combination of creatine’s multifaceted functions [079].
Creatine supplementation (CS) has been reported to increase body mass and improve performance in high-intensity, short-duration exercise tasks. Research on CS, most of which has come into existence since 1994, has been the focus of several qualitative reviews, but only one meta-analysis, which was conducted with a limited number of studies. One study compared the effects of CS on effect size (ES) for body composition (BC) variables (mass and lean body mass), duration and intensity of the exercise task, type of exercise task (single, repetitive, laboratory, field, upper-body, lower-body), CS duration (loading, maintenance), and subject characteristics (gender, training status). A search of MEDLINE and SPORTDiscus using the phrase "creatine supplementation" revealed 96 English-language, peer-reviewed papers (100 studies), which included randomized group formation, a placebo control, and human subjects who were blinded to treatments. ES was calculated for each body composition and performance variable. Small, but significant ES were reported for BC (n=163), ATP-PCr (n=17), G (n=135), and O (n=69). ES was greater for change in BC following a loading-only CS regimen compared to a maintenance regimen for repetitive-bout compared to single-bout exercise, and for upper-body exercise compared to lower and total body exercise. ES for laboratory-based tasks (e.g. isometric/isotonic/isokinetic exercise) were greater than those observed for field-based tasks (e.g. running, swimming). There were no differences in BC or performance ES between males and females or between trained and untrained subjects. It was concluded that ES was greater for changes in lean body mass following short-term CS, repetitive-bout laboratory-based exercise tasks ≤ 30 s (e.g. isometric, isokinetic, and isotonic resistance exercise), and upper-body exercise. CS does not appear to be effective in improving running and swimming performance. There is no evidence in the literature of an effect of gender or training status on ES following CS [080].

Contradicting results up to 2007

A 2003 meta-analysis showed individuals ingesting creatine, combined with resistance training, obtain on average +8 and +14 percent more performance on maximum (1RM) or endurance strength (maximal repetitions at a given percent of 1RM) respectively than the placebo groups. However, contradicting studies have reported no effects of creatine supplementation on strength performance. These conflicting results can be explained by the possibility that the supplemented groups were formed by a greater amount of non-responders or even because creatine supplementation was administered on the training days only (3 times a week). This strategy has not been adequately tested as effective in middle aged and older men for maintaining post loading elevated creatine stores. A quantitative, comprehensive scientific summary and view of knowledge up to 2007 on the effects of creatine supplementation in athletes and active people was published in a 100 citation review position paper by the International Society of Sports Nutrition. More recent literature has provided greater insight into the anabolic/performance enhancing mechanisms of creatine supplementation suggesting that these effects may be due to satellite cell proliferation, myogenic transcription factors and insulin-like growth factor-1 signalling. Collectively, in spite of a few controversial results, it seems that creatine supplementation combined with resistance training would amplify performance enhancement on maximum and endurance strength as well muscle hypertrophy [035].

Hormonal effects

In one study, the effect of short-term creatine supplementation on the growth hormone, testosterone, and cortisol response to heavy resistance training was investigated. According to a double-blind crossover study design, 11 healthy young male volunteers underwent a 1-h standardized heavy resistance training session (3 series of 10RM; 12 exercises), both before (pretest) and after (posttest) 5 d of either placebo (P, maltodextrine) or creatine (CR; 20 g/d,
5 d) supplementation. A 5-wk washout period separated the treatments. Thirty minutes before each training session, CR subjects ingested 10 g of creatine monohydrate (CR) while P subjects received placebo. Venous blood was sampled before, immediately after, and 30 and 60 min after the training session. The exercise-induced increase of serum growth hormone was not altered by acute creatine intake and was similar in P and CR. The weight training session, either or not in conjunction with acute or chronic creatine intake, did not significantly impact on serum testosterone. However, serum cortisol during recovery tended to be higher in CR than in P. It is concluded that short-term creatine supplementation does not alter the responses of growth hormone, testosterone, and cortisol to a single bout of heavy resistance training [081].

**Effects on repeated bouts of supramaximal exercise**

The aim of one study was to investigate the effects of creatine supplementation on performance during the repeated bouts of supramaximal exercise. Twenty-three untrained young males participated in the study. A double blind design was used to create the creatine and placebo groups. Wingate test was performed 5 times with 90 g/kg body weight load with 2-min intervals. Peak power, mean power (MP), fatigue index (FI) were calculated. Capillary blood samples for lactate analysis were taken during the initial rest period and soon after the fifth Wingate test. For 6 days the creatine group (n=12) ingested 5 g creatine monohydrate, the placebo group (n=11) a flavored drink without creatine monohydrate 4 times daily. On the 7th day, the Wingate tests were repeated, as was the 1st day. In the creatine group, MP in the 3rd and 4th Wingate test, in the placebo group FI in the 1st and 2nd Wingate test significantly increased. While the total power output obtained from the five Wingate tests increased 8 percent whereas there was no change in the placebo group. It is concluded that creatine supplementation enhances total power output during the repeated bouts of supramaximal exercise separated by short resting intervals [082].

**Effect of creatine on myostatin during resistance training**

Myostatin is a catabolic regulator of skeletal muscle mass. The purpose of one study was to determine the effect of resistance training for 8 weeks in conjunction with creatine supplementation on muscle strength, lean body mass, and serum levels of myostatin and growth and differentiation factor-associated serum protein-1 (GASP-1). In a double-blinded design 27 healthy male subjects were assigned to control (CON), resistance training plus placebo (RT+PL) and resistance training+creatine supplementation (RT+CR) groups. The protocol consisted of 3 days per week of training for 8 weeks, each session including three sets of 8-10 repetitions at 60-70 percent of 1 RM for whole-body exercise. Blood sampling, muscular strength testing and body composition analysis (full body DEXA) were performed at 0, 4th and 8th weeks. Myostatin and GASP-1 was measured. Resistance training caused significant decrease in serum levels of myostatin and increase in that of GASP-1. Creatine supplementation in conjunction with resistance training lead to greater decreases in serum myostatin, but had not additional effect on GASP-1. The effects of resistance training on serum levels of myostatin and GASP-1, may explain the increased muscle mass that is amplified by creatine supplementation [083].

**Effect of creatine loading on long-term sprint exercise performance and metabolism**

One study examined whether creatine (Cr) supplementation could enhance long-term repeated-sprint exercise performance of approximately 80 min in duration. Fourteen active, but not well-trained, male subjects initially performed 10 sets of either 5 or 6 x 6 s maximal bike sprints, with varying recoveries (24, 54, or 84 s between sprints) over a period of 80 min. Work done (kJ) and peak power (W) were recorded for each sprint, and venous blood was collected preexercise and on four occasions during the exercise challenge. Muscle biopsies
(vastus lateralis) were obtained preexercise as well as 0 min and 3 min postexercise. Subjects were then administered either 20 g/d Cr.H₂O (n=7) or placebo (n=7) for 5 d. Urine samples were collected for each 24 h of the supplementation period. Subjects were then retested using the same procedures as in test 1. Total work done increased significantly from 251.7 ± 18.4 kJ presupplementation to 266.9 ± 19.3 kJ (6 % increase) after Cr ingestion. No change was observed for the placebo group (254.0 ± 10.4 kJ to 252.3 ± 9.3 kJ). Work done also improved significantly during 6 x 6 s sets with 54-s and 84-s recoveries and approached significance in 5 x 6 s sets with 24-s recovery in the Cr condition. Peak power was significantly increased in all types of exercise sets after Cr loading. No differences were observed for any performance variables in the placebo group. Resting muscle Cr and PCr concentrations were significantly elevated after 5 d of Cr supplementation (Cr: 48.9 %; PCr: 12.5 %). Phosphocreatine levels were also significantly higher immediately and 3 min after the completion of exercise in the Cr condition. The results of this study indicate that Cr ingestion (20 g/day x 5 d) improved exercise performance during 80 min of repeated-sprint exercise, possibly due to an increased TCr store and improved PCr replenishment rate [084].

**Creatine loading, resistance exercise performance, and muscle mechanics**

It was tested the null hypothesis that creatine monohydrate loading (20 g per day for 7 days, n=18) would not alter resistance exercise performance, isometric strength, or in vivo contractile properties of the quadriceps femoris muscle compared with loading with placebo (n=13) in resistance-trained subjects. For the entire study group, the 1 repetition maximum (1RM) and 5-set performance (the number of repetitions) for unilateral, dynamic knee extension increased slightly (2 % and 5 %, respectively) after dietary supplementation, and these responses did not differ by condition. Maximal voluntary isometric torque and the rate of torque development did not change. During electromyostimulation, torque development and relaxation time were also unaffected. The data suggest that creatine loading does not augment unilateral strength or multiset resistance exercise performance for knee extensions compared with placebo loading [085].

**Creatine effects on periodized, off-season resistance-training program**

The periodized resistance-training model has not been well documented in the literature. Further research is needed to determine if periodized resistance training in conjunction with creatine supplementation can cause changes in strength, performance, total body weight, girth, and lean muscle mass. Therefore, the purpose of this investigation was to determine the effects of periodized resistance training in conjunction with low-dose (LD) and high-dose (HD) creatine supplementation on strength, body composition, and anaerobic muscular endurance. Subjects were divided into 3 groups: LD, HD, and placebo (P). Testing took place pre-, mid-, and postsupplementation for the following: weight, body composition (fat-free mass and fat mass), 1 repetition maximum squat, and anaerobic muscular endurance testing. Results revealed no significant differences in either creatine group when compared with the P group. However, significant differences were noted over time. These data suggest that 10 weeks of periodized resistance training was effective for causing changes in strength, body composition, and anaerobic muscular endurance [086].

**Effects on isometric bench-press performance in resistance-trained humans**

The purpose of one study was to investigate the effects of creatine (Cr) supplementation on force generation during an isometric bench-press in resistance-trained men. Thirty-two resistance-trained men were matched for peak isometric force and assigned in double-blind fashion to either a Cr or placebo group. Subjects performed an isometric bench-press test involving five maximal isometric contractions before and after 5 d of Cr (20 g/d Cr + 180 g/d dextrose) or placebo (200 g/d dextrose). Body composition was measured before and after
supplementation. Subjects completed 24-h urine collections throughout the study period; these were subsequently analyzed to provide total Cr and creatinine excretion. The amount of Cr retained over the supplementation period was $45 \pm 18$ g, with an estimated intramuscular Cr storage of $43 (13-61)$ mmol/kg dry weight muscle. Four subjects in the Cr group were classified as "nonresponders" ($< 21$ mmol/kg dry weight muscle increase following Cr supplementation) and the remaining 17 subjects were classed as "responders". For the Cr group, peak force and total force pre- or post-supplementation were not different from placebo. However, when the analysis was confined to the responders, both the change in peak force and the change in total force post-supplementation were significantly greater compared with the placebo group. For the Cr group, estimated Cr uptake was inversely correlated with training status. Cr significantly increased body weight ($84.1 \pm 8.6$ kg pre- vs $85.3 \pm 8.3$ kg post-supplementation) and fat-free mass ($71.8 \pm 6.0$ kg pre- vs $72.6 \pm 6.0$ kg post-supplementation), with the magnitude of increase being significantly greater in the responder group than in the placebo group. Five days of Cr supplementation increased body weight and fat-free body mass in resistance-trained men who were classified as responders. Peak force and total force during a repeated maximal isometric bench-press test were also significantly greater in the responders compared to the placebo group [087].

**Short-term resistance training**

To determine the effects of creatine supplementation during short-term resistance training overreaching on performance, body composition, and resting hormone concentrations, 17 men were randomly assigned to supplement with 0.3 g/kg per day of creatine monohydrate (CrM: n=9) or placebo (P: n=8) while performing resistance exercise (5 days/week for 4 weeks) followed by a 2-week taper phase. Maximal squat and bench press and explosive power in the bench press were reduced during the initial weeks of training in P but not CrM. Explosive power in the bench press, body mass, and lean body mass (LBM) in the legs were augmented to a greater extent in CrM by the end of the 6-week period. A tendency for greater 1-RM squat improvement was also observed in CrM. Total testosterone (TT) and the free androgen index (TT/SHBG) decreased in CrM and P, reaching a nadir at week 3, whereas sex hormone binding globulin (SHBG) responded in an opposite direction. Cortisol significantly increased after week 1 in CrM (+29 %), and returned to baseline at week 2. Insulin was significantly depressed at week 1 (-24 %) and drifted back toward baseline during weeks 2-4. Growth hormone and IGF-I levels were not affected. Therefore, some measures of muscular performance and body composition are enhanced to a greater extent following the rebound phase of short-term resistance training overreaching with creatine supplementation and these changes are not related to changes in circulating hormone concentrations obtained in the resting, postabsorptive state. In addition, creatine supplementation appears to be effective for maintaining muscular performance during the initial phase of high-volume resistance training overreaching that otherwise results in small performance decrements [088].

**In older males**

Age-related sarcopenia and dynapenia have negative effects on strength and the ability to perform activities of daily living. Resistance training (RT) increases muscle mass and strength in older adults and is an established countermeasure for sarcopenia and dynapenia and creatine may enhance this effect. It was aimed to determine whether the addition of Cr to RT increased gains in muscle mass, strength and function in older adults over RT alone by conducting a systematic review and meta-analysis. Pubmed and Healthstar databases were searched. Randomized, placebo (PL) controlled trials that involved older adults supplemented with Cr and including RT regimes (>6 weeks) were included. Data were analyzed using fixed or random (if data were heterogeneous) effects meta-analysis using RevMan 5. The meta-analysis comprised 357 older adults (64 ± 5, 64 ± 5 years, creatine and placebo groups, respectively) with 13 ± 5 weeks of RT. Cr+RT increased total body mass
and fat free mass with no effect on fat mass as compared with RT alone. Cr+RT increased chest press and leg press 1RM to a greater extent than RT alone, with no difference in effect on knee extension or biceps curl 1RM, isokinetic or isometric knee extension peak torque. Cr+RT had a greater effect than RT alone on the 30s chair stand test. It was concluded that retention of muscle mass and strength is integral to healthy aging. The results from this meta-analysis are encouraging in supporting a role for Cr supplementation during RT in healthful aging by enhancing muscle mass gain, strength and functional performance; however, the limited number of studies indicates further work is needed [089].

Effects of creatine on anaerobic exercise

Creatine has demonstrated neuromuscular performance enhancing properties on short duration, predominantly anaerobic, intermittent exercises. It has been observed enhanced neuromuscular function of the elbow flexors in both electrically induced and voluntary contractions but not on endurance performance after 4 loading doses of 5 g creatine plus 15 g maltodextrin for 5/d in young, moderately trained men. Creatine supplementation may facilitate the reuptake of Ca\(^{2+}\) into the sarcoplasmic reticulum by the action of the Ca\(^{2+}\) adenosine triphosphatase pump, which could enable force to be produced more rapidly through the faster detachment of the actomyosin bridges. A previous meta-analysis reported an overall creatine supplementation effect size of 0.24 ± 0.02 for activities lasting ≤30 s. (primarily using the ATP-phosphocreatine energy system). For this short high-intensity exercise, creatine supplementation resulted in a 7.5 ± 0.7 percent increase from base line which was greater than the 4.3 ± 0.6 percent improvement observed for placebo groups. When looking at the individual selected measures for anaerobic performance the greatest effect of creatine supplementation was observed on the number of repetitions which showed an ES of 0.64 ± 0.18. Furthermore, an increase from base line of 45.4 ± 7.2 percent compared to 22.9 ± 7.3 percent for the placebo group was observed. The second greatest ES was on the weight lifted at 0.51 ± 0.16 with an increase from base line of 13.4 ± 2.7 percent for the placebo group and 24.7 ± 3.9 percent for the creatine group. The possible effect of creatine supplementation on multiple high intensity short duration bouts (<30 s) have shown an effect size not statistically significant from 0. This would indicate that creatine supplementation might be useful to attenuate fatigue symptoms over multiple bouts of high-intensity, short duration exercise. The ES of creatine on anaerobic endurance exercise (>30-150s), primarily using the anaerobic glycolysis energy system, was 0.19 ± 0.05 with an improvement from baseline of 4.9 ± 1.5 percent for creatine and -2.0 ± 0.6 percent for the placebo [035].

Creatine supplementation and upper extremity anaerobic response in females

The purpose of one study was to investigate the influence of creatine monohydrate (CrH\(_2\)O) on upper extremity anaerobic response in strength-trained females involved in overhead sports. Two movements were utilized in this evaluation: elbow flexion and shoulder internal rotation. Subjects were pair-matched and assigned to receive placebo (n=13) or 25 g CrH\(_2\)O (n=11) for 7 days. Pre- and post-treatment measurements included peak concentric and eccentric isokinetic torque, isotonic 1RM, and fatigue during elbow flexion; isotonic 1RM, FAT, and peak velocity during internal rotation; and body weight. MANOVAs revealed significant interaction between treatment and trial for elbow flexion but not for internal rotation or weight. Univariate analysis indicated a significantly greater change in elbow flexion fatigue following CrH\(_2\)O than following placebo. Thus, creatine monohydrate did not influence peak elbow flexion or internal rotation strength, internal rotation work to fatigue, or internal rotation velocity, but was associated with greater work capacity during fatiguing elbow flexion. These data suggest that CrH\(_2\)O may enhance upper extremity work capacity, but this enhancement may not extend to the muscles primarily responsible for overhead sports performance [090].
Endurance

Although creatine supplementation has been shown to be more effective on predominantly anaerobic intermittent exercise, there is some evidence of its positive effects on endurance activities as well. From a meta-analysis, it would appear that the ergogenic potential for creatine supplementation on predominantly aerobic endurance exercise diminishes as the duration of the activity increases over 150s. However it is suggested that creatine supplementation may cause a change in substrate utilization during aerobic activity possibly leading to an increase in steady state endurance performance. Despite that, the effects of creatine supplementation on endurance performance have been questioned by some studies. In addition, of the concern related to the dosage used in many studies, it could be possible that the potential benefits of creatine supplementation on endurance performance were more related to effects of anaerobic threshold localization [035].

The effect of oral creatine supplementation on aerobic and anaerobic performance was investigated in 16 elite male rowers during 7-day endurance training. Before and after the daily ingestion of 20 g creatine monohydrate for 5 days (Cr-Group, n=8) or placebo (Pl-Group, n=8), subjects performed two exercise tests on a rowing ergometer: (a) incremental exercise consisting of 3-min stage durations and increased by 50 W until volitional exhaustion; (b) an all-out anaerobic exercise performed against a constant load of 7 W/kg. Heart rate and blood lactate concentrations were determined during exercise and recovery. Maximal power output did not significantly differ after the treatment in either group. The mean individual lactate threshold rose significantly after Cr treatment from 314.3 ± 5.0 W to 335.6 ± 7.1 W, as compared with 305.0 ± 6.9 W and 308.9 ± 5.9 W, before and after placebo ingestion, respectively. During the anaerobic test, the athletes supplemented with creatine were able to continue rowing longer than Pl-Group. No significant differences were found between groups in blood LA after the all-out exercise. The results indicate that in elite rowers, creatine supplementation improves endurance (expressed by the individual lactate threshold) and anaerobic performance, independent of the effect of intensive endurance training [091].

To determine whether creatine monohydrate supplementation would improve performance during a submaximal treadmill run interspersed with high-intensity intervals, 15 college soccer players (8 women, 7 men) received either creatine or a maltodextrin placebo at 0.3 g/kg body mass per day for 6 days. The speed of the treadmill was constant at 160.8 m/min, and every 2 minutes the grade was elevated to 15 percent. Each hill segment was 1 minute long. At the end of the 20-minute protocol, the treadmill was again elevated to 15 percent and held there until volitional exhaustion occurred. There was a significant treatment effect of creatine supplementation on body mass in the men; however, no significant differences were observed in the women. There were no treatment effects on time to exhaustion, ratings of perceived exertion, or blood lactate concentration. There was a tendency for blood lactate levels to be lower after short-term creatine supplementation in the women, but this was not statistically significant. Based on these results, it appears that creatine supplementation does not improve performance in submaximal running interspersed with high-intensity intervals [092].

Effects on jumping, sprinting or cycling

When performance is assessed based on intensity and duration of the exercises, there is contradictory evidence relative to both continuous and intermittent endurance activities. However, activities that involve jumping, sprinting or cycling generally show improved sport performance following creatine ingestion [018].
Creatine reduces muscle inosine monophosphate depletion

Creatine (Cr) supplementation has been shown to attenuate increases in plasma ammonia and hypoxanthine during intense endurance exercise lasting 1 h, suggesting that Cr supplementation may improve muscle energy balance (matching of ATP resynthesis to ATP demand) during such exercise. It was hypothesized that Cr supplementation would improve muscle energy balance (as assessed by muscle inosine monophosphate (IMP) accumulation) during intense endurance exercise. Seven well-trained men completed two experimental trials involving approximately 1 h of intense endurance exercise (cycling 45 min at 78 % of VO2peak followed by completion of 251kJ as quickly as possible (performance ride)). Subjects ingested approximately 42 g/d dextrose for 5 d before the first experimental trial (CON), then approximately 21 g Cr monohydrate plus approximately 21 g/d dextrose for 5 d before the second experimental trial (CREAT). Trials were ordered because of the long washout time for Cr. Subjects were blinded to the order of the trials. Creatine supplementation significantly increased muscle total Cr. No difference was seen between treatments in any measured muscle or blood metabolite after the first 45 min of exercise. Despite the performance ride completion time being similar in the two treatments (approximately 14 min, approximately 86 % of VO2 peak), IMP at the end of the performance ride was significantly lower in CREAT than in CON. It was concluded that raising muscle total Cr content before exercise appears to improve the ability of the muscle to maintain energy balance during intense aerobic exercise, but not during more moderate exercise intensities.

Endurance followed by sprint

The effects of creatine supplementation on muscle metabolism and exercise performance during a simulated endurance road race were investigated. Twelve adult male endurance-trained cyclists completed a simulated road race on a cycle ergometer (Lode), consisting of a two-hour cycling bout at 60 percent of peak aerobic capacity (VO2peak) with three 10-second sprints performed at 110 percent VO2peak every 15 minutes. Cyclists completed the 2-hr cycling bout before and after dietary creatine monohydrate or placebo supplementation (3 g/day for 28 days). Muscle biopsies were taken at rest and five minutes before the end of the two-hour ride. There was a 25 percent increase in resting muscle total creatine and 38 percent increase in muscle creatine phosphate in the creatine group. Plasma glucose, blood lactate, and respiratory exchange ratio during the 2-hour ride, as well as VO2peak, were not affected by creatine supplementation. Submaximal oxygen consumption near the end of the two-hour ride was significantly decreased by approximately 10 percent by creatine supplementation. Changes in plasma volume from pre- to post-supplementation were significantly greater in the creatine group than the placebo group at 90 minutes of exercise. The time of the final sprint to exhaustion at the end of the 2-hour cycling bout was not affected by creatine supplementation. Power output for the final sprint was increased by about 33 percent in both groups (creatine vs placebo not significant). It can be concluded that although creatine supplementation may increase resting muscle total creatine, muscle creatine phosphate, and plasma volume, and may lead to a reduction in oxygen consumption during submaximal exercise, creatine supplementation does not improve sprint performance at the end of endurance cycling exercise.

Few studies have focused on the metabolic changes induced by creatine supplementation. This study investigated the effects of creatine supplementation on plasma and urinary metabolite changes of athletes after endurance and sprint running. Twelve male athletes (20 ± 1 year) performed two identical (65-70 % maximum heart rate reserved) 60 min running exercises (endurance trial) before and after creatine supplementation (12 g creatine monohydrate/day for 15 days), followed by a 5-day washout period. Subsequently, they performed two identical 100 m sprint running exercises (power trial) before and after 15 days
of creatine supplementation in accordance with the supplementary protocol of the endurance trial. Body composition measurements were performed during the entire study. Plasma samples were examined for the concentrations of glucose, lactate, branched-chain amino acids (BCAAs), free-tryptophan (f-TRP), glutamine, alanine, hypoxanthine, and uric acid. Urinary samples were examined for the concentrations of hydroxyproline, 3-methylhistidine, urea nitrogen, and creatinine. Creatine supplementation significantly increased body weights of the athletes of endurance trial. Plasma lactate concentration and ratio of f-TRP/BCAAs after recovery from endurance running were significantly decreased with creatine supplementation. Plasma purine metabolites (the sum of hypoxanthine and uric acid), glutamine, urinary 3-methylhistidine, and urea nitrogen concentrations tended to decrease before running in trials with creatine supplements. After running, urinary hydroxyproline concentration significantly increased in the power trial with creatine supplements. The findings suggest that creatine supplementation tended to decrease muscle glycogen and protein degradation, especially after endurance exercise. However, creatine supplementation might induce collagen proteolysis in athletes after sprint running [095].

Effects on blood lactate
To determine the effects of creatine supplementation on blood lactate during incremental cycling exercise 13 male subjects (23 ± 2 years) performed a maximal, incremental cycling test to exhaustion before (Pre) and after (Post) 6 d of creatine supplementation (4 doses/d of 5 g creatine + 15 g glucose). Blood lactate was measured at the end of each exercise stage during the protocol, and the lactate threshold was determined as the stage before achieving 4 mmol/L. Lactate concentrations during the incremental test were analyzed using a 2 (condition) × 6 (exercise stage) repeated-measures ANOVA. Differences in power at lactate threshold, power at exhaustion, and total exercise time were determined by paired t tests. Lactate concentrations were reduced during exercise after supplementation, demonstrating a significant condition effect. There was a tendency for increased power at the lactate threshold. Total time to fatigue approached significant increases as did maximal power output. The findings demonstrate that creatine supplementation decreases lactate during incremental cycling exercise and tends to raise lactate threshold. Therefore, creatine supplementation could potentially benefit endurance athletes [096].

Effect of creatine loading on oxygen uptake during a 1-km cycling time trial
For the first time, it was investigated the effects of altering cellular metabolic capacitance, via a 5-d creatine (Cr) loading protocol (20 g/d), on oxygen uptake (VO₂), accumulated oxygen deficit, muscle recruitment, and performance during a 1-km cycling time trial. In a double-blind, randomized, placebo-controlled design, 19 amateur cyclists were allocated to a Cr (n=10) or placebo (n=9) group, and performed a 1-km cycling time trial before and after the supplementation period. Body mass was significantly increased in the Cr group, but not in the placebo group. Participants adopted an "all-out" pacing strategy in both groups. However, Cr loading reduced VO₂ immediately after the beginning (12th to 23th seconds), and this was accompanied by a reduced aerobic and increased anaerobic contribution. The VO₂ mean response time was slower (pre: 17.2 ± 5.6 s vs post: 19.9 ± 4.6 s), the total O₂ uptake was reduced (pre: 4.64 ± 0.59 L vs post: 4.47 ± 0.53 L), and the oxygen deficit was increased (pre: 0.82 ± 0.27 L vs post: 0.98 ± 0.25 L) after Cr loading. No differences were observed in the placebo group for these variables. Plasma lactate and integrated electromyography were not altered in either group, nor was the time to complete the trial (Cr group: pre: 89.1 ± 6.7 s vs post 89.1 ± 6.2 s and placebo group: pre 85.9 ± 4.9 s vs post 87.0 ± 5.4 s). Cr loading slows the VO₂ response and increases the anaerobic contribution during a 1-km cycling time trial [097].
Creatine for endurance in female soccer players

To investigate the effects of a six-week plyometric training and creatine supplementation intervention on maximal-intensity and endurance performance in female soccer players during in-season training a randomized, double-blind, placebo-controlled trial was performed. Young (age 22.9 ± 2.5y) female players with similar training load and competitive background were assigned to a plyometric training group receiving placebo (PLACEBO, n=10), a plyometric training group receiving creatine supplementation (CREATINE, n=10) or a control group receiving placebo without following a plyometric program (CONTROL, n=10). Athletes were evaluated for jumping, maximal and repeated sprinting, endurance and change-of-direction speed performance before and after six weeks of training. After intervention the CONTROL group did not change, whereas both plyometric training groups improved jumps (ES=0.25-0.49), sprint (ES=0.35-0.41), repeated sprinting (ES=0.48-0.55), endurance (ES=0.32-0.34) and change-of-direction speed performance (ES=0.46-0.55). However, the CREATINE group improved more in the jumps and repeated sprinting performance tests than the CONTROL and the PLACEBO groups. Adaptations to plyometric training may be enhanced with creatine supplementation [098].

Cardiorespiratory responses

Creatine supplementation alters the response to a graded cycle ergometer test

To determine the effects of creatine supplementation on cardiorespiratory responses during a graded exercise test (GXT) 36 trained adults (20 male, 16 female; 21-27 years old) performed two maximal GXTs on a cycle ergometer. The first GXT was done in a nonsupplemented condition, and the second GXT was done following 7 days of ingesting either 5 g creatine monohydrate, encased in gelatin capsules, four times daily (CS, 13 male, 6 female), or the same number of glucose capsules (PL, 7 male, 10 female). CS significantly improved total test time [pre-CS = 1217 s, mean (std. dev.) versus post-CS = 1289], while PL administration had no effect on total test time. In addition, both oxygen consumption (VO₂) and heart rate at the end of each of the first five GXT stages were significantly lower after CS, but were unchanged after PL. Moreover, the ventilatory threshold occurred at a significantly greater VO₂ for CS. Neither CS nor PL had an effect on peak VO₂. Apparently, CS can alter the contributions of the different metabolic systems during the initial stages of a GXT. Thus, the body is able to perform the sub-maximal workloads at a lower oxygen cost with a concomitant reduction in the work performed by the cardiovascular system [099].

Increased enhances oxygen uptake during alternating intensity exercise

The main purpose of one study was to measure the total oxygen consumed, accumulation of blood metabolites, and performance during alternating intensity exercise before and after a period of creatine (Cr) loading in well-trained humans. Fourteen males were randomly assigned to two groups of seven males and were tested before and after 5 d of placebo (PL) or Cr monohydrate (CR) loading (20 g/d). Oxygen uptake was measured using a breath-by-breath system during bicycle exercise alternating every 3 min between bouts at 30 percent and 90 percent of the maximal power output to exhaustion. Blood samples were also obtained at rest, before the end of each cycling load, at exhaustion, and 5-min postexercise. The oxygen consumed during 1-90 percent and 2-90 percent was larger after CR. Blood ammonia accumulation at the end of 1-90 percent and 3-30 percent was lower after CR, whereas plasma uric acid accumulation was lower at exhaustion and 5-min postexercise. Time to exhaustion increased from 29.9 ± 3.8 to 36.5 ± 5.7 min after CR, whereas it remained the same after PL. The results indicate that Cr feeding increases the capacity of human muscle to perform work during alternating intensity contraction, possibly as a
consequence of increased aerobic phosphorylation and flux through the creatine kinase system [100].

Effect on strength and power

The purpose of one study was to examine the effects of 7 days of supplementation with 20 g/day of creatine monohydrate (CM) on mean power (MP) and peak power (PP) from the Wingate anaerobic test (WAnT), body weight (BW), 1-repetition maximum (1RM) bilateral leg extension (LE) strength, and 1RM bench press (BP) strength. The study used a randomized, double-blind, placebo-controlled design. Twenty-two men (age 22 ± 2 years) were randomly assigned to either a supplement (SUPP; n=10) or placebo (PLAC; n=12) group. The SUPP group ingested 20 g/d of CM powder for 7 days, whereas the PLAC ingested 20 g/d of maltodextrin powder. Measurements for the PLAC and SUPP groups included BW, PP, and MP from two 30-second WAnTs (separated by 7 minutes), and 1RM strength for LE and BP. Testing was conducted before (PRE) and after (POST) 7 days of ingesting either the supplement or placebo. The results of this study indicated that there was a significant increase from PRE to POST testing in MP for the SUPP group (5 %) but not for the PLAC group (-0.3 %). There were no between-group differences, however, for 1RM LE and 1RM BP strength. Furthermore, there were no changes in PP or BW for either group. The findings of this study indicated that loading with 20 g/d of CM for 7 days increased MP (5.4 % increase) from the WAnT, but it had no effect on strength (1RM LE and 1RM BP), PP, or BW [101].

Creatine monohydrate (CrH2O) supplementation has been demonstrated to increase skeletal muscle power output in men. However, its effect upon women is not as clearly defined. This study investigated the effect of oral creatine supplementation upon muscle function, thigh circumference, and body weight in women. Twenty-two consenting college-age women were assigned to 1 of 2 groups matched for dietary and exercise habits, phase of menstrual cycle, and fat-free mass (FFM). After familiarization with testing procedures, pretrial measures of muscle function (5 repetitions 60 deg/s and 50 repetitions 180 deg/s were conducted during maximal voluntary concentric contraction of the preferred quadriceps muscle using an isokinetic dynamometer. Subjects then ingested 0.5 g/kg FFM of either CrH2O or placebo (one fourth dosage 4 times daily) in a double-blind design for 5 days. Resistance exercise was prohibited. After the ingestion phase was completed, all measures were repeated at the same time of day as during pretrials. Statistical analysis revealed time to peak torque in quadriceps extension decreased from pre-test values of 255 ± 11 ms to post-test values of 223 ± 3 ms; average power in extension increased from 103 ± 7 W pre-test to 112 ± 7 W post-test; and, during flexion, average power increased from 59 ± 5 W pre-test to 65 ± 5 W post-test in the creatine group as compared to controls. FFM, percent body fat, midquadriceps circumference, skinfold thickness of the measured thigh, and total body weight did not change for both groups between trials. It was concluded that CrH2O improves muscle performance in women without significant gains in muscle volume or body weight [102].

Critical reviews of the scientific literature, including a meta-analysis and a monograph generally indicate that creatine supplementation may increase muscular strength and endurance as documented by enhanced performance in 1-repetition maximum strength tests, increased number of repetitions in various isotonic and isokinetic resistance exercise tasks, and increased work output during maximal short term (6-30 seconds) cycle ergometer tasks. In general, activities that involve sprinting, jumping or cycling performance show improved performance following creatine supplementation, but the beneficial effects appear to be less consistent. For example, using a standard creatine loading protocol with well-trained male sprinters as subjects, it was reported significant improvements in 100-meter sprint velocity and time to complete 6 intermittent 60-meter sprints. It was also reported
significant increases in peak power and total work production in 10 sets of multiple 6-second bike sprints with varying periods of recovery in an 80-minute time frame following creatine supplementation. Conversely, it was reported no significant improvement in a 70-meter shuttle run sprint power test by well-trained tennis players and no beneficial effects on repeated 10-second skating sprints in ice-hockey players. Some research findings have a direct application to sports competition, such as an increased 1-RM performance in weight lifting and faster 100-meter sprint run times. The laboratory findings for other types of exercise performance are also rather strong, and do support a possible application to actual field competitions. For example, findings of increased muscle power output during intermittent sprint exercises may be applicable to football (soccer) and other sports associated with high-intensity intermittent sprinting. In one such study, it was studied the effects of creatine supplementation on an exercise test protocol designed to simulate match play in soccer (football). The test involved 5 blocks of 11-minute exercise involving sprint running, agility runs, and a precision ball-kicking drill interspersed with recovery walks, jogs and runs. Creatine supplementation improved performance in some repeated sprint and agility tasks even though the subjects increased body mass, but the creatine had no effect on ball kicking accuracy. Thus, creatine supplementation might improve speed in repetitive sprints, important for many sports, but may not necessarily enhance sports skills. In support of this latter point, several studies reported no significant effects of creatine loading on tennis skill performance as measured by power and precision of their serves [030].

Little effect on maximal strength

When maximal force or strength (dynamic or isotonic contractions) is the outcome measure following Cr ingestion, it generally appears that Cr does significantly impact force production regardless of sport, sex or age. The evidence is much more equivocal when investigating isokinetic force production and little evidence exists to support the use of Cr for isometric muscular performance [018].

Dietary creatine (Cr) supplementation has been shown to enhance muscular strength and endurance. This study determined the effects of Cr supplementation on performance of military training tasks. Two groups (Cr and placebo [Pl]) of 13 male soldiers each performed 3 consecutive military obstacle course runs (approximately 3 minutes over 7 obstacles with a 2-minute rest between runs) followed by a rifle marksmanship task on 3 occasions (T(1), T(2), and T(3)), each separated by 5 days. They also completed a bench press protocol (5 sets to failure at 70% of 1 repetition maximum) and answered the Profile of Mood States questionnaire during each test session. Testing was done 3 times. No supplementation was given before T(1). Supplementation was provided using sports bars, with both groups receiving Pl bars between T(1) and T(2), whereas from T(2) to T(3) the Cr group consumed 24 g per day of Cr monohydrate in sports bars and the Pl group consumed an equal amount (kilocalories) of Pl sports bars. Creatine usage resulted in a significant (14 %) increase in total bench press repetitions, but no difference between groups in obstacle course run times for the 3 runs from T(2) to T(3). Marksmanship or mood was not affected by Cr supplementation. An increase of 1.4 kg in body mass and a 0.5 percent decrease in percent body fat were observed after Cr ingestion. Creatine supplementation over 5 days improved performance during a controlled strength test but did not significantly improve military obstacle course performance [103].

Improvement of strength? A meta-analysis

Oral creatine is the most widely used nutritional supplement among athletes. The purpose of one study was to investigate whether creatine supplementation increases maximal strength and power in healthy adults through a meta-analysis of existing literature. It was searched MEDLINE (1966-2000) and the Cochrane Controlled Trials Register (through June 2001) to
locate relevant articles. It was reviewed conference proceedings and bibliographies of identified studies. An expert in the field was contacted for sources of unpublished data. Randomized or matched placebo controlled trials comparing creatine supplementation with placebo in healthy adults were considered. Presupplementation and postsupplementation change in maximal weight lifted, cycle ergometry sprint peak power, and isokinetic dynamometer peak torque were measured. Sixteen studies were identified for inclusion. The summary difference in maximum weight lifted was 6.85 kg (95% confidence interval 5.24 to 8.47) greater after creatine than placebo for bench press and 9.76 kg (95% confidence interval 3.37 to 16.15) greater for squats; there was no difference for arm curls. In 7 of 10 studies evaluating maximal weight lifted, subjects were young men (younger than 36 years) engaged in resistance training. There was no difference in cycle ergometer or isokinetic dynamometer performance. It was concluded that oral creatine supplementation combined with resistance training increases maximal weight lifted in young men. There is no evidence for improved performance in older individuals or women or for other types of strength and power exercises. Also, the safety of creatine remains unproven. Therefore, until these issues are addressed, its use cannot be universally recommended [104].

Effects of oral creatine on muscular strength and body composition

The purpose of one investigation was to examine the effects of 6 week of oral creatine supplementation during a periodized program of arm flexor strength training on arm flexor IRM, upper arm muscle area, and body composition. Twenty-three male volunteers with at least 1 yr of weight training experience were assigned in a double blind fashion to two groups (Cr, n=10; Placebo, n=13) with no significant mean pretest one repetition maximum (IRM) differences in arm flexor strength. Cr ingested 5 g of creatine monohydrate in a flavored, sucrose drink four times per day for 5 d. After 5 d, supplementation was reduced to 2 g/d. Placebo ingested a flavored, sucrose drink. Both drinks were 500 mL and made with 32 g of sucrose. IRM strength of the arm flexors, body composition, and anthropometric upper arm muscle area (UAMA) were measured before and after a 6-week resistance training program. Subjects trained twice per week with training loads that began at 6RM and progressed to 2RM. IRM for Cr increased from (mean ± SD) 42.8 ± 17.7 kg to 54.7 ± 14.1 kg, while IRM for placebo increased from 42.5 ± 15.9 kg to 49.3 ± 15.7 kg. At post-test IRM was significantly greater for Cr than for Placebo. Body mass for Cr increased from 86.7 ± 14.7 kg to 88.7 ± 13.8 kg. Fat-free mass for Cr increased from 71.2 ± 10.0 kg to 72.8 ± 10.1 kg. No changes in body mass or fat-free mass were found for placebo. There were no changes in fat mass and percent body fat for either group. UAMA increased 7.9 cm² for Cr and did not change for placebo. It was concluded that creatine supplementation during arm flexor strength training lead to greater increases in arm flexor muscular strength, upper arm muscle area, and fat-free mass than strength training alone [105].

Fatigue sustained during short-term, high-intensity exercise in humans is associated with the inability of skeletal muscle to maintain a high rate of anaerobic ATP production from phosphocreatine hydrolysis. Ingestion of creatine monohydrate at a rate of 20 g/day for 5-6 days was shown to increase the total creatine concentration of human skeletal muscle by approximately 25 mmol/kg dry mass, some 30 percent of this in phosphorylated form as phosphocreatine. A positive relation was then shown between muscle creatine uptake and improvements in performance during repeated bouts of maximal exercise. However, there is no evidence that increasing intake > 20-30 g/day for 5-6 days has any potentiating effect on creatine uptake or performance. In individuals in whom the initial total creatine concentration already approached 150 mmol/kg dry mass, neither creatine uptake nor an effect on phosphocreatine resynthesis or performance was found after supplementation. Loss of ATP during heavy anaerobic exercise was found to decline after creatine ingestion, despite an increase in work production. These results suggest that improvements in performance are due to parallel improvements in ATP resynthesis during exercise as a consequence of
increased phosphocreatine availability. Creatine uptake is augmented by combining creatine supplementation with exercise and with carbohydrate ingestion [106].

**Strength loss after eccentric contractions is unaffected by creatine supplementation**

One study's objective was to determine whether 14 days of dietary creatine supplementation preceding an injurious bout of eccentric contractions affect the in vivo strength loss of mouse anterior crural muscles. Three groups of nine mice each were fed a meal diet for 14 days, one group at each of three levels of creatine supplementation (i.e., 0, 0.5, and 1 % creatine). Electrically stimulated concentric, isometric, and eccentric contraction torques produced about the ankle were measured both before and after a bout of 150 eccentric contractions. Tibialis anterior muscle creatine concentration was significantly increased by the supplementation, being 12% higher in the mice fed the 1 percent creatine diet compared with control mice. After the bout of eccentric contractions, the reductions in torque (i.e. 46-58 %) were similar for the isometric contraction, all eccentric contractions, and the slow (i.e. <200 (o)/s) concentric contractions; above 200 (o)/s, the percent reduction in concentric torque increased progressively to 85-88 percent at 1,000-1,200 (o)/s. However, there was no effect of creatine supplementation on the isometric torque loss or on the torque loss at any eccentric or concentric angular velocity. In conclusion, a moderate increase in muscle creatine concentration induced by dietary supplementation in mice does not affect the strength loss after eccentric contractions [107].

**Local effects on muscles**

**Effects of creatine supplementation on isometric force-time curve characteristics**

To assess the effects of creatine monohydrate on isometric force-time curve parameters of sedentary college males aged 18-25 years a double-blind study randomly assigned subjects to either a treatment (with creatine (Cr)) group (n=11) or placebo group (P) (n=8). The Cr group received 20 g/day of Cr for the first 5 d, in 5-g doses, four times daily (loading period) followed by a 5 g/dose for the next 5 d (maintenance phase) and then no Cr ingestion for 7 d (washout period). Each 5-g dose was mixed with 250 mL of Gatorade. The P group received a placebo (cornstarch) following the exact same dosage regimen and protocol as the Cr group. All subjects were sedentary and had not used any nutritional supplements for 6 months before the study. Measurements of isometric force production of four muscle groups (elbow flexors and extensors; knee flexors and extensors) were characterized by a number of force-time parameters including strength (MF), time to maximal force (TMF), rate of force development (MRFD), and intermittent endurance (total impulse (TI) and percent force decrement (PFD)). Testing was done at pretreatment, after the 10-d loading and maintenance phases, and after the washout phase. Repeated measures ANOVA indicated no significant group effect for any muscle group concerning the maximal strength parameters and only two significant time effects for the knee flexors during MF and MRFD. Similarly, there were no significant group effects for any muscle group during the endurance trials; however, there was a significant time effect concerning TI for each muscle group tested. The findings indicate that oral supplementation with creatine monohydrate in untrained males does not positively influence isometric strength but may enhance intermittent isometric muscular endurance [108].

**Creatine supplementation and muscular adaptation to resistive overload**

One study examined the influence of dietary creatine (CR) supplementation upon mechanical and hypertrophic responses to a well-defined conditioning stimulus provided by electromyostimulation (EMS). Eighteen resistance-trained subjects were assigned CR or a
placebo (PL) in a randomized, double-blind fashion. After CR loading (20 g/d for 7 d), CR supplementation (5 g/d) or PL was continued for 8 weeks. During supplementation, EMS (3-5 sets of 10 coupled eccentric and concentric actions) was applied to the left m. quadriceps femoris (QF) twice weekly while subjects continued voluntary resistance training of both lower limbs unsupervised. Cross-sectional area (CSA) of each QF was assessed with magnetic resonance imaging (MRI). Torque during EMS was analyzed to assess muscle loading and fatigue resistance. Maximal torque and the torque time integral increased markedly over training. These responses reflected activation of more muscle as EMS current was increased (about 16 %), greater recovery between sets, and less fatigue during sets over training. CR did not influence these responses. In accord with these results, the increase in CSA for the stimulated QF (11 %) was comparable for CR and PL. CSA in the nonstimulated QF increased 5 percent in CR but did not change in PL. It was concluded that CR supplementation did not augment the mechanical or hypertrophic response to a precisely measured conditioning stimulus that attenuated but did not ameliorate fatigue. It was suggested that enhanced fatigue resistance may not explain the apparent ergogenic effect of CR during voluntary training [109].

**Creatine supplementation and lower limb strength performance**

Creatine is the most widely used supplementation to increase strength performance. However, the few meta-analyses are more than 10 years old and suffer from inclusion bias such as the absence of randomization and placebo, the diversity of the inclusion criteria (aerobic/endurance, anaerobic/strength), no evaluation on specific muscles or group of muscles, and the considerable amount of conflicting results within the last decade. The objective of one systematic review was to evaluate meta-analyzed effects of creatine supplementation on lower limb strength performance. It was conducted a systematic review and meta-analyses of all randomized controlled trials comparing creatine supplementation with a placebo, with strength performance of the lower limbs measured in exercises lasting less than 3 min. The search strategy used the keywords “creatine supplementation” and “performance”. Dependent variables were creatine loading, total dose, duration, the time-intervals between baseline (T0) and the end of the supplementation (T1), as well as any training during supplementation. Independent variables were age, sex, and level of physical activity at baseline. It was conducted that meta-analyses at T1, and on changes between T0 and T1. Each meta-analysis was stratified within lower limb muscle groups and exercise tests. It was included 60 studies (646 individuals in the creatine supplementation group and 651 controls). At T1, the effect size (ES) among stratification for squat and leg press were, respectively, 0.336 (95 % confidence interval 0.047 to 0.625) and 0.297 (95 % confidence interval 0.098 to 0.496). Overall quadriceps ES was 0.266 (95 % confidence interval 0.150 to 0.381). Global lower limb ES was 0.235 (95 % confidence interval 0.125 to 0.346). Meta-analysis on changes between T0 and T1 gave similar results. The meta-regression showed no links with characteristics of population or of supplementation, demonstrating the creatine efficacy effects, independent of all listed conditions. It was concluded that creatine supplementation is effective in lower limb strength performance for exercise with a duration of less than 3 min, independent of population characteristic, training protocols, and supplementary doses and duration [110].

**Effect of oral creatine supplementation on isokinetic torque production**

One study was conducted to examine the effect of oral creatine supplementation on the decline in peak isokinetic torque of the quadriceps muscle group during an endurance test. Twenty-three active, but untrained, male subjects performed isokinetic strength tests on a Cybex II dynamometer at 180 degrees/s. The protocol consisted of pre- and post-tests with five sets of 30 maximum volitional contractions with a 1-min rest period between sets. Subjects returned to perform the posttest after 5 days of placebo (4 x 6 g glucose/d, n=12) or
creatine (4 x 5 g creatine + 1 g glucose/d, n=11) supplementation. Supplements and testing were administered in a double blind fashion. Peak torque was measured during each contraction and the 30 contractions were averaged for each set. A three-way mixed ANOVA with one between factor (placebo vs creatine) and two within factors (pre/post supplementation and sets 1-5) revealed no significant interactions. The placebo versus creatine main effect was also nonsignificant, whereas the pre/post and set effects were significant. Peak torque increased (approximately 3 %) from pre- to post-testing, but the absolute magnitude of the differences is unlikely to be of any practical significance. Peak torque decreased from sets 1 to 4, whereas sets 4 and 5 were not different. A priori contrasts comparing the creatine group's performance pre vs post test for the fourth and fifth sets were nonsignificant. Based on within and between group comparisons, it was not possible to detect an ergogenic effect of oral creatine supplementation on the decline in peak torque during isokinetic exercise at 180 degrees/s [111].

Minimal effect on electromyographic fatigue threshold

The purpose of one study was to examine the effects of 5 days of creatine loading on the electromyographic fatigue threshold (EMG FT) in college-age men. Sixteen men participated in this double-blind study and were randomly placed into either placebo (n=8) or creatine (5 g dicitrate citrate plus 10 g of flavored fructose powder per packet; n=8) loading groups. Each participant ingested 1 packet 4 times/d, totaling 20 g/d for 5 days (loading). Before and after loading, each participant performed a discontinuous cycle-ergometer test to determine his EMG FT, using bipolar surface electrodes placed on the vastus lateralis of the right thigh. Four 60-s work bouts (ranging from 200 to 400 W) were completed. Adequate rest was given between bouts to allow for the participants' heart rate to drop within 10 beats of their resting HR. The EMG amplitude was averaged over 5-s intervals for each 60-s work bout. Resulting slopes from each successive work resulted in a nonsignificant interaction for supplement and time. In addition, a significant increase in weight was observed in the creatine group. These data suggest that there was a minimal influence of creatin loading on EMG FT for the participants in this study [112].

Effects of creatine supplementation on skeletal muscle hypertrophy

It has been observed greater improvements on 1RM, lean body mass, fiber cross sectional area and contractile protein in trained young males when resistance training was combined with a multi-nutrient supplement containing 0.1 g/kg/d of creatine, 1.5 g/kg/d of protein and carbohydrate compared with protein alone or a protein carbohydrate supplement without the creatine. These findings were novel because at the time no other research had noted such improvements in body composition at the cellular and sub cellular level in resistance trained participants supplementing with creatine. When creatine supplementation is combined with heavy resistance training, muscle insulin like growth factor (IGF-1) concentration has been shown to increase. Compared to placebo, creatine groups produced greater increments in IGF-1 (78 % vs 55 %) and body mass (2.2 vs 0.6 kg). Additionally, vegetarians within the supplemented group had the largest increase of lean mass compared to non vegetarian (2.4 and 1.9 kg, respectively). Changes in lean mass were positively correlated to the modifications in intramuscular total creatine stores which were also correlated with the modified levels of intramuscular IGF-1. The authors suggested that the rise in muscle IGF-1 content in the creatine group could be due to the higher metabolic demand created by a more intensely performed training session. These amplifying effects could be caused by the increased total creatine store in working muscles. Even though vegetarians had a greater increase in high energy phosphate content, the IGF-1 levels were similar to the amount observed in the non vegetarian groups. These findings do not support the observed correlation pattern by which a low essential amino acid content of a typical vegetarian diet should reduce IGF-1 production [035].
The objective of one study was to determine if additional dietary protein improves the lean tissue deposition and carcass merit of pigs supplemented creatine monohydrate in combination with a high glycemic carbohydrate (dextrose). Forty-eight crossbred barrows and gilts (91 kg) were blocked by sex assigned to 1 of 12 pens (4 pigs/pen, 16 pigs/treatment). Treatments included: control (CON; basal diet consisting of a ground corn-soybean base), combination diet (COMBO; basal diet supplemented with 0.92 % creatine monohydrate and 2.75 % dextrose), and a combination high protein diet (COMBOHP; COMBO formulated to contain a minimum of 16 % crude protein). Barrows on the COMBOHP gained the least 10th rib fat and expressed the highest percentage fat-free carcass lean after 28 days on test. No significant treatment differences were noted in the fat and lean tissue accretion of gilts. Treatments had no affect the meat quality parameters of barrow and gilt carcasses [113].

Effect on skeletal muscle metabolism in physical exercise

Creatine is the object of growing interest in the scientific literature. This is because of the widespread use of creatine by athletes, on the one hand, and to some promising results regarding its therapeutic potential in neuromuscular disease on the other. In fact, since the late 1990s, many studies have examined the effects of creatine supplementation on exercise performance. This article reviews the literature on creatine supplementation as an ergogenic aid, including some basic aspects relating to its metabolism, pharmacokinetics and side effects. The use of creatine supplements to increase muscle creatine content above approximately 20 mmol/kg dry muscle mass leads to improvements in high-intensity, intermittent high-intensity and even endurance exercise (mainly in nonweight-bearing endurance activities). An effective supplementation scheme is a dosage of 20 g/day for 4-6 days, and 5 g/day thereafter. Based on recent pharmacokinetic data, new regimens of creatine supplementation could be used. Although there are opinion statements suggesting that creatine supplementation may be implicated in carcinogenesis, data to prove this effect are lacking, and indeed, several studies showing anticarcinogenic effects of creatine and its analogues have been published. There is a shortage of scientific evidence concerning the adverse effects following creatine supplementation in healthy individuals even with long-term dosage. Therefore, creatine may be considered as a widespread, effective and safe ergogenic aid [114].

Effect of exogenous creatine supplementation on muscle PCr metabolism

$^{31}$P NMR was used to assess the influence of two weeks creatine supplementation (21 g/d) on resting muscle PCr concentration, on the rate of PCr repletion (R(depl)), and on the half-time of PCr repletion ($t_{1/2}$). Body mass (BM) and volume of body water compartments were also estimated by impedance spectroscopy. Fourteen healthy male subjects (21 years) participated in this double-blind study. PCr was measured using a surface coil placed under the calf muscle, at rest and during two exercise bout the duration of which was 1 min. They were interspaced by a recovery of 10 min. The exercises comprised of 50 plantar flexions-extensions against weights corresponding to 40 and 70 percent of maximal voluntary contraction (MVC), respectively. Creatine supplementation increased resting muscle PCr content by approximately 20 percent. R(depl) was also increased by approximately 15 and approximately 10 percent during 40 and 70 percent MVC exercises, respectively. No change was observed in R(repl) and $t_{1/2}$. BM and body water compartments were not influenced. These results indicate that during a standardized exercise more ATP is synthesized by the CK reaction when the pre-exercise level in PCr is higher, giving some support to the positive effects recorded on muscle performance [115].
Effect of creatine loading on neuromuscular fatigue threshold

The purpose of one investigation was to determine the effect of creatine (Cr) loading on the onset of neuromuscular fatigue by monitoring electromyographic fatigue curves from the vastus lateralis muscle using the physical working capacity at the fatigue threshold (PWC_{FT}) test. Using a double-blind random design, 15 women athletes (mean age 19) from the university crew team received a placebo (n=8; 20 g glucose) or Cr (n=7; 5 g Cr monohydrate + 20 g glucose) four times per day for 5 consecutive days. Analysis of covariance was used to analyze the data (covaried for presupplementation PWC_{FT} values). The adjusted mean postsupplementation PWC_{FT} value for the Cr group (mean = 186 W) was significantly higher than that of the placebo group (mean = 155 W). These findings suggest that Cr loading may delay the onset of neuromuscular fatigue [116].

Contractile properties, fatigue and recovery are not influenced by short-term creatine

There have been several studies on the effect of short-term creatine (Cr) supplementation on exercise performance, but none have investigated both voluntary and stimulated muscle contractions in the same experiment. Fourteen moderately active young men (19-28 years) were randomly assigned, in a double blind manner, to either a creatine (Cr) or placebo (P) group. The subjects supplemented their regular diet 4 times a day for 5 days with either 5 g Cr + 5 g maltodextrin (Cr group), or 5 g maltodextrin (P group). Isometric maximal voluntary contraction (MVC), muscle activation, as assessed using the modified twitch interpolation technique, electrically stimulated contractile properties, electromyography (EMG), endurance time and recovery from fatigue were measured in the elbow flexors. The fatigue protocol involved both voluntary and stimulated contractions. Following supplementation there was a significant weight gain in the Cr group (1.0 kg), whereas the P group did not change. For each group, pre-supplementation measures were not significantly different from post-supplementation for MVC, twitch and tetanic tensions at rest, time to peak tension, half-relaxation time and contraction duration. Prior to Cr supplementation time to fatigue was 10 ± 4 min for both groups, and following supplementation there was a non-significant increase of 1 min in each group. MVC force, muscle activation, EMG, stimulated tensions and durations were similar for the Cr and P groups over the course of the fatigue protocol and did not change after supplementation. Furthermore, recovery of MVC, stimulated tensions and contractile speeds did not differ as a result of Cr supplementation. These results indicate that short-term Cr supplementation does not influence isometric elbow flexion force, muscle activation, stimulated contractile properties, or delay time to fatigue or improve recovery [117].

Effect of low doses

Effect of continuous low dose creatine on force, power, and total work

Dietary supplementation (SUP) has become a significant part of athletic training. Studies indicate that creatine (Cr) can enhance short-duration, high-intensity activities. This study examined the effect of 21 days of low dose Cr SUP (approximately 7.7 g/day) and resistance training on force output, power output, duration of mean peak power output, and total work performed until fatigue. A double-blind protocol was used, where an individual, who was not part of any other aspect of the study, randomly assigned subjects to creatine and placebo groups. Forty-one male university athletes were randomly assigned to either Cr (n=20) or placebo (n=21) SUP. On the first and last day of the study, subjects were required to perform concentric bench press movements until exhaustion on an isokinetic dynamometer. The dynamometer was hard-wired to a personal computer, which provided force, velocity, and duration measures. Force and power output until fatigue, were used to determine total work,
force-time, and power-time relationships. ANOVA results revealed that the Cr subjects performed more total work until fatigue, experienced significantly greater improvements in peak force and peak power, and maintained elevated mean peak power for a longer period of time. These results indicate that Cr SUP can significantly improve factors associated with short-duration, high-intensity activity [118].

Effects of low doses of creatine on strength and urinary creatinine concentration

To compare the effects of low doses of creatine and creatine loading on strength, urinary creatinine concentration, and percentage of body fat division IA collegiate football players took creatine monohydrate for 10 weeks during a sport-specific, periodized, off-season strength and conditioning program. One-repetition maximum (1-RM) squat, urinary creatinine concentrations, and percentage of body fat were analyzed. Twenty-five highly trained, Division IA collegiate football players with at least 1 year of college playing experience. It was tested strength with a 1-RM squat exercise before, during, and after creatine supplementation. Percentage of body fat was measured by hydrostatic weighing before and after supplementation. Urinary creatinine concentration was measured via light spectrophotometer at 0, 1, 3, 7, 14, 21, 28, 35, 42, 48, 56, and 63 days. An analysis of variance with repeated measures was computed to compare means for all variables. Creatine supplementation had no significant group, time, or interaction effects on strength, urinary creatinine concentration, or percentage of body fat. However, significant time effects were found for 1-RM squat and fat-free mass in all groups. The data suggest that creatine monohydrate in any amount does not have any beneficial ergogenic effects in highly trained collegiate football players. However, a proper resistance training stimulus for 10 weeks can increase strength and fat-free mass in highly trained athletes [119].
EFFECTS OF CREATINE IN SPECIFIED SPORTS

Running

Runners advantage (RA) creatine (Cr) serum has been marketed to increase running performance. To test this claim, cross-country runners completed baseline testing (BASE), an outdoor 5,000-m run followed by treadmill VO2max testing on the same day. Subjects repeated testing after ingesting 5 ml of RA (n=13) containing 2.5 g of Cr or placebo (n=11). Heart rate (HR), rating of perceived exertion (RPE), and run time were recorded. With RA VO2max was higher versus BASE yet the magnitude of the increase was within the coefficient of variation of VO2max. No effect of RA on maximal HR was exhibited, yet VO2max and duration of incremental exercise were significantly higher versus BASE. VO2max was similar in PL and BASE. With RA, the 5,000-m time was unchanged, and RPE was lower versus BASE. These data do not support the ergogenic claims of RA in its current form and dose [120].

Twenty-nine (17 men, 12 women) collegiate track and field athletes were randomly divided into a creatine monohydrate (CM, n=10) group, creatine monohydrate and glutamine (CG, n=10) group, or placebo (P, n=9) group. The CM group received 0.3 g creatine.kg body mass per day for 1 week, followed by 0.03 g creatine.kg body mass per day for 7 weeks. The CG group received the same creatine dosage scheme as the CM group plus 4 g glutamine/day. All 3 treatment groups participated in an identical periodized strength and conditioning program during preseason training. Body composition, vertical jump, and cycle performances were tested before (T1) and after (T2) the 8-week supplementation period. Body mass and lean body mass (LBM) increased at a greater rate for the CM and CG groups, compared with the P treatment. Additionally, the CM and CG groups exhibited significantly greater improvement in initial rate of power production, compared with the placebo treatment. These results suggest CM and CG significantly increase body mass, LBM, and initial rate of power production during multiple cycle ergometer bouts [121].

In sprinting

Creatine is an ergogenic aid used in individual and team sports. The aim of this study is to analyze the effect of monohydrate creatine supplementation on physical performance during 6 consecutive maximal speed 60 meter races, and the changes induced in some characteristic biochemical and ventilatory parameters. One study was carried out on nineteen healthy and physically active male volunteers, and randomly distributed into two groups: Group C received a supplement of creatine monohydrate (20 g/day for 5 days) and group P received placebo. Tests were performed before and after supplementation. No significant changes were observed in weight or body water measured by bioimpedance or the sum of 7 skinfold or performance during the 60 meter races. Group C showed a statistically significant increase in plasma creatinine from 69.8 ± 12.4 to 89.3 ± 12.4 micromol/L. In group C in the second control day (after creatine supplementation), expiratory volume V(E), O2 uptake and CO2 production were lower after 2 minutes of active recovery period. These results indicate that creatine monohydrate supplementation does not appear to improve the performance in 6 consecutive 60 meter repeated races but may modify ventilatory dynamics during the recovery after maximal effort [122].

The purpose of this study was to examine the influence of short-term creatine (Cr) supplementation on exercise-induced transverse relaxation time (T2) and sprint performance during maximum intermittent cycling exercise using the muscle functional magnetic resonance imaging (mFMRI) technique. Twelve men were divided into a Cr supplementation group (the Cr group, taking 4 x (5 g Cr monohydrate + 2.5 g maltodextrin)/day), or a placebo
supplementation group (the P group, taking 4 x 7.5 g maltodextrin/day). The allocation to the groups was based on cycling tests and the subject's physical characteristics, and thus was not randomized. A double-blind research design was employed for a 5-day supplementation period. MfMR images of the right thigh were collected at rest and immediately after two, five, and ten 6-s sprint bouts of maximum intermittent cycling exercise with a 30-s recovery interval between sets. Before and after supplementation, blood was taken to calculate lactate accumulation, and the muscle volume of the thigh was determined by MRI. Following supplementation, there was significant body mass gain in the Cr group, whereas the P group did not change. The exercise-induced T2, blood lactate levels and sprint performance were not affected by Cr supplementation in any sprint bouts. These results suggest that short-term Cr supplementation does not influence short duration repetitive sprint performance and muscle activation and/or metabolic state during sprint cycling evaluated by mfMRI of the skeletal muscle in humans [123].

One study examined the impact of short-term (7-day), high-dose (0.35 g/kg/day) oral creatine monohydrate supplementation (CrS) on single sprint running performance (40 m, <6 seconds) and on intermittent sprint performance in highly trained sprinters. Nine subjects completed the double-blind cross-over design with 2 supplementation periods (placebo and creatine) and a 7-week wash-out period. A test protocol consisting of 40-m sprint runs was performed, and running velocity was continuously recorded over the total distance. The maximal sprint performance, the relative degree of fatigue at the end of intermittent sprint exercise (6 x 40 m, 30-second rest interval), as well as the degree of recovery (120-second passive rest) remained unchanged following CrS. There were no significant changes related to CrS in absolute running velocity at any distance between start and finish (40 m). It was concluded that no ergogenic effect on single or repeated 40-m sprint times with varying rest periods was observed in highly trained athletes [124].

Most research on creatine has focused on short-term creatine loading and its effect on high-intensity performance capacity. Some studies have investigated the effect of prolonged creatine use during strength training. However, studies on the effects of prolonged creatine supplementation are lacking. In the present study, we have assessed the effects of both creatine loading and prolonged supplementation on muscle creatine content, body composition, muscle and whole-body oxidative capacity, substrate utilization during submaximal exercise, and on repeated supramaximal sprint, as well as endurance-type time-trial performance on a cycle ergometer. Twenty subjects ingested creatine or a placebo during a 5-day loading period (20 g/day) after which supplementation was continued for up to 6 weeks (2 g/day). Creatine loading increased muscle free creatine, creatine phosphate (CrP) and total creatine content. The subsequent use of a 2 g/day maintenance dose, as suggested by an American College of Sports Medicine Roundtable, resulted in a decline in both the elevated CrP and total creatine content and maintenance of the free creatine concentration. Both short- and long-term creatine supplementation improved performance during repeated supramaximal sprints on a cycle ergometer. However, whole-body and muscle oxidative capacity, substrate utilization and time-trial performance were not affected. The increase in body mass following creatine loading was maintained after 6 weeks of continued supplementation and accounted for by a corresponding increase in fat-free mass. This study provides definite evidence that prolonged creatine supplementation in humans does not increase muscle or whole-body oxidative capacity and, as such, does not influence substrate utilization or performance during endurance cycling exercise. In addition, our findings suggest that prolonged creatine ingestion induces an increase in fat-free mass [125].

**Creatine supplementation improves sprint performance in male sprinters**

The object of one study was to evaluate the effect of creatine (Cr) supplementation in well trained male sprinters. The study was performed as a single blind test on 18 sprinters at a local competition level. During the last two years a substantial part of their training had
consisted of a series of maximal sprints with short rest periods to improve their fatigue resistance. The participants consumed either 20 g Cr+20 g glucose per day (Cr group, n=9) or 40 g glucose per day (placebo group, n=9), divided into 4 equal dosages. The effect of Cr on sprint performance was evaluated in two tests, 1 x 100 m sprint and an intermittent 6x60 m sprint. Cr supplementation increased the 100 m sprint velocity and reduced the total time of 6 intermittent 60 m sprints whereas no changes were observed in the placebo group. The sprint velocity was significantly increased in 5 out of 6 intermittent 60 m sprints. Venous blood was drawn 5 min after finishing the final intermittent 60 m run. Plasma lactate, Cr and serum creatinine (Crn) were all increased in the Cr group compared to presupplementation values; no changes were observed in the placebo group. The improved sprint performance suggests an increased availability of energy substrate for performing work, possibly as a result of increased skeletal muscle creatine phosphate (PCr) [126].

Multiple sprint running

The aim of one study was to examine the effects of short-term creatine monohydrate supplementation on multiple sprint running performance. Using a double-blind research design, 42 physically active men completed a series of 3 indoor multiple sprint running trials (15 x 30 m repeated at 35-second intervals). After the first 2 trials (familiarization and baseline), subjects were matched for fatigue score before being randomly assigned to 5 days of either creatine (4/day x 5 g creatine monohydrate + 1 g maltodextrin) or placebo (4/day x 6 g maltodextrin) supplementation. Sprint times were recorded via twin-beam photocells, and earlobe blood samples were drawn to evaluate posttest lactate concentrations. Relative to placebo, creatine supplementation resulted in a 0.7 kg increase in body mass and a 0.4 percent reduction in body fat. There were no significant between-group differences in multiple sprint measures of fastest time, mean time, fatigue, or posttest blood lactate concentration. Despite widespread use as an ergogenic aid in sport, the results of the study suggest that creatine monohydrate supplementation conveys no benefit to multiple sprint running performance [127].

Sprint performance enhancement after one-week creatine supplementation

In order to test whether an improvement of maximal sprinting speed after creatine (Cr) supplementation was due to the increase of stride frequency, stride length or both, 7 subjects ran 4 consecutive sprints after 1 week of placebo or Cr supplementation. Both were assessed by a triaxial accelerometer. Compared to the placebo, Cr induced an increase of running speed (+1.4 %) and stride frequency (+1.5 %), but not of stride length. The drop in performance following repeated sprints was partially prevented by Cr. In conclusion, exogenous Cr enhanced sprinting performance by increasing SF. This result may be related to the recent findings of shortening in muscular relaxation time after Cr supplementation [128].

No acute effects of short-term creatine muscle properties and sprint performance

In a double-blind, placebo, controlled study, it was investigated the acute effects of short-term oral creatine supplementation (20 g/day for 6 days) on muscle activation, fatigue and recovery of the m. quadriceps femoris during electrical stimulation, and on maximal performance during sprint cycling. The quadriceps muscles of 23 well-trained rowers were stimulated at different frequencies (10, 20, 50, 100, 150 and 200 Hz). Furthermore, 40 repetitive, electrically stimulated (duration 220 ms, stimulation frequency 150 Hz) concentric contractions were imposed at a constant angular velocity of 180 degrees/s over a range of 50 degrees (from 90 to 140 degrees knee angle), each extension/flexion cycle lasting 1200 ms. To determine recovery, torque was measured at 20, 50, 80, 120, 180 and 300 s after the last contraction. In addition, two maximal 30-s sprints were performed on a cycle ergometer
with 4 min rest in between. Following short-term creatine supplementation, body mass increased from 85.7 kg to 87.3 kg. Creatine supplementation had no effect on maximal voluntary isometric torque and muscle activation, or on fatigue and recovery of dynamic exercise. There was also no significant effect on peak power, time to peak power and work to peak power, or total work during both sprints on the cycle ergometer. It was concluded that short-term oral creatine supplementation resulted in increased body mass, but did not enhance muscle performance or maximal output during sprint cycling [129].

Similar ergogenic effect in sprinters and long-distance runners?

The aim of the study was to determine whether creatine malate (CML) supplementation results in similar ergogenic effect in sprinters and long-distance runners. The other goal was to compare changes in body composition, physical performance and hormone levels after six-week training in athletes, divided into subgroups supplemented with creatine malate or taking placebo. Six-week supplementation combined with physical training induced different effects in athletes. Significantly higher increases in relative and absolute peak power and total work were found in sprinters compared to other groups. Except for growth hormone, post-exercise venous blood serum hormone levels exhibited no statistically significant differences in athletes. After CML loading period, a significant increase in growth hormone was found in the group of sprinters. It was concluded that a significant ergogenic effect was found in sprinters, which was reflected by the increase in anaerobic exercise indices and morphological indices and elevated growth hormone level, after graded exercise testing. The significant increase in the distance covered during graded test was only observed in supplemented long-distance runners, whereas no significant changes in maximal oxygen uptake, relative peak power and relative total work were noticed. This could be caused by later anaerobic threshold appearance in exercise test to exhaustion [130].

Biking

One study aimed to determine the effects of different acute creatine loadings (ACRL) on repeated cycle sprints. Twenty-eight active subjects divided into the control (n=7) and the experimental (n=21) group. The exercise protocol comprised three 30s Anaerobic Wingate Tests (AWT) interspersed with six minutes recovery, without any supplements ingested and following placebo and creatine ingestion, according to each ACRL (40g, 100g and 135g throughout a four-day period). Blood and urinary creatine levels were also determined from the experimental group for each ACRL. Protein intake (across all groups) was held constant during the study. There were no changes in protein intake or performance of the control group. For the experimental group creatine supplementation produced significant increases in body mass (83), blood, and urinary creatine. No significant differences were found between the non-supplement and placebo condition. Creatine supplementation produced an average improvement of 0.7 percent, 11.8 percent and 11.1 percent for the 40 g, 100 g and 135 g ACRL respectively. However, statistics revealed significant differences only for the 100g and 135g ACRL. Mean ± SD values for the 100g ACRL for mean and minimum power were 612 ± 180 W placebo versus 693 ± 221 W creatine and 381 ± 35 W placebo versus 415 ± 11 W creatine accordingly. For the 135g ACRL the respective performance values were 722 ± 215 W placebo versus 810 ± 240 W creatine and 405 ± 59 W placebo vs 436 ± 30 W creatine. These data indicate that a 100 g compared to 40 g ACRL produces a greater potentiation of performance whilst, greater quantities of creatine ingestion (135 g ACRL) can not provide a greater benefit [131].

A double-blind study was performed to evaluate the effects of oral creatine-pyruvate administration on exercise performance in well-trained cyclists. Endurance and intermittent sprint performance were evaluated before (pretest) and after (posttest) one week of creatine-
pyruvate intake (Cr(pyr), 2 x 3.5 g/d, n=7) or placebo (PL, n=7). Subjects first performed a 1-hour time trial during which the workload could be adjusted at 5-min intervals. Immediately they did five 10-sec sprints interspersed by 2-min rest intervals. Tests were performed on an individual race bicycle that was mounted on an ergometer. Steady-state power production on average was about 235-245 W, which corresponded to blood lactate concentrations of 4-5 mmol/L and heart rate in the range of 160-170 beats/min. Power outputs as well as blood lactate levels and heart rates were similar between Cr(pyr) and PL at all times. Total work performed during the 1-h trial was 872 ± 44 KJ in PL versus 891 ± 51 KJ in CR pyr. During the intermittent sprint test power peaked at about 800-1000 watt within 2-3 sec, decreasing by 15-20 percent towards the end of each sprint. Peak and mean power outputs were similar between groups at all times. Peak lactate concentrations after the final sprint were approximately 11 mmol/L in both groups during both the pretest and the posttest. It is concluded that one week of creatine-pyruvate supplementation at a rate of 7 g/day does not beneficially impact on either endurance capacity or intermittent sprint performance in cyclists [132].

Effect of creatine loading on oxygen uptake during cycling

For the first time, it was investigated the effects of altering cellular metabolic capacitance, via a 5-day creatine (Cr) loading protocol (20 g/day), on oxygen uptake (VO_{2}), accumulated oxygen deficit, muscle recruitment, and performance during a 1-km cycling time-trial. In a double-blind, randomized, placebo-controlled design, nineteen amateur cyclists were allocated to a Cr (n=10) or placebo (n=9) group, and performed a 1-km cycling time-trial before and after the supplementation period. Body mass was significantly increased in the Cr group, but not in the placebo group. Participants adopted an "all-out" pacing strategy in both groups. However, Cr loading reduced VO_{2} immediately after the beginning (12th to 23th seconds), and this was accompanied by a reduced aerobic and increased anaerobic contribution. The VO_{2mean} response time was slower, the total O_{2} uptake was reduced and the oxygen deficit was increased after Cr loading. No differences were observed in the placebo group for these variables. Plasma lactate and integrated electromyography were not altered in either group, nor was the time to complete the trial. It was concluded that Cr loading slows the VO_{2} response and increases the anaerobic contribution during a 1-km cycling time trial [133].

Effect of recovery interval on multiple-bout sprint cycling

The purpose of this study was to examine the effect of varying recovery intervals on multiple-bout, short-duration, high-intensity cycling efforts of adult men supplemented with creatine (Cr) or a placebo (Pl). Thirty subjects underwent 3 trials of a maximal cycling protocol (T(0), T(1), T(2)). T(0) included VO_{2max} testing and familiarization with the sprint cycling protocol. T(1) consisted of 8 15-second bouts of sprint cycling exercise. Subjects were randomly assigned to recovery interval groups (1 minute, 3 minutes, 6 minutes), and Cr or Pl groups (0.3 g/kg/d). Posttesting (T(2)) took place 7 days after T(1) and consisted of an identical protocol as during T(1). Changes in mean power (MP), peak power (PP), and fatigue index (FI) were compared between trials. MP was significantly increased in Cr 1-minute, Cr 3-minute, and Pl 6-minute groups. Significant PP increases were demonstrated in Cr 1-minute and Pl 6-minute groups, and FI significantly increased in Pl 1-minute group. Results indicate that Cr supplementation is effective in improving recovery from repeated sprint cycling performances when the recovery interval is of a short (< 6 minutes) duration [134].
Rowing

This study investigated the effect of creatine monohydrate (Cr) supplementation on performance and training volume in rowers. Twenty-two rowers trained with continuous and interval rowing and resistance training 4 and 2 days/week, respectively, for 6 weeks. Cr supplementation consisted of a 5-day load (0.3 g/kg/day) followed by a 5-week maintenance dose (0.03 g/kg/day) while training. Five days of Cr loading did not change body composition, repeated interval rowing performance, 2,000-m rowing times, or strength performance. Five additional weeks of training with a maintenance dose of Cr or placebo significantly improved body composition, VO$_{2\text{max}}$, 2,000-m rowing times, repeated power interval performance, and strength to a similar extent in both groups. Subjects training with Cr did not perform more repetitions per set of strength exercise nor produce or maintain higher power outputs during repeated rowing sessions. Cr supplementation did not increase performance or training volume over a placebo condition in rowers that performed a combined high intensity rowing and strength program [135].

Track and field

Usage and education of track and field throwers in American universities

The purpose of one study was to analyze the level of creatine use along with the perceived benefits and barriers of creatine use among collegiate athletes who participate in throwing events within the sport of track and field. A total of 258 throwers from National Collegiate Athletic Association Division I institutions completed an online survey regarding creatine. The results provided baseline levels of creatine use and allowed for the analysis of factors related to athletic conference affiliation. Results indicate that creatine use remains to be a common (33 %) practice among throwers with significantly higher levels of use among Football Bowl Subdivision (FBS) conference athletes (45 %) than Football Championship Subdivision (FCS) conference athletes (29 %). The most common reasons for using creatine included a desire to improve/increase: strength (83 %), recovery time (69 %), and performance (61 %). The most common perceived obstacles included contamination/quality control (40 %), cost (33 %), inconvenience (17 %), and cramping (14 %). A desire for additional education and training was noted through an expression of interest (56 %) with significantly higher levels of interest from FBS athletes (66 %) than FCS athletes (52 %). However, the athletic departments provide nutritional supplement counseling at only 27 percent of the schools. Although the access to full-time nutritionist counsel was available at 57 percent of the schools, there was a significant difference between FBS schools (73.7%) and FCS schools (51.7%) [136].

Football

One investigation examined the effects of creatine (Cr) supplementation on intermittent high-intensity exercise activities specific to competitive soccer. On two occasions 7 d apart, 17 highly trained male soccer players performed a counter-movement jump test (CMJT), a repeated sprint test (RST) consisting of six maximal 15-m runs with a 30-s recovery, an intermittent endurance test (IET) consisting of forty 15-s bouts of high-intensity running interspersed by 10-s bouts of low-intensity running, and a recovery CMJT consisting of three jumps. After the initial testing session, players were evenly and randomly included in a CREATINE (5 g of Cr, four times per day for 6 d) or a PLACEBO group (same dosage of maltodextrins) using a double-blind research design. The CREATINE group's average 5-m and 15-m times during the RST were consistently faster after the intervention. Neither group showed significant changes in the CMJT or the IET. The CREATINE group's recovery CMJT
performance relative to the resting CMJT remained unchanged postsupplementation, whereas it tended to decrease in the PLACEBO group. In conclusion, acute Cr supplementation favorably affected repeated sprint performance and limited the decay in jumping ability after the IET in highly trained soccer players. Intermittent endurance performance was not affected by Cr [137].

One study investigated the effects of acute creatine (Cr) supplementation on the performance of elite female soccer players undertaking an exercise protocol simulating match play. On two occasions, 7 days apart, 12 players performed 5 x 11-min exercise testing blocks interspersed with 1 min of rest. Each block consisted of 11 all-out 20-m running sprints, 2 agility runs, and 1 precision ball-kicking drill, separated by recovery 20-m walks, jogs, and runs. After the initial testing session, subjects were assigned to either a CREATINE (5 g of Cr, 4 times per day for 6 days) or a PLACEBO group (same dosage of a glucose polymer) using a double-blind research design. Body mass (BM) increased (61.7 ± 8.9 to 62.5 ± 8.9 kg) in the CREATINE group; however, no change was observed in the PLACEBO group. No overall change in 20-m sprint times and agility run times were observed, although the CREATINE group achieved faster post-supplementation times in sprints 11, 13, 14, 16, 21, 23, 25, 32, and 39, and agility runs 3, 5, and 8. The accuracy of shooting was unaffected in both groups. In conclusion, acute Cr supplementation improved performance of some repeated sprint and agility tasks simulating soccer match play, despite an increase in BM [138].

The purpose of one study was to examine the effects of acute creatine-monohydrate supplementation on soccer-specific performance in young soccer players. Twenty young male soccer players participated in the study and were matched and allocated to 2 randomly assigned trials: ingesting creatine-monohydrate supplement (3 x 10 g doses) or placebo for 7 days. Before and after the supplementation protocol, each subject underwent a series of soccer-specific skill tests: dribble test, sprint-power test, endurance test, and vertical jump test. Specific dribble test times improved significantly in the creatine group after supplementation protocol. Sprint-power test times were significantly improved after creatine-monohydrate supplementation as well as vertical jump height in creatine trial. Furthermore, dribble and power test times, along with vertical jump height, were superior in creatine versus placebo trial at post-supplementation performance. There were no changes in specific endurance test results within or between trials. There were no between-trial differences in the placebo trial. The main finding of the present study indicates that supplementation with creatine in young soccer players improved soccer-specific skill performance compared with ingestion of placebo [139].

Handball

To determine the effects of creatine (Cr) supplementation (20 g/d during 5 d) on maximal strength, muscle power production during repetitive high-power-output exercise bouts (MRPB), repeated running sprints, and endurance in handball players. Nineteen trained male handball players were randomly assigned in a double-blind fashion to either creatine (n=9) or placebo (n=10) group. Before and after supplementation, subjects performed one-repetition maximum half-squat (1RM(HS)) and bench press (1RM(BP)), 2 sets of MRPB consisting of one set of 10 continuous repetitions (R10) followed by 1 set until exhaustion (R(max)), with exactly 2-min rest periods between each set, during bench-press and half-squat protocols with a resistance equal to 60 and 70% of the subjects’ 1RM, respectively. In addition, a countermovement jumping test (CMJ) interspersed before and after the MRPB half-squat exercise bouts and a repeated sprint running test and a maximal multistage discontinuous incremental running test (MDRT) were performed. Cr supplementation significantly increased body mass, number of repetitions performed to fatigue, and total average power output
values in the R(max) set of MRPB during bench press (21 % and 17 %, respectively) and half-squat (33 % and 20 %, respectively), the 1RM(HS) (11 %), as well as the CMJ values after the MRPB half-squat (5 %), and the average running times during the first 5 m of the six repeated 15-m sprints (3 %). No changes were observed in the strength, running velocity, or body mass measures in the placebo group during the experimental period. It was concluded that short-term Cr supplementation leads to significant improvements in lower-body maximal strength, maximal repetitive upper- and lower-body high-power exercise bouts, and total repetitions performed to fatigue in the R(max) set of MRPB, as well as enhanced repeated sprint performance and attenuated decline in jumping ability after MRPB in highly trained handball players. Cr supplementation did not result in any improvement in upper-body maximal strength and in endurance running performance [140].

Ice-hockey

Creatine monohydrate supplementation is beneficial for enhancing high-intensity exercise performance, especially activities that involve repeated sprints. Creatine monohydrate supplementation is common in ice-hockey players. The purpose of one study was to determine the effect of creatine monohydrate supplementation on sprint skating performance in Junior B and collegiate ice-hockey players. Seventeen ice-hockey players were randomly assigned to receive creatine (0.3 g/kg body mass/day for 5 days) or placebo. Before and after supplementation players performed repeated sprints to exhaustion on a skating treadmill (repeated 10-s sprints; 30-s rest between sprints) while blood lactate was simultaneously collected. The time to exhaustion on the treadmill test was calculated as total amount of time, including partial intervals, before the player reached exhaustion. Players were also tested for peak torque and average power during knee extension/flexion (3 sets of 10 reps; 60-s rest between sets) on an isokinetic dynamometer at 60 degrees/s. The change in time to exhaustion from before to after supplementation averaged 21 ± 7 s in the creatine group and 22 ± 13 s in the placebo group, with no differences between groups. Likewise, there were no differences between groups for changes in isokinetic peak torque and average power. There were no differences between groups over time for blood lactate changes during the repeated sprints on the treadmill. It was concluded that creatine was not effective for improving performance in these ice-hockey players [141].

Rugby

The purpose of the one study was to examine player-movement patterns to determine total distance covered during competitive Rugby League match play using global positioning systems (GPSs) and to examine pre, during, and postmatch creatine kinase (CK) and endocrine responses to competitive Rugby League match play. Seventeen elite rugby league players were monitored for a single game. Player movement patterns were recorded using portable GPS units. Saliva and blood samples were collected 24 hours prematch, 30 minutes prematch, 30 minutes postmatch, and then at 24-hour intervals for a period of 5 days postmatch to determine plasma CK and salivary testosterone, cortisol, and testosterone to cortisol ratio (T:C). The change in the dependent variables at each sample collection time was compared to 24-hour prematch measures. Backs and forwards traveled distances 5,747 ± 1,095 and 4,774 ± 1,186 m, respectively, throughout the match. Cortisol and CK increased significantly from 30 minutes prematch to 30 minutes postmatch. Creatine kinase increased significantly postmatch, with peak CK concentration measured 24 hours postmatch (889.25 + 238.27 UL). Cortisol displayed a clear pattern of response with significant elevations up to 24 hours postmatch, compared with 24 hours prematch. The GPS was able to successfully provide data on player-movement patterns during competitive rugby league match play. The CK and endocrine profile identified acute muscle damage and a catabolic state associated
with Rugby League match play. A return to normal T:C within 48 hours postmatch indicates that a minimum period of 48 hours is required for endocrine homeostasis postcompetition. Creatine kinase remained elevated despite 120 hours of recovery postmatch identifying that a prolonged period of at least 5 days modified activity is required to achieve full recovery after muscle damage during competitive Rugby League match play [142].

Rugby union football requires muscular strength and endurance, as well as aerobic endurance. Creatine supplementation may enhance muscular performance, but it is unclear if it would interfere with aerobic endurance during running because of increased body mass. The purpose of one study was to determine if creatine supplementation during 8 weeks of a season of rugby union football can increase muscular performance, without negatively affecting aerobic endurance. Rugby union football players were randomized to receive 0.1 g.kg(-1).d(-1) creatine monohydrate (n=9) or placebo (n=9) during 8 weeks of the rugby season. Players practiced twice per week for approximately 2 h per session and played one 80 min game per week. Before and after the 8 weeks, players were measured for body composition (air displacement plethysmography), muscular endurance (number of repetitions at 75% of one repetition maximum (1 RM) for bench press and leg press), and aerobic endurance (Leger shuttle-run test with 1 min stages of progressively increasing speed). There were time main effects for body mass, fat mass, and a trend for an increase in lean tissue mass, with no differences between groups. The group receiving creatine supplementation had a greater increase in the number of repetitions for combined bench press and leg press tests compared with the placebo group. There were no changes in either group for aerobic endurance. Creatine supplementation during a rugby union football season is effective for increasing muscular endurance, but has no effect on body composition or aerobic endurance [143].

The benefits of creatine (CR) supplementation are well documented, particularly during repeated bouts of high-intensity muscular activity. Most published experiments use mass-supported (cycle ergometry) activities as a means of evaluating creatine’s efficacy, therefore minimizing any possible adverse effects of increased body mass associated with CR supplementation. One study aimed to use both mass-supported and mass-dependent activities to assess the effectiveness of acute CR supplementation on a group of highly trained rugby players. A randomized, double-blind, crossover research design was utilized, with subjects receiving 20 g/d x 5 d of both CR and a glucose placebo (PL). Subjects were assessed via 10 x 6-second Wingate test and a 10 x 40-m sprint test on separate days, presupplementation and postsupplementation. A 28-d washout period separated the two treatments. No significant treatment or treatment by test interaction effects were observed for peak or minimum power output (W), peak or minimum running velocity (m/s), or fatigue index (%). No significant differences were found postsupplementation for body mass and percentage body fat. Although statistical significance was not achieved for any of the measured parameters, there were small improvements in performance that may be of benefit to rugby players [144].

Swimming

The objective of one study was to determine the effect of creatine supplementation on performance and body composition of swimmers. Eighteen swimmers were evaluated in terms of post-performance lactate accumulation, body composition, creatine and creatinine excretion, and serum creatinine concentrations before and after creatine or placebo supplementation. No significant differences were observed in the marks obtained in swimming tests after supplementation, although lactate concentrations were higher in placebo group during this period. In the creatine-supplemented group, urinary creatine, creatinine, and body mass, lean mass and body water were significantly increased, but no
significant difference in muscle or bone mass was observed. These results suggest that creatine supplementation cannot be considered to be an ergogenic supplement ensuring improved performance and muscle mass gain in swimmers [145].

One study demonstrated the effect of low dose creatine supplement (10 g per day) on the sprinting time in the last 50 meters of 400 meters swimming competition, as well as the effect on exertion. Nineteen swimmers in the experimental group received creatine monohydrate 5 g with orange solution 15 g, twice per day for 7 days and nineteen swimmers in the control group received the same quantity of orange solution. The results showed that the swimmers who received creatine supplement lessened the sprinting time in the last 50 meters of 400 meters swimming competition than the control group. The results of Wingate test (anaerobic power, anaerobic capacity and fatigue index) compared between pre and post supplementation. There was significant difference at in the control group from training effect whereas there was significant difference at from training effect and creatine supplement in the experiment group. Therefore, the creatine supplement in amateur swimmers in the present study enhanced the physical performance up to the maximum capacity [146].

Creatine (Cr) supplementation has yielded inconsistent results when applied to competitive swimming. To further define the role of Cr, we tested the hypothesis that a Cr supplementation group of Division III swimmers would demonstrate enhanced performance when compared with placebo. In order to test this hypothesis, 8 male and 7 female collegiate Division III swimmers were assigned in a random, double-blind manner into either a Cr supplementation group (0.3 g Cr/kg body mass) or a placebo group. Loading was maintained for 5 days followed by a 9-day period where Cr-supplemented subjects consumed 2.25 g Cr regardless of body weight. A 50- and 100-yards sprint was performed prior to and following the supplementation regimens. The Cr supplementation group decreased their finish times in both the 50- and 100-yards sprints. Support of the hypothesis suggests that Cr supplementation for swimming events is effective for singular effort sprints of 50 and 100 yd in Division III athletes [147].

To determine whether 4 weeks of oral creatine (Cr) supplementation could enhance single freestyle sprint and swim bench performance in experienced competitive junior swimmers, 10 young men and 10 young women (17 years) participated in a 27-day supplementation period and pre- and posttesting sessions. In session 1 (presupplementation testing), subjects swam one 50-m freestyle and then (after approximately 5 minutes of active recovery) one 100-m freestyle at maximum speed. Blood lactate was measured before and 1 minute after each swim trial. Forty-eight hours later, height, mass, and the sum of 6 skinfolds were recorded, and a Biokinetic Swim Bench total work output test (2 x 30-second trials, with a 10-minute passive recovery in between) was undertaken. After the pretests were completed, participants were divided into 2 groups (n=10, Cr; and n=10, placebo) by means of matched pairs on the basis of gender and 50-m swim times. A Cr loading phase of 20 g/d for 5 days was then instituted, followed by a maintenance phase of 5 g/d for 22 days. Postsupplementation testing replicated the presupplementation tests. Four weeks of Cr supplementation did not influence single sprint performance in the pool or body mass and composition. However, 30-second swim bench total work scores for trial 1 and trial 2 increased after Cr but not placebo ingestion. Postexercise blood lactate values were not different after supplementation for the 50- and 100-m sprint trials either within or between groups. It was concluded that 4 weeks of Cr supplementation did not significantly improve single sprint performance in competitive junior swimmers, but it did enhance swim bench test performance [148].

One study demonstrated the effect of low dose creatine supplement (10 g per day) on the sprinting time in the last 50 meters of 400 meters swimming competition, as well as the effect on exertion. Nineteen swimmers in the experimental group received creatine monohydrate 5 g with orange solution 15 g, twice per day for 7 days and nineteen swimmers in the control
group received the same quantity of orange solution. The results showed that the swimmers who received creatine supplement lessened the sprinting time in the last 50 meters of 400 meters swimming competition than the control group. The results of Wingate test (anaerobic power, anaerobic capacity and fatigue index) compared between pre and post supplementation. There was significant difference in the control group from training effect whereas there was significant difference from training effect and creatine supplement in the experiment group. Therefore, the creatine supplement in amateur swimmers in the present study enhanced the physical performance up to the maximum capacity [149].

Well trained swimmers

One study was conducted to examine the effects of oral creatine supplementation on training for competition in 20 elite swimmers. Subjects performed a maximal sprint test (8 x 50 yd (45.72 m), T1) before loading with creatine (Cr, 20 g/d Cr monohydrate for 5 d), 1 week later (T2), and following a 22- to 27-week period of training and competition (T3). Following T2, subjects supplemented with either Cr (3 g + glucose 7 g/d) or placebo (glucose 10 g/d; double blind) for the remainder of the 22- to 27-wk season and then both groups supplemented once more with 20 g/d Cr monohydrate for 5 d before their major competition. Venous and capillary blood samples were obtained pre- and posttest during the repeated sprint tests to determine blood metabolites and hormones. Competition times were recorded, and changes in subjects’ best times were used to compare the effect of training and supplementation on competitive performance. Mean competition times in the Cr and control groups changed by 1.90 + 1.91 and 0.72 + 1.64 percent for short course (SC, 25-m pool) and by 0.14 + 1.14 and -0.59 + 0.82 percent long course (LC, 50-m pool), respectively. No differences between groups were found in blood metabolites, although the human growth hormone (hGH) response to repeated sprints was blunted following Cr loading (T1, 30.42 + 14.60 and 28.95 + 18.27 microg/L; T2, 21.48 + 13.96 and 14.24 + 7.32 microg/L for Cr and control groups, respectively). It was concluded that no statistically significant differences in performance were observed between groups after long-term maintenance during training, although small differences were observed that might be meaningful for elite performers [150].

The addition of carbohydrate (CHO) to an acute creatine (Cr) loading regimen has been shown to increase muscle total creatine content significantly beyond that achieved through creatine loading alone. However, the potential ergogenic effects of combined Cr and CHO loading have not been assessed. The purpose of this study was to compare swimming performance, assessed as mean swimming velocity over repeated maximal intervals, in high-performance swimmers before and after an acute loading regimen of either creatine alone (Cr) or combined creatine and carbohydrate (Cr + CHO). Ten swimmers of international caliber were recruited and were randomized to 1 of 2 groups. Each swimmer ingested five 5 g doses of creatine for 4 days, with the Cr + CHO group also ingesting approximately 100 g of simple CHO 30 minutes after each dose of creatine. Performance was measured on 5 separate occasions: twice at "baseline" (prior to intervention, to assess the repeatability of the performance test), within 48 hours after intervention, and then 2 and 4 weeks later. All subjects swam faster after either dietary loading regimen; however, there was no difference in the extent of improvement of performance between groups. In addition, all swimmers continued to produce faster swim times for up to 4 weeks after intervention. The findings suggest that no performance advantage was gained from the addition of carbohydrate to a creatine-loading regimen in these high-caliber swimmers [151].

It was evaluated the effect of Creatine (Cr) supplementation on muscle fatigue and physiological indices after intermittent swimming bouts in trained swimmers. Sixteen healthy non-elite swimmers (19 ± 4 years, 75 ± 12 kg) were randomly assigned into two groups of either Cr supplementation or placebo and performed six repeated sprints swimming bouts of 50-m departing every 120 seconds. The Cr group was supplemented 4 times a day for 6
days. Blood lactate, Creatine Kinase (CK), creatinine, heart rate, best repeated sprint (RSb) and mean repeated sprint (RSm) times, and percentage of speed decrement (%Dec) were measured at the various phases of swimming bouts. Repeated measure ANOVA and independent t-student tests showed CK and blood lactate concentration increased gradually after the third and sixth swimming bouts. % Dec in Cr group was significantly lower after 3rd swimming bout, also heart rate in Cr group was associated with lower increase in HR mean compared to placebo. These results suggest that Cr supplementation may improve swimming performance and reduce increased blood lactate levels following intermittent sprint swimming bouts. In conclusion Cr supplementation in trained swimmers may improve anaerobic performance and heart rate variations independent of the effect of intensive sprint swimming bouts [152].

**Female swimmers**

Creatine supplementation (CS) has been reported to increase body weight and improve performance during high intensity, short duration, exercise tasks. However, none of the published studies has investigated the influence of CS on performance related hydrodynamic variables during swimming. To investigate the effect of oral CS on swimming velocity, body composition and hydrodynamic variables during the period of final preparation of competitive junior female swimmers. In a double blind and randomized manner, 16 female swimmers, were supplemented with 20 g/day of creatine monohydrate (CS group), or a maltodextrin placebo (PL group) for 21 days. Just pre- and post-21 days of supplementation, subjects performed 2x25 swimming bouts at maximum velocity with a 3 min recovery between bouts. The variables measured were 25 m swimming velocity (MSV(25)); active drag force (D(f)); hydrodynamic coefficient (C(x)); power output (P(o)). Body measures were also analysed: body weight (kg), fat-mass (% FAT), body water (% H2O), and fat free mass (FFM). Significant differences were observed in hydrodynamic values: the CS group showed a significant reduction (approximately 25 %), in D(f), C(x) and P(o) values, when comparing pretest with post-test. No differences were found in variables related to body composition and performance between CS group and PL group, as well as for CS group during the experimental period. These data suggest that 21 days of CS produced significant effects on gross and/or propelling efficiency during swimming in female athletes. However, CS did not influence performance, body weight and body composition [153].

**Junior swimmers**

The objective of this study was to determine whether creatine supplementation could improve mechanical power output, and swimming performance in highly trained junior competitive fin swimmers. Sixteen male fin swimmers (age: 16 + 2 years) were randomly and evenly assigned to either a creatine (4x5 g/day creatine monohydrate for 5 days) or placebo group (same dose of a dextrose-ascorbic acid placebo) in a double-blind research. Before and after creatine the average power output was determined by a Bosco-test and the swimming time was measured in two maximal 100 m fin swims. After five days of creatine the average power of one minute continuous rebound jumps increased by 20 percent. The lactate concentration was significantly less after 5 minutes restitution at the second measurement in both groups. The swimming time was significantly reduced in both first and second sessions of swimming in the creatine group, but remained almost unchanged in the placebo group. The results of this study indicate that five day creatine supplementation enhances the dynamic strength and may increase anaerobic metabolism in the lower extremity muscles, and improves performance in consecutive maximal swims in highly trained adolescent fin swimmers [154].
Weightlifting

A large number of studies have been published on creatine supplementation over the last decade. Many studies show that creatine supplementation in conjunction with resistance training augments gains in muscle strength and size. The underlying physiological mechanism(s) to explain this ergogenic effect remain unclear. Increases in muscle fiber hypertrophy and myosin heavy chain expression have been observed with creatine supplementation. Creatine supplementation increases acute weightlifting performance and training volume, which may allow for greater overload and adaptations to training. Creatine supplementation may also induce a cellular swelling in muscle cells, which in turn may affect carbohydrate and protein metabolism. Several studies point to the conclusion that elevated intramuscular creatine can enhance glycogen levels but an effect on protein synthesis/degradation has not been consistently detected. As expected there is a distribution of responses to creatine supplementation that can be largely explained by the degree of creatine uptake into muscle. Thus, there is wide interest in methods to maximize muscle creatine levels. A carbohydrate or carbohydrate/protein-induced insulin response appears to benefit creatine uptake. In summary, the predominance of research indicates that creatine supplementation represents a safe, effective, and legal method to enhance muscle size and strength responses to resistance training [155].

Taekwondo

Taekwondo (TKD) is a combat sport, which has also been proposed as a fitness program, with a strong anaerobic component. Creatine (Cr) supplementation is used to improve both anaerobic exercise performance and body composition. Therefore, Cr supplementation could be beneficial in TKD. To determine the effect of Cr supplementation (50 mg/kg body wt) on body composition, anaerobic power and blood chemistry in young male TKD practitioners 10 male TKD practitioners (age 20 ± 2 years) participated in a placebo-controlled, double blind, crossover study. Body composition (DEXA), anaerobic power (Wingate Test), blood lactate and blood chemistry were measured before and after supplementation. Differences between data before and after supplementation were calculated for each treatment (Cr and Placebo) and were compared using the Wilcoxon signed-rank test. Fat mass (kg) decreased after placebo and increased following Cr intake. Serum triglyceride concentration (mg/mL) increased after Cr and decrease with placebo. No changes were found in others parameters. Thus, Cr supplementation may increase fat mass and serum triglycerides concentration in young male TKD practitioners without improvement in anaerobic power. Cr supplementation appears to be safe, but athletes should be careful when they want to loss fat [156].

Wrestling

The aim of one study was to test the hypothesis that creatine supplementation with concomitant carbohydrate ingestion during recovery period after rapid body mass reduction accelerates the restoration of body mass and physical performance in well-trained wrestlers. A double-blind, placebo-controlled cross-over study was conducted on five young healthy male wrestlers, who reduced their body mass by 4.5-5.3 percent in two series of investigations separated by one month. During 17 hrs recovery period they consumed controlled diet supplemented in random order with glucose (GL trial) or with glucose plus creatine (GL+CR trial). The capacity of the subjects to perform submaximal and maximal (Wmax) intensity work was measured using 5 min intermittent intensity test exercise at the Cybex II device before (Test 1) and after body mass loss (Test 2), also after the recovery (Test 3) on both trials. There was no effect of treatment on the extent of body mass regain during 17 hrs recovery. A significant increase (19.2 %) in Wmax from Test 2 to Test 3 was
observed in GL+CR trial whereas no change was evident with GL treatment. A strong correlation was established between the whole body creatine retention and the extent of change in $W_{\text{max}}$ from Test 2 to Test 3. The results suggest that creatine supplementation with concomitant glucose ingestion during 17 hrs recovery from rapid body mass loss does not accelerate the restoration of body mass but still stimulates the regain of physical performance in maximal intensity efforts in well-trained wrestlers [157].

The purpose of one study was to investigate the effect of high dose oral creatine supplementation on anaerobic capacity of elite wrestlers in a comparative randomized design. A Wingate anaerobic tests of the participants were taken on 20 active international level wrestlers participated (22 to 27 years old). The daily dosage of creatine or placebo was divided into 4 equal amounts ($5 \times 4 = 20$ g). Every 5 g of supplement was dissolved in 250 ml water and it was given to participants 1 hour before breakfast, lunch, dinner, and workout session. Measures: subjects underwent a 30-s Wingate Anaerobic tests until exhaustion in pre- and post-tests. After the pretest measurements were completed, participants were classified as creatine (Cr., $n=10$) and placebo (Pl., $n=10$) groups with regard to their average anaerobic power scores obtained during the test. Results of paired $t$-test revealed that there was no significant change in placebo group between pre- and post-test in average and peak anaerobic power. However, average and peak power mean scores obtained from post-test were significantly higher than pretest for creatine group. Results of the independent $t$-test also indicated that the mean gained scores of creatine group in average and peak power were significantly higher than placebo group. Thus, the study demonstrated that short-term high dose oral creatine supplementation has an ergogenic effect on anaerobic capacity of elite wrestlers [158].

No impact on upper-body anaerobic power in trained wrestlers

Creatine (CR) is considered an effective nutritional supplement having ergogenic effects, which appears more pronounced in upper-body compared to lower-body exercise. Nevertheless, results regarding the impact of CR loading on repeated high-intensity arm-cranking exercise are scarce and in some cases conflicting. Interestingly, few of the conducted studies have structured their research designs to mimic real world sporting events. Therefore, our purpose was to address the hypothesis that CR ingestion would increase anaerobic power output in consecutive upper-body intermittent sprint performance (UBISP) tests designed to simulate wrestling matches on a competition-day. In a double-blind, placebo-controlled, parallel-group study, 20 trained wrestlers were assigned to either placebo or CR supplemented group ($0.3$ g/kg of body mass per day). Four 6-min UBISP tests interspersed with 30-min recovery periods were performed before (trial 1) and after 5 days (trial 2) of supplementation. Each test consisted of six 15-s periods of arm-cranking at maximal executable cadence against resistance of $0.04$ kg/kg body mass interspersed with 40-s unloaded easy cranking periods and 5-s acceleration intervals ($T_1$-$T_4$). Mean power (MP), peak power (PP), fatigue index and heart rate parameters were measured during UBISP tests. Also, body weight and hydration status were assessed. Principle measures were statistical analysed with mixed-model ANOVAs. Mean individual CR consumption in the CR group was $24.8 \pm 2.5$ g/d. No significant differences occurred in body mass or hydration status indices between the groups or across trials. MP, PP and fatigue index responses were unaffected by supplementation; although, a significant reduction in MP and PP did occurred from $T_1$ to $T_4$ in both trial 1 and 2. Overall heart rate responses in the tests tended to be higher in the CR than PLC group; but, trends in responses in trials and tests were comparable. These results suggest that 5-day CR supplementation has no impact on upper-body muscle anaerobic power output in consecutive UBISP anaerobic tests mimicking wrestling matches on a competition day [159].
Tennis

Creatine supplementation is popular among tennis players but it is not clear whether it actually enhances tennis performance. To examine the effects of creatine supplementation on tennis specific performance indices. In a randomised, double blind design, 36 competitive male tennis players (24 creatine, mean age 23 years; 12 placebo, 23 years) were tested at baseline, after six days of creatine loading, and after a maintenance phase of four weeks (14 creatine, 10 placebo). Serving velocity (10 serves), forehand and backhand velocity (three series of 5x8 strokes), arm and leg strength (bench press and leg press), and intermittent running speed (three series of five 20 metre sprints) were measured. Compared with placebo, neither six days nor five weeks of creatine supplementation had a significant effect on serving velocity; forehand velocity, or backhand velocity. There was also no significant effect of creatine supplementation on repetitive sprint power after 5, 10, and 20 metres, or in the strength of the upper and lower extremities. It was concluded that creatine supplementation is not effective in improving selected factors of tennis specific performance and should not be recommended to tennis players [160].

Squash

The purpose of one study was to determine the effects of oral creatine supplementation on high intensity, intermittent exercise performance in competitive squash players. Nine squash players performed an on-court "ghosting" routine that involved 10 sets of 2 repetitions of simulated positional play, each set interspersed with 30 s passive recovery. A double blind, crossover design was utilised whereby experimental and control groups supplemented 4 times daily for 5 d with 0.075 g/kg body mass of creatine monohydrate and maltodextrine, respectively, and a 4 weeks washout period separated the crossover of treatments. The experimental group improved mean set sprint time by 3.2 ± 0.8 percent over and above the changes noted for the control group. Sets 2 to 10 were completed in a significantly shorter time following creatine supplementation compared to the placebo condition. In conclusion, these data support existing evidence that creatine supplementation improves high intensity, intermittent exercise performance. In addition, the present study provides new evidence that oral creatine supplementation improves exercise performance in competitive squash players [161].
POSSIBLE SIDE EFFECTS

Overview

The use of creatine (Cr) as a nutritional supplement to aid athletic performance has gained widespread popularity among athletes. However, concerns have recently been expressed over potentially harmful effects of short and long term Cr supplementation on health. Forty eight young healthy subjects were randomly allocated to three experimental protocols aimed at elucidating any potential health risks associated with five days (20 g/day) to nine weeks (3 g/day) of Cr supplementation. Venous blood samples were collected before and after periods of Cr supplementation and were analysed for some haematological indices, and for indices of hepatic, muscular, and renal dysfunction. All measured indices were well within their respective normal range at all times. Serum creatinine concentration tended to be increased the day after Cr supplementation. However, values had returned to baseline six weeks after the cessation of supplementation. These increases were probably attributable to increased creatinine production rather than renal dysfunction. No indication of impairment to the haematological indices measured, hepatic function, or muscle damage was apparent after Cr supplementation. These data provide evidence that there are no obvious adverse effects of acute or more chronic Cr supplementation on the haematological indices measured, nor on hepatic, muscle, and renal function. Therefore there is no apparent health risk associated with Cr supplementation to healthy people when it is ingested in quantities that have been scientifically proven to increase muscle Cr stores [162].

The consumption of oral creatine monohydrate has become increasingly common among professional and amateur athletes. Despite numerous publications on the ergogenic effects of this naturally occurring substance, there is little information on the possible adverse effects of this supplement. The objectives of this review are to identify the scientific facts and contrast them with reports in the news media, which have repeatedly emphasised the health risks of creatine supplementation and do not hesitate to draw broad conclusions from individual case reports. Exogenous creatine supplements are often consumed by athletes in amounts of up to 20 g/day for a few days, followed by 1 to 10 g/day for weeks, months and even years. Usually, consumers do not report any adverse effects, but body mass increases. There are few reports that creatine supplementation has protective effects in heart, muscle and neurological diseases. Gastrointestinal disturbances and muscle cramps have been reported occasionally in healthy individuals, but the effects are anecdotal. Liver and kidney dysfunction have also been suggested on the basis of small changes in markers of organ function and of occasional case reports, but well controlled studies on the adverse effects of exogenous creatine supplementation are almost nonexistent. It was investigated liver changes during medium term (4 weeks) creatine supplementation in young athletes. None showed any evidence of dysfunction on the basis of serum enzymes and urea production. Short term (5 days), medium term (9 weeks) and long term (up to 5 years) oral creatine supplementation has been studied in small cohorts of athletes whose kidney function was monitored by clearance methods and urine protein excretion rate. We did not find any adverse effects on renal function. One review was not intended to reach conclusions on the effect of creatine supplementation on sport performance, but we believe that there is no evidence for deleterious effects in healthy individuals. Nevertheless, idiosyncratic effects may occur when large amounts of an exogenous substance containing an amino group are consumed, with the consequent increased load on the liver and kidneys. Regular monitoring is compulsory to avoid any abnormal reactions during oral creatine supplementation [163].
Statement of the International Society of Sports Nutrition

The International Society of Sports Nutrition’s position statement on creatine monohydrate states that there is no scientific evidence of side effects or adverse effects when creatine is used appropriately. They therefore conclude that if used properly, creatine is an acceptable nutritional supplement and ergogenic aid for young athletes to use. No adverse effects were reported in a study of young healthy individuals after supplementation with creatine from 7 d to 10 weeks. Creatine is excreted by the kidney, which led to the hypothesis that creatine supplementation may be detrimental to renal function. Several studies have looked at serum creatinine levels during creatine loading but have not reported significant increases in serum creatinine in younger healthy populations. Slight increases in serum creatinine levels have been reported with larger doses of creatine during the loading phase, although not statistically significant. However, increases in urinary creatinine excretion and decreases in total urine volume output have been reported during creatine loading. The decrease in urinary output is thought to result in fluid retention and weight gain during the initial phases of creatine supplementation. Creatine has been reported to result in weight gain and water retention during short-term use. In the literature, there is a case report of a 20-year-old man with interstitial nephritis as a result of creatine supplementation. However, the individual in the case was taking loading doses of creatine (20 g/d) over a 4-week period instead of the recommended and well-studied loading phase of 5 to 7 d. A longer study of creatine supplementation in nonathletes with other medical comorbidities also did not show evidence of renal problems [014].

Few side effects overall

Use of creatine has become widespread among sportsmen and women, although there are no conclusive evidences concerning possible health risks of long-term creatine supplementation. To investigate long-term effects of creatine monohydrate supplementation on clinical parameters related to health, 18 professional basketball players of the first Spanish Basketball League participated in the present longitudinal study. The subjects were ingesting 5 g creatine monohydrate daily during three competition seasons. Blood was collected in the morning after an overnight fast, five times during each of the three official competition seasons of the first National Basketball League (September 1999-June 2000, September 2000-June 2001 and September 2001-June 2002) and the European League. Standard clinical examination was performed for 16 blood chemistries. The plasma concentrations of all clinical parameters did not alter significantly during the analyzed time frames of creatine supplementation. All of these parameters were, with the exception of creatinine and creatine kinase, within their respective clinical ranges at all time points. The data shows that low-dose supplementation with creatine monohydrate did not produce laboratory abnormalities for the majority of the parameters tested [164].

Effects on the liver

Despite published allegations of detrimental effects of oral creatine supplementation on liver metabolism, studies on humans have not shown any significant increase in plasma urea, nor liver enzyme activity, during 5 years of creatine supplementation [004].

It was present a case of acute cholestatic liver injury associated with the combination of whey protein and creatine supplements. The difficulty of diagnosing drug-induced liver injury is emphasized. The patient was a healthy, 27-year-old man who presented with painless jaundice. He had no occupational exposures to solvents, was not taking prescription medications, and did not use recreational drugs or alcohol. He was an enthusiastic weight-
lifter and had been taking creatine for 8 to 9 months and whey protein supplements for 4 weeks prior to the development of symptoms [165].

**Effects on the brain**

The role of creatine together with carbohydrates and proteins is to provide a neuroprotective function by enhancing the energy metabolism in the brain tissue, promoting antioxidant activities, improving cerebral vasculature and protecting the brain from hyperosmotic shock by acting as a brain cell osmolyte. Creatine can provide other neuroprotective benefits through stabilisation of mitochondrial membranes, stimulation of glutamate uptake into synaptic vesicles and balance of intracellular calcium homeostasis [035].

**Effects on the heart**

One project aimed to determine whether creatine (Cr) supplementation affects cardiovascular structure and function and to examine its effect on aerobic power. Eighteen males undertook aerobic testing on a cycle ergometer and echocardiographic assessment of the heart. The experimental group (n=9) ingested 20g/day of Cr for seven days followed by 10g/day for a further 21 days. The control group (n=9) followed an identical protocol ingesting a placebo for the same period. Assessment was performed pre-, mid- (seven days) and post-testing (28 days). A MANOVA with repeated measures was used to test for group differences before and after supplementation. The Cr group demonstrated a significant increase in body mass for the pre-mid (1.0 ± 0.6 kg) and the pre-post (1.5 ± 0.7 kg) testing occasions. Submaximal VO2 decreased significantly from the pre-mid and pre-post testing occasions by between 5 to 11 percent with Cr supplementation at workloads of 75 W and 150 W. Other oxygen consumption measures and exercise time to exhaustion, for the Cr group, showed decreasing trends that approached significance. Additionally, there was a significant pre-post decrease in maximum heart rate of 4 percent. There were no changes in any of the echocardiographic or blood pressure measures for either group. The present results suggest short term Cr supplementation has no detectable negative effect on cardiac structure or function. Additionally, Cr ingestion improves submaximal cycling efficiency. These results suggest that the increase in efficiency may be related to peripheral factors such an increase in muscle phosphocreatine, rather than central changes [166].

**Atrial fibrillation**

Atrial fibrillation in young patients without structural heart disease is rare. Therefore, when the arrhythmia is present in this population, reversible causes must be identified and resolved. Thyroid disorders, illicit drug or stimulant use, and acute alcohol intoxication are among these causes. It was reported a case of a 30-year-old Caucasian man who came to the emergency department in atrial fibrillation with rapid ventricular response. His medical history was unremarkable, except for minor fractures of the fingers and foot. Thyroid-stimulating hormone, magnesium, and potassium levels were within normal limits, urine drug screen was negative, and alcohol use was denied. However, when the patient was questioned about use of herbal products and supplements, the use of creatine monohydrate was revealed. The patient was admitted to the hospital, anticoagulated with unfractionated heparin, and given intravenous diltiazem for rate control and intravenous amiodarone for rate and rhythm control. When discharged less than 24 hours later, he was receiving metoprolol and aspirin, with follow-up plans for echocardiography and nuclear imaging to assess perfusion. Exogenous creatine is used by athletes to theoretically improve exercise performance. Vegetarians may also take creatine to replace what they are not consuming from meat, fish, and other animal products. Previous anecdotal reports have linked creatine
to the development of arrhythmia. Clinicians must be diligent when interviewing patients about their drug therapy histories and include questions about their use of herbal products and dietary supplements. In addition, it is important to report adverse effects associated with frequently consumed supplements and herbal products to the Food and Drug Administration and in the literature [167].

**Water retention**

There is no definitive evidence that CrS causes gastrointestinal, renal, and/or muscle-cramping complications. An investigation from 2003 examined the effects of long-term CrS (up to 21 months) on clinical markers of health status in 98 athletes. A loading phase of 16 g/day for 5 days was followed by a maintenance dose averaging 5 g/day thereafter, with a comprehensive urinary and blood chemistry panel determined. The results indicate that long-term CrS (up to 21 months) does not appear to adversely affect markers of health status in athletes undergoing intense training in comparison to athletes who do not take CrS. The only significant side effect reported in the literature is that of weight gain within the first few days which is likely due to water retention related to creatine uptake in the muscle [002].

**Increased body weight**

The purpose of this study was to determine the effect of 30 days of single-dose creatine supplementation with phosphate salts on body weight and anaerobic working capacity in men. Using a double-blind design, 32 men randomly received 1 serving of either 5 g creatine + 4 g phosphate (n=17) or 20 g of dextrose as placebo (n=15) for 30 days. Results showed no significant differences between groups for working capacity at any time point; however, body weight was significantly increased at 10 days in the creatine group (1.0 kg) versus placebo (0.0 kg), and remained elevated for the duration of the study [168].

Allegations about side effects of creatine supplementation by athletes have been published in the popular media and scientific publications. One of the purported effects of oral creatine supplementation is increased muscle mass. A review of the literature reveals a 1.0 to 2.3 percent increase in body mass, which is attributed to fat-free mass and, more specifically, to skeletal-muscle mass. Although it is unlikely that water retention can completely explain these changes, increase in muscle-protein synthesis has never been observed after creatine supplementation. Indirect evidence based on mRNA analyses suggests that transcription of certain genes is enhanced. Although the effect of creatine on muscle-protein synthesis seems irrefutable according to advertising, this allegation remains under debate in the scientific literature [169].

**Renal effects**

Although oral creatine supplementation is very popular among athletes, no prospective placebo-controlled studies on the adverse effects of long-term supplementation have yet been conducted. It was performed a double-blind, placebo-controlled trial of creatine monohydrate in patients with the neurodegenerative disease amyotrophic lateral sclerosis, because of the neuroprotective effects it was shown to have in animal experiments. The purpose of this paper is to compare the adverse effects, and to describe the effects on indirect markers of renal function of long-term creatine supplementation. 175 subjects (age 58) were randomly assigned to receive creatine monohydrate 10 g daily or placebo during an average period of 310 days. After one month, two months and from then on every fourth month, adverse effects were scored using dichotomous questionnaires, plasma urea concentrations were measured, and urinary creatine and albumin concentrations were
determined. No significant differences in the occurrence at any time of adverse effects due to creatine supplementation were found (23 % nausea in the creatine group, vs 24 % in the placebo group, 19 % gastro-intestinal discomfort in the creatine group, vs 18 % in the placebo group, 35 % diarrhoea in the creatine group, vs 24 % in the placebo group). After two months of treatment, oedematous limbs were seen more often in subjects using creatine, probably due to water retention. Severe diarrhoea (n=2) and severe nausea (n=1) caused 3 subjects in the creatine group to stop intake of creatine, after which these adverse effects subsided. Long-term supplementation of creatine did not lead to an increase of plasma urea levels or to a higher prevalence of micro-albuminuria (5.4 % before treatment vs 1.8 % at the end of treatment) [170].

Creatine is a popular supplement used by athletes in an effort to increase muscle performance. The purpose of this review was to assess the literature evaluating the effects of creatine supplementation on renal function. A PubMed search was conducted to identify relevant articles using the keywords, creatine, supplementation, supplements, renal dysfunction, ergogenic aid and renal function. Twelve pertinent articles and case reports were identified. According to the existing literature, creatine supplementation appears safe when used by healthy adults at the recommended loading (20 g/day for five days) and maintenance doses (≤3 g/day). In people with a history of renal disease or those taking nephrotoxic medications, creatine may be associated with an increased risk of renal dysfunction. One case report of acute renal failure was reported in a 20-year-old man taking 20 gm/day of creatine for a period of four weeks. There are few trials investigating the long-term use of creatine supplementation in doses exceeding 10 gm/day. Furthermore, the safety of creatine in children and adolescents has not been established. Since creatine supplementation may increase creatinine levels, it may act as a false indicator of renal dysfunction. Future studies should include renal function markers other than serum creatinine and creatinine clearance [171].

With regard to athletes attempting to improve their performance, at the present time creatine monohydrate is clearly the most widely used dietary supplement or ergogenic aid. Loading doses as high as 20 g/d are typical among athletes. The majority (> 90 %) of the creatine ingested is removed from the plasma by the kidney and excreted in the urine. Despite relatively few isolated reports of renal dysfunction in persons taking creatine, the studies completed to date suggest that in normal healthy individuals the kidneys are able to excrete creatine, and its end product creatinine, in a manner that does not adversely alter renal function. This situation would be predicted to be different in persons with impaired glomerular filtration or inherent renal disease. The question of whether long-term creatine supplementation (i.e. months to years) has any deleterious affects on renal structure or function can not be answered at this time. The limited number of studies that have addressed the issue of the chronic use of creatine have not seen remarkable changes in renal function. However, physicians should be aware that the safety of long-term creatine supplementation, in regard to the effects on the kidneys, cannot be guaranteed. More information is needed on possible changes in blood pressure, protein/albumin excretion, and glomerular filtration in athletes who are habitual users of this compound [172].

The purpose of one study was to determine the effect of long-term Cr supplementation on blood parameters reflecting liver and kidney function. Twenty-three members of an NCAA Division II American football team (ages 19-24 years) with at least 2 years of strength training experience were divided into a Cr monohydrate group (CrM, n=10) in which they voluntarily and spontaneously ingested creatine, and a control group (n=13) in which they took no supplements. Individuals in the CrM group averaged regular daily consumption of 5 to 20 g for 0.25 to 5.6 years. Venous blood analysis for serum albumin, alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, bilirubin, urea, and creatinine produced no significant differences between groups. Creatinine clearance was estimated from serum creatinine and was not significantly different between groups. Within the CrM
group, correlations between all blood parameters and either daily dosage or duration of supplementation were nonsignificant. Therefore, it appears that oral supplementation with CrM has no long-term detrimental effects on kidney or liver functions in highly trained college athletes in the absence of other nutritional supplements [173].

No reports have observed a modification of the glomerular filtration rate, nor the presence of microalbuminuria. All values remained within the normal range adapted for the age range [004].

Creatine supplements are approved and considered relatively safe, but there have been a few case reports of renal dysfunction associated with their use. It was presented a case of a patient who developed acute renal failure and lactic acidosis while using creatine and metformin simultaneously [174].

It was reported on the short-term effects of creatine supplementation on kidney function in a young man with a single kidney and mildly decreased glomerular filtration rate (GFR). A 20-year-old man who had undergone unilateral nephrectomy and presented with mildly decreased GFR without kidney damage underwent a trial with 35 days of creatine supplementation (20 g/d for 5 days followed by 5 g/d for the next 30 days) and had his kidney function monitored. After the intervention, 51Cr-EDTA clearance, proteinuria, and electrolyte levels were unchanged. Albuminuria, serum urea level, and estimated creatinine clearance were decreased, whereas serum creatinine level was slightly increased, falsely suggesting kidney function impairment. This prospective report suggests that short-term creatine supplementation may not affect kidney function in an individual with a single kidney, mild decreased GFR, and ingesting a high-protein diet (i.e. 2.8 g/kg/d). This finding has great relevance considering that creatine-induced kidney disease has been a growing concern, even for healthy people [175].

Although there have not been significant increases in serum creatinine with creatine supplementation, there have been other concerns about the effects of creatine loading on the kidney. Creatine can be metabolized to methylamine and subsequently formaldehyde during urinary excretion. Both methylamine and formaldehyde are known cytotoxic substances raising concerns about potential harmful effects to the kidney with long-term use. Studies have shown significant increases in both methylamine and formaldehyde after short-term creatine supplementation at loading doses. Further studies are needed to further evaluate the potential harm to the kidney related to the increases in urinary methylamine and formaldehyde levels, particularly with longer term use and high loading doses of creatine. In the literature, there are case reports of young healthy individuals developing acute liver failure when one of the dietary supplements they were ingesting was creatine. However in these cases, the individuals were taking large doses of creatine in addition to several other dietary supplements for weight training. When creatine has been studied in isolation and at acceptable doses, there have been no significant adverse effects to the liver [014].

Since creatine does result in a decrease in urinary volume and water retention during supplementation, concerns arose that athletes could develop problems staying hydrated and regulating body temperature. Creatine supplementation increases intracellular volume with increased cellular water volume. A study evaluated lower extremity anterior compartment pressures after heat-stressed exercise, and it did find transient asymptomatic increases in compartment pressures with creatine supplementation compared to placebo. There is a case report of compartment syndrome occurring with large doses of creatine, with subsequent resolution with cessation. In 1998, the University of Tennessee football team had many of their football players develop cramping during a game, after the team instituted a creatine supplementation program. The number of athletes was disproportional to historic values of athletes cramping, which caused the linkage to creatine usage. Further studies have since
shown no increase in the incidence of cramping in college football players taking creatine. Several studies also have reported no issues with heat tolerance or hydration status with creatine supplementation [014].

The aim of one study was to determine the effects of creatine supplementation on kidney function in resistance-trained individuals ingesting a high-protein diet. A randomized, double-blind, placebo-controlled trial was performed. The participants were randomly allocated to receive either creatine (20 g/d for 5 d followed by 5 g/d throughout the trial) or placebo for 12 weeks. All of the participants were engaged in resistance training and consumed a high-protein diet (i.e., ≥ 1.2 g/Kg/d). Subjects were assessed at baseline (Pre) and after 12 weeks (Post). Glomerular filtration rate was measured by 51Cr-EDTA clearance. Additionally, blood samples and a 24-h urine collection were obtained for other kidney function assessments. No significant differences were observed for 51Cr-EDTA clearance throughout the trial. Creatinine clearance, serum and urinary urea, electrolytes, proteinuria, and albuminuria remained virtually unchanged. It was concluded that a 12-week creatine supplementation protocol did not affect kidney function in resistance-trained healthy individuals consuming a high-protein diet; thus reinforcing the safety of this dietary supplement [176].

It has been claimed that oral creatine supplementation might have potential cytotoxic effects on healthy consumers by increasing the production of methylamine and formaldehyde. Despite this allegation, there has been no scientific evidence obtained in humans to sustain or disprove such a detrimental effect of this widely used ergogenic substance. Twenty young healthy men ingested 21 g of creatine monohydrate daily for 14 consecutive days. Venous blood samples and 24-h urine were collected before and after the 14th day of supplementation. Creatine and creatinine were analyzed in plasma and urine, and methylamine, formaldehyde, and formate were determined in 24-h urine samples. Oral creatine supplementation increased plasma creatine content 7.2-fold and urine output 141-fold with no effect on creatinine levels. Twenty-four-hour urine excretion of methylamine and formaldehyde increased, respectively, 9.2-fold and 4.5-fold after creatine feeding, with no increase in urinary albumin output. The investigation shows that short-term, high-dose oral creatine supplementation enhances the excretion of potential cytotoxic compounds, but does not have any detrimental effects on kidney permeability. This provides indirect evidence of the absence of microangiopathy in renal glomeruli [177].

*Long-term effects in rats*

The aim of one study was to evaluate the long-term effects of oral creatine supplementation on renal function and body composition (fat and lean mass) in an experimental model. Male Wistar rats were supplemented with creatine (2 g/kg of food) for 10 weeks in combination with treadmill exercise, 12 m/min, 1 h/day (CREAT + EX, n=12) or not (CREAT, n=10), and compared with exercised animals without creatine supplementation (EX, n=7) and CONTROL animals, n=7. Body composition and bone mineral density (BMD) were determined by dual x-ray absorptiometry and glomerular filtration rate (GFR) and renal plasma flow (RPF) were measured by inulin and paraaminohippurate clearance, respectively. At the end of the study (post), CREAT+EX presented higher lean mass and lower fat mass than CREAT, EX or CONTROL. Post lean/fat mass ratio was higher than baseline only in CREAT + EX. Post BMD was significantly higher than baseline in all groups. GFR and RPF were lower in CREAT versus CONTROL. It was concluded that creatine supplement in combination with exercise increased the proportion of lean mass more than EX or CREAT alone. The use of creatine alone induced an important and significant reduction of both RPF and GFR [178].
Elevating of serum creatinine

It was described a case in which serum creatinine is elevated due to the use of creatine ethyl ester. One week after withdrawal, the plasma creatinine had normalised. There are two types of creatine products available: creatine ethyl ester (CEE) and creatine monohydrate (CM). Plasma creatinine is not elevated in all creatine-using subjects. CEE, but not CM, is converted into creatinine in the gastrointestinal tract. As a result the use of CEE may be associated with elevated plasma creatinine levels. Since plasma creatinine is a widely used marker for renal function, the use of CEE may lead to a false assumption of renal failure [055].

Safety recommendations

It was advised that high-dose (>3-5 g/day) creatine supplementation should not be used by individuals with pre-existing renal disease or those with a potential risk for renal dysfunction (diabetes, hypertension, reduced glomerular filtration rate). A pre-supplementation investigation of kidney function might be considered for reasons of safety, but in normal healthy subjects appears unnecessary [179].

Diarrhoea

The main aim of one study was to investigate the effects of two different creatine-supplementation protocols on incidence of gastrointestinal (GI) distress in top-level athletes. Data were collected from 59 top-level male soccer players who were allocated in a double-blind design to three randomly assigned trials: ingesting creatine supplement (2 x 5-g doses or 1 x 10-g dose) or placebo for 28 days. In order to assess potential side effects of the supplementation regimen, all subjects were instructed to report any adverse effects of supplementation on their GI system. Survey questions covered perceived side effects on GI system linked with creatine supplementation. In all three treatment groups, the most frequent GI complaints were diarrhoea (39 %), stomach upset (24 %), and belching (17 %). It was not found a significant difference between incidence of GI distress symptoms between 5 gram doses and the placebo group after the survey. Yet, significant differences were found for incidence of diarrhoea between the 5 and 10 gram groups (29 % vs 56 %, respectively). Moreover, diarrhoea was significantly more frequent in the 10 gram group as compared with the placebo group (56 % vs 35 %). There is no reason to believe that short-term oral creatine supplementation for 28 days has any detrimental effect on the GI tract if taken in a recommended amount (10 g per day in two equal doses). The risk of diarrhoea may be increased, however, following intake of 10 grams of creatine per single serving [180].

Muscle cramping

Anecdotal reports from athletes have claimed that creatine supplementation may induce muscle cramps. However, it appears that muscle cramping might be due to the intensity of exercise rather than creatine supplementation itself [004].

Long-term use

There have been a few reported renal health disorders associated with creatine supplementation. These are isolated reports in which recommended dosages are not followed or there is a history of previous health complaints, such as renal disease or those
taking nephrotoxic medication aggravated by creatine supplementation. Specific studies into creatine supplementation, renal function and/or safety conclude that although creatine does slightly raise creatinine levels there is no progressive effect to cause negative consequences to renal function and health in already healthy individuals when proper dosage recommendations are followed. Urinary methylamine and formaldehyde have been shown to increase due to creatine supplementation of 20 g/d; this however did not bring the production outside of normal healthy range and did not impact on kidney function. A retrospective study, that examined the effects of long lasting (0.8 to 4 years) CM supplementation on health markers and prescribed training benefits, suggested that there is no negative health effects (including muscle cramp or injuries) caused by long term CM consumption. In addition, despite many anecdotal claims, it appears that creatine supplementation would have positive influences on muscle cramps and dehydration. Creatine was found to increase total body water possibly by decreasing the risk of dehydration, reducing sweat rate, lowering core body temperature and exercising heart rate. Furthermore, creatine supplementation does not increase symptoms nor negatively affect hydration or thermoregulation status of athletes exercising in the heat. Additionally, CM ingestion has been shown to reduce the rate of perceived exertion when training in the heat. It is prudent to note that creatine supplementation has been shown to reduce the body’s endogenous production of creatine, however levels return to normal after a brief period of time when supplementation ceases. However, long term effects are unknown, therefore safety cannot be guaranteed. Whilst the long term effects of creatine supplementation remain unclear, no definitive certainty of either a negative or a positive effect upon the body has been determined for many health professionals and national agencies [035].

Potential side effects of long-term use of creatine are unknown. Since the majority of the creatine ingested is removed from the plasma by the kidneys and excreted in the urine, concerns have particularly been related to a possible effect on renal function, and especially in subjects with impaired renal capacity. However, in one study it was not any effect of creatine supplementation on kidney function in healthy athletes. Further concerns are the possibility for muscle dysfunction and the association between supplementation and heat illness, because creatine may increase intracellular water and dilute electrolytes. However, increased prevalence of muscle injury and cramping or heat illness has not been reported in creatine users versus nonusers, but long-term studies are lacking. Because creatine affects fluid balance, users should be advised to pay attention to fluid need in hot climates. Further, because commercially marketed creatine products do not meet the same quality control standards as pharmaceuticals, there is always a concern of impurities or doses higher or lower than those on the labeling. Intake of large doses of creatine can reduce endogenous synthesis, probably via feedback regulation, but the enzymes involved in creatine synthesis seem to be reactivated when supplementation is discontinued. Finally, despite the fact that creatine is normally found in cardiac muscle, brain, and testes, these areas remain essentially unstudied with respect to oral creatine supplementation. The effect on these organ systems also needs to be included in long-term, randomized, controlled studies [070].

One investigation assessed the effects of a 9-weeks regimen of creatine monohydrate (Cr x H₂O) supplementation coupled with resistance training on body composition and neuromuscular performance in NCAA Division I football athletes. Twenty-five subjects were randomly assigned in a double-blind, randomized placebo-controlled design, to a treatment (Cr, n=9), placebo (P, n=8), or control group (C, n=8). The Cr group received 20 g/d of creatine for the first 5 d in 5-g doses, four times daily, followed by 5 g/d for the remainder of the study. Each 5-g dose was mixed with 500 mL of glucose solution (Gatorade®). The P group received a placebo (sodium phosphate monohydrate; NaH₂PO₄ x H₂O) following the exact protocol as the Cr group. The C group received no supplementation. All subjects resistance trained 4 d/wk. Measurements of neuromuscular performance and body composition were made pre- and post-training after supplementation while monitoring dietary intakes. Repeated measures ANOVA indicated significant differences occurred between the
Cr group and the other two groups (P and C) for total body weight, lean body mass, cell hydration, strength, peak torque at 300 degrees/s knee flexion, percent torque decrement, and anaerobic power and capacity. However, percent body fat, peak torque during both knee flexion and extension at 60 and 180 degrees/s, peak torque at 300 degrees/s during knee extension, global muscular strength (power clean), and extracellular fluid remained statistically unchanged for all groups. The findings indicate that creatine, supplemented concurrently with resistance and anaerobic training, may positively affect cell hydration status and enhance performance variables further than augmentation seen with training alone [181].

Long-term safety of creatine supplementation has been questioned. One retrospective study was performed to examine markers related to health, the incidence of reported side effects and the perceived training benefits in athletes supplementing with creatine monohydrate. Twenty-six athletes (18 M and 8 F) from various sports were used as subjects. Blood was collected between 7:00 and 8:30 a.m. after a 12-h fast. Standard clinical examination was performed for CBC and 27 blood chemistries. Testosterone, cortisol, and growth hormone were analyzed using an ELISA. Subjects answered a questionnaire on dietary habits, creatine supplementation, medical history, training history, and perceived effects of supplementation. Body mass was measured using a medical scale, body composition was estimated using skinfolds, and resting heart rate and blood pressure were recorded. Subjects were grouped by supplementation length or no use: Gp1 (control) = no use (n=7; 3 F, 4 M); Gp2 = 0.8-1.0 yr (n=9; 2 F, 7 M); and Gp3 = 1(+) (n=10; 3 F, 7 M). Creatine supplementation ranged from 0.8 to 4 years. Mean loading dose for Gp2 and Gp3 was 13.7 ± 10.0 and the maintenance dose was 9.7 ± 5.7. Group differences were analyzed using one-way ANOVA. Expected gender differences were observed. Of the comparisons made among supplementation groups, only two differences for creatinine and total protein were noted. All group means fell within normal clinical ranges. There were no differences in the reported incidence of muscle injury, cramps, or other side effects. These data suggest that long-term creatine supplementation does not result in adverse health effects [182].

**Increased production of formaldehyde**

Creatine is alleged to be an ergogenic aid to enhance sports performance and recently became a popular sports nutrition supplement. Although short-term supplementation of creatine has not been associated with major health risks, the safety of prolonged use has caused some concern. The present study demonstrates that creatine is metabolized to methylamine, which is further converted to formaldehyde by semicarbazide-sensitive amine oxidase (SSAO). Formaldehyde is well known to cross-link proteins and DNAs, and known to be a major environmental risk factor. SSAO-mediated production of toxic aldehydes has been recently proposed to be related to pathological conditions such as vascular damage, diabetic complications, nephropathy, etc. Chronic administration of a large quantity of creatine can increase the production of formaldehyde, which may potentially cause serious unwanted side-effects [183].

**Cytotoxicity**

Experimentally, an excess conversion of creatine to sarcosine may result in cytotoxic agents such as methylamine. However, in humans taking up to 20 g creatine per day for 2 weeks urine methylamine excretion remains largely under the upper limit for healthy individuals [004].

The potential effects (production of heterocyclic amines) of mutagenicity and carcinogenicity
induced by creatine supplementation have been claimed by a French Sanitary Agency (AFSSA), which might put consumers at risk. Even if there is a slight increase (within the normal range) of urinary methylamine and formaldehyde excretion after a heavy load of creatine (20 g/day) this is without effect on kidney function. The search for the excretion of heterocyclic amines remains a future task to definitively exclude the unproved allegation made by some national agencies [184].

Even if there is a slight increase in mutagenic agents (methylamine and formaldehyde) in urine after a heavy load of creatine (20 g/day), their excretion remains within a normal range. No data are currently available regarding the potential production of heterocyclic amines with creatine supplementation. In summary, the major risk for health is probably associated with the purity of commercially available creatine [169].

*Cancer?*

Animal data suggest a link with cancer after long term exposure. The precise composition of creatine supplements is unclear: contamination is possible, and other substances, especially doping agents, are sometimes added. Taking creatine supplements is inadvisable [185].

*Children*

The safety of creatine in the pediatric and adolescent population is lacking appropriate research. In addition, studies that have shown benefit in resistance training have not included subjects less than 18 years of age. Therefore creatine supplementation in athletes less than 18 years old needs further research before it should be recommended [014].

*Chronic exposition to individuals with chronic disease*

Even if there are no health risks induced by oral creatine supplementation, it is safer to remain cautious when this substance is administered chronically. It is advised that creatine supplementation should not be used by individuals with pre-existing renal disease or those with a potential risk of renal dysfunction (diabetes, hypertension, reduced glomerular filtration rate). Regular check-ups should be undertaken to monitor potential dysfunction, which could appear with some individuals less prone to compensate any homeostatic imbalance. Great care should also be taken as far as the purity of exogenous creatine supplements is concerned. Analytical tests must prove their unique nutraceutical composition, as safety is not assured in some preparations [004].

*Safety levels*

Creatine monohydrate (creatine) has become an increasingly popular ingredient in dietary supplements, especially sports nutrition products. A large body of human and animal research suggests that creatine does have a consistent ergogenic effect, particularly with exercises or activities requiring high intensity short bursts of energy. Human data are primarily derived from three types of studies: acute studies, involving high doses (20 g/d) with short duration (< 1 week), chronic studies involving lower doses (3-5 g/d) and longer duration (1 year), or a combination of both. Systematic evaluation of the research designs and data do not provide a basis for risk assessment and the usual safe Upper Level of Intake (UL) derived from it unless the newer methods described as the Observed Safe Level (OSL) or Highest Observed Intake (HOI) are utilized. The OSL risk assessment method indicates that
the evidence of safety is strong at intakes up to 5 g/d for chronic supplementation, and this level is identified as the OSL. Although much higher levels have been tested under acute conditions without adverse effects and may be safe, the data for intakes above 5 g/d are not sufficient for a confident conclusion of long-term safety [186].

**Contaminants**

A major point that related to the quality of creatine products is the amount of creatine ingested in relation to the amount of contaminants present. During the production of creatine from sarcosine and cyanamide, variable amounts of contaminants (dicyandiamide, dihydrotriazines, creatinine, ions) are generated and, thus, their tolerable concentrations (ppm) must be defined by specific toxicological researches [187].
EFFECT OF CREATINE IN COMBINATION WITH OTHER ORAL SUBSTANCES

Combination of creatine with glucose

Consuming carbohydrate with creatine appears to increase muscle creatine stores significantly more than creatine supplementation alone [030].

Creatine (Cr) is a guanidine compound which is naturally synthesised in the liver, kidney, and pancreas from amino acids arginine, glycine, and methionin. Cr can also be obtained through diet, especially from meat and fish. Dietary Cr is taken up to tissues, including skeletal muscle. Enhancement in muscle Cr content facilitates growth in lean body mass, strength, and high intensity exercise performance. It also enhances fluid retention and provides the improvement in thermoregulation during exercise in the heat. As a consequence of these findings, Cr supplementation has become popular amongst recreational and professional athletes. In vitro and in vivo studies demonstrated that Cr uptake by rat skeletal muscle is increased by the presence of insulin. In humans, carbohydrate (CHO) ingestion, aimed at rising plasma insulin concentration, has been demonstrated to enhance both whole body Cr retention and skeletal muscle Cr accumulation [059].

It has been suggested that muscle Cr accumulation is maximized when about100 g of simple sugars is ingested with a typical 5 g dose of Cr supplement. The amount of glucose required can be expected to increase available CHO intake above the habitually consumed level. Safety of such a high CHO intake while applying Cr supplementation protocols has not been investigated yet. Some studies have demonstrated that increase in CHO intake may have detrimental impact on concentration of plasma lipids not only in sedentary but also in well-trained individuals. For example, in distance runners consumption of a high CHO diet for two weeks increased concentration of plasma triglycerides (TAG) and total and LDL-cholesterol and reduced concentration of HDL-cholesterol. These results were supported by another study which examined the effects of 12-week high CHO diet on plasma lipids in 32 endurance-trained cyclists and found a significant increase in plasma concentration of total cholesterol and TAG. A study also showed that in 9 trained males after 5 days on diet providing 70 percent of energy from CHO, plasma concentration of TAG was increased and concentration of HDL-cholesterol decreased. Based on population studies, these changes would be expected to increase the risk of coronary heart disease. Due to these possible detrimental changes in plasma lipids induced by high glucose (Glu) intake consumed with Cr containing supplements, there has been a search for alternative agents that stimulate insulin secretion and thus may be expected to enhance skeletal muscle Cr uptake [059].

The addition of carbohydrate in the form of simple sugars to creatine supplements is central. The study aimed to determine whether ingestion of glucose simultaneously with Cr and glycerol (Cr/Gly) supplement is detrimental to plasma lipids of endurance-trained individuals and find out whether modification arising can be attenuated by replacing part of the Glu with alpha lipoic acid (Ala). Twenty-two endurance-trained cyclists were randomized to receive Cr/Gly/Glu (11.4 g Cr-H₂O, 1 g Gly/kg BM, and 150 g Glu) or Cr/Gly/Glu/Ala (11.4 g Cr-H₂O, 1 g Gly/kg BM, 100 g Glu, and 1 g Ala) for 7 days. Fasting concentration of TAG increased significantly after supplementation with Cr/Gly/Glu (before: 0.9 ± 0.2 mmol/L; after: 1.3 ± 0.4 mmol/L) and Cr/Gly/Glu/Ala (before: 0.8 ± 0.2 mmol/L; after: 1.2 ± 0.5 mmol/L) but changes were not different between the groups. Supplementation significantly increased the TAG to HDL-cholesterol ratio but had no effect on fasting concentration of total, HDL-, and LDL-cholesterol and insulin resistance. Thus, addition of Glu to Cr containing supplements enhances plasma TAG concentration and the TAG to HDL-cholesterol ratio and this enhancement cannot be attenuated by partial replacement of Glu with Ala [059].
Combination of creatine with carbohydrate and protein

Studies attributing gains in strength and lean body mass (LBM) to creatine monohydrate (CrM) during resistance exercise (RE) training have not assessed these changes alongside cellular and subcellular adaptations. Additionally, CrM-treated groups have seldom been compared with a group receiving a placebo similar in nitrogen and energy. The purpose of one study was to examine the effects of a CrM-containing protein-carbohydrate (PRO-CHO) supplement in comparison with a supplement containing a similar amount of nitrogen and energy on body composition, muscle strength, fiber-specific hypertrophy, and contractile protein accrual during RE training. In a double-blind, randomized protocol, resistance-trained males were matched for strength and placed into one of three groups: protein (PRO), PRO-CHO, or the same PRO-CHO supplement (1.5 g/kg body weight and day) containing CrM (Cr-PRO-CHO) (0.1 g/kg body weight and day). Assessments were completed the week before and after a 10-wk structured, supervised RE program: strength (1RM, three exercises), body composition (DEXA), and vastus lateralis muscle biopsies for determination of muscle fiber type (I, IIa, IIx), cross-sectional area (CSA), contractile protein, and creatine content. Cr-PRO-CHO provided greater improvements in 1RM strength. At least 40 percent of the strength improvements could be attributed to hypertrophy of muscle involved in this exercise. Cr-PRO-CHO also resulted in greater increases in LBM, fiber CSA, and contractile protein compared with PRO and PRO-CHO. In RE-trained participants, supplementation with Cr-PRO-CHO provided greater muscle hypertrophy than an equivalent dose of PRO-CHO, and this response was apparent at three levels of physiology (LBM, fiber CSA, and contractile protein content) [188].

Although creatine can be bought commercially as a standalone product it is often found in combination with other nutrients. A prime example is the combination of creatine with carbohydrate or protein and carbohydrate for augmenting creatine muscle retention mediated through an insulin response from the pancreas. The addition of 10 g of creatine to 75 g of dextrose, 2 g of taurine, vitamins and minerals, induced a change in cellular osmolarity which in addition to the expected increase in body mass, seems to produce an up regulation of large scale gene expression (mRNA content of genes and protein content of kinases involved in osmosensing and signal transduction, cytoskeleton remodelling, protein and glycogen synthesis regulation, satellite cell proliferation and differentiation, DNA replication and repair, RNA transcription control, and cell survival). Similar findings have also been reported for creatine monohydrate supplementation alone when combined with resistance training. A commercially available pre-workout formula comprised of 2.05 g of caffeine, taurine and glucuronolactone, 7.9 g of L-leucine, L-valine, L-arginine and L-glutamine, 5 g of di-creatine citrate and 2.5 g of beta-alanine mixed with 500 ml of water taken 10 minutes prior to exercise has been shown to enhance time to exhaustion during moderate intensity endurance exercise and to increase feelings of focus, energy and reduce subjective feelings of fatigue before and during endurance exercise due to a synergistic effect of the before mentioned ingredients [019].

Combination of creatine and carbohydrate or cinnamon

The insulin response following carbohydrate ingestion enhances creatine transport into muscle. Cinnamon extract is promoted to have insulin-like effects, therefore this study examined if creatine co-ingestion with carbohydrates or cinnamon extract improved anaerobic capacity, muscular strength, and muscular endurance. Active young males (n=25; 24 ± 3 years) were stratified into 3 groups: (1) creatine only (CRE); (2) creatine+ 70 g carbohydrate (CHO); or (3) creatine+ 500 mg cinnamon extract (CIN), based on anaerobic capacity (peak power·kg⁻¹) and muscular strength at baseline. Three weeks of supplementation consisted of a 5 d loading phase (20 g/d) and a 16 d maintenance phase (5
g/d). Pre- and post-supplementation measures included a 30-s Wingate and a 30-s maximal running test (on a self-propelled treadmill) for anaerobic capacity. Muscular strength was measured as the one-repetition maximum 1-RM for chest, back, quadriceps, hamstrings, and leg press. Additional sets of the number of repetitions performed at 60 percent 1-RM until fatigue measured muscular endurance. All three groups significantly improved Wingate relative peak power, and muscular strength for chest, back, and leg press. Only the CRE and CIN group improved total muscular endurance. No differences existed between groups post-supplementation. These findings demonstrate that three different methods of creatine ingestion lead to similar changes in anaerobic power, strength, and endurance [189].

Combination of creatine-dextrose versus protein-dextrose

Creatine supplementation during resistance exercise training has been reported to induce greater increases in fat-free mass (FFM), muscle fiber area, and strength when compared with a placebo. It was recently shown that timing of nutrient delivery in the postexercise period can have positive effects on whole body protein turnover [Roy BD et al., Med Sci Sports Exerc 2000; 32:1412-8]. It was tested the hypothesis that a postexercise protein-carbohydrate supplement would result in similar increases in FFM, muscle fiber area, and strength as compared with creatine monohydrate (CM), during a supervised 2-month resistance exercise training program in untrained men. Young healthy male subjects were randomized to receive either CM and glucose (n=11; CM 10 g + glucose 75 g [CR-CHO] (CELL-Tech)) or protein and glucose (n=8; casein 10 g + glucose 75 g [PRO+CHO]), using double-blinded allocation. Participants performed 8 weeks of whole body split-routine straight set weight training, 1 h/d, 6 d/week. Measurements, pre- and post-training were made of fat-free mass (FFM; DEXA), total body mass, muscle fiber area, isokinetic knee extension strength (45 and 240 degrees.s(-1)), and 1 repetition maximal (1RM) strength for 16 weight training exercises. Total body mass increased more for CR-CHO (+4.3 kg, 5.4 %) as compared with PRO-CHO (+1.9 kg, 2.4 %) and FFM increased after training but was not significantly different between the groups (CR-CHO = +4.0 kg, 6.4 %; PRO-CHO = +2.6 kg, 4.1 %). Muscle fiber area increased similarly after training for both groups (approximately 20 %). Training resulted in an increase in 1RM for each of the 16 activities (range 14.2-39.9 %), isokinetic knee extension torque, with no treatment effects upon any of the variables. It was concluded that postexercise supplementation with PRO-CHO resulted in similar increases in strength after a resistance exercise training program as compared with CR-CHO. However, the greater gains in total mass for the CR-CHO group may have implications for sport-specific performance [190].

Combination of creatine and whey protein

Studies that have attributed gains in lean body mass to dietary supplementation during resistance exercise (RE) training have not reported these changes alongside adaptations at the cellular and subcellular levels. Therefore, the purpose of one study was to examine the effects of two popular supplements – whey protein (WP) and creatine monohydrate (CrM) (both separately and in combination) – on body composition, muscle strength, fiber-specific hypertrophy (i.e. type I, IIA, IIX), and contractile protein accrual during RE training. In a double-blind randomized protocol, resistance-trained males were matched for strength and placed into one of four groups: creatine/carbohydrate (CrCHO), creatine/whey protein (CrWP), WP only, or carbohydrate only (CHO) (1.5 g/kg body weight per day). All assessments were completed the week before and after an 11-week structured, supervised RE program. Assessments included strength (1RM, three exercises), body composition (DEXA), and vastus lateralis muscle biopsies for determination of muscle fiber type (I, IIA, IIX), cross-sectional area (CSA), contractile protein, and creatine (Cr) content.
Supplementation with CrCHO, WP, and CrWP resulted in significantly greater 1RM strength improvements (three of three assessments) and muscle hypertrophy compared with CHO. Up to 76 percent of the strength improvements in the squat could be attributed to hypertrophy of muscle involved in this exercise. However, the hypertrophy responses within these groups varied at the three levels assessed (i.e. changes in lean mass, fiber-specific hypertrophy, and contractile protein content). Although WP and/or CrM seem to promote greater strength gains and muscle morphology during RE training, the hypertrophy responses within the groups varied. These differences in skeletal muscle morphology may have important implications for various populations and, therefore, warrant further investigation [191].

The purpose of one study was to assess muscular adaptations during 6 weeks of resistance training in 36 males randomly assigned to supplementation with whey protein (W; 1.2 g/kg/day), whey protein and creatine monohydrate (WC; 0.1 g/kg/day), or placebo (P; 1.2 g/kg/day maltodextrin). Measures included lean tissue mass by dual energy x-ray absorptiometry, bench press and squat strength (1-repetition maximum), and knee extension/flexion peak torque. Lean tissue mass increased to a greater extent with training in WC compared to the other groups, and in the W compared to the P group. Bench press strength increased to a greater extent for WC compared to W and P. Knee extension peak torque increased with training for WC and W, but not for P. All other measures increased to a similar extent across groups. Continued training without supplementation for an additional 6 weeks resulted in maintenance of strength and lean tissue mass in all groups. Males that supplemented with whey protein while resistance training demonstrated greater improvement in knee extension peak torque and lean tissue mass than males engaged in training alone. Males that supplemented with a combination of whey protein and creatine had greater increases in lean tissue mass and bench press than those who supplemented with only whey protein or placebo. However, not all strength measures were improved with supplementation, since subjects who supplemented with creatine and/or whey protein had similar increases in squat strength and knee flexion peak torque compared to subjects who received placebo [192].

Combination of creatine and beta-alanine

The aim of one study was to investigate the effects of beta alanine and/or creatine supplementation on performance during repeated bouts of supramaximal exercise in sedentary men. Forty-four untrained healthy men (aged 20-22 years, weight: 68-72 kg, height: 174-178 cm) participated in the present study. After performing the Wingate Test (WAnT) for three times in the baseline exercise session, the subjects were assigned to one of four treatment groups randomly: 1) placebo (P; 10 g maltodextrose); 2) creatine (Cr; 5 g creatine plus 5 g maltodextrose); 3) beta-alanine (beta-ALA; 1.6 g beta alanine plus 8.4 g maltodextrose); and 4) beta-alanine plus creatine (beta-ALA+Cr; 1.6 g beta alanine plus 5 g creatine plus 3.4 g maltodextrose). Participants were given the supplements orally twice a day for 22 consecutive days, then four times a day for the following 6 days. After 28 days, the second exercise session was applied during which peak power (PP) and mean power (MP) were measured and fatigue index (FI) was calculated. PP and MP decreased and FI increased in all groups during exercise before and after the treatment. During the postsupplementation session PP2 and PP3 increased in creatine supplemented group (from 642.7 ± 148.6 to 825.1 ± 205.2 in PP2 and from 522.9 ± 117.5 to 683.0 ± 148.0 in PP3, respectively). However, MP increased in beta-ALA+Cr during the postsupplementation compared to presupplementation in all exercise sessions (from 586.2 ± 55.4 to 620.6 ± 49.6 in MP1, from 418.1 ± 37.2 to 478.3 ± 30.3 in MP2 and from 362.0 ± 41.3 to 399.1 ± 3 in MP3, respectively). FI did not change with beta alanine and beta alanine plus creatine supplementation during the postsupplementation exercise session. It was concluded that
beta-alanine and beta alanine plus creatine supplementations have strong performance enhancing effect by increasing mean power and delaying fatigue Index during the repeated WAnT [193].

**Combination of creatine and bicarbonate**

Creatine and sodium bicarbonate supplementation independently increase exercise performance, but it remains unclear whether combining these 2 supplements is more beneficial on exercise performance. The purpose of one study was to evaluate the impact of combining creatine monohydrate and sodium bicarbonate supplementation on exercise performance. Thirteen healthy, trained men (21) completed 3 conditions in a double-blinded, crossover fashion: (a) Placebo (Pl; 20 g maltodextrin), (b) Creatine (Cr; 20 g), and (c) Creatine plus sodium bicarbonate (Cr + Sb). Each condition consisted of supplementation for 2 days followed by a 3-week washout. Peak power, mean power, relative peak power, and bicarbonate concentrations were assessed during six 10-second repeated Wingate sprint tests on a cycle ergometer with a 60-second rest period between each sprint. Compared with Pl, relative peak power was significantly higher in Cr (4 %) and Cr + Sb (7 %). Relative peak power was significantly lower in sprints 4-6, compared with that in sprint 1, in both Pl and Cr. However, in Cr + Sb, sprint 6 was the only sprint significantly lower compared with sprint 1. Pre-Wingate bicarbonate concentrations were significantly higher in Cr + Sb (10 %), compared with in Pl and Cr, and mean concentrations remained higher after sprint 6, although not significantly. Combining creatine and sodium bicarbonate supplementation increased peak and mean power and had the greatest attenuation of decline in relative peak power over the 6 repeated sprints. These data suggest that combining these 2 supplements may be advantageous for athletes participating in high-intensity, intermittent exercise [194].

One study examined the effect of simultaneous supplementation of creatine and sodium bicarbonate on consecutive maximal swims. Sixteen competitive male and female swimmers completed, in a randomized order, 2 different treatments (placebo and a combination of creatine and sodium bicarbonate) with 30 days of washout period between treatments in a double-blind crossover procedure. Both treatments consisted of placebo or creatine supplementation (20 g per day) in 6 days. In the morning of the seventh day, there was placebo or sodium bicarbonate supplementation (0.3 g per kg body weight) during 2 hours before a warm-up for 2 maximal 100-m freestyle swims that were performed with a passive recovery of 10 minutes in between. The first swims were similar, but the increase in time of the second versus the first 100-m swimming time was 0.9 seconds less in the combination group than in placebo. Mean blood pH was higher in the combination group than in placebo after supplementation on the test day. Mean blood pH decreased similarly during the swims in both groups. Mean blood lactate increased during the swims, but there were no differences in peak blood lactate between the combination group and placebo. The data indicate that simultaneous supplementation of creatine and sodium bicarbonate enhances performance in consecutive maximal swims [195].

**Combination of creatine with ribose and glutamine**

The purpose of one study was to examine the effects of a combination of effervescent creatine, ribose, and glutamine on muscular strength (MS), muscular endurance (ME) and body composition (BC) in resistance-trained men. Subjects were 28 men (age 22) who had 2 or more years of resistance-training experience. A double blind, randomized trial was completed involving supplementation or placebo control and a progressive resistance-training program for 8 weeks. Dependent measures were assessed at baseline and after 8 weeks of resistance training. Both groups significantly improved MS and ME while the
supplement group significantly increased body weight and fat-free mass. Control decreased body fat and increased fat-free mass. This study demonstrated that the supplement group did not enhance MS, ME, or BC significantly more than control after an 8-week resistance-training program [196].

**Combination of creatin with magnesium**

It was tested the hypotheses that, compared with a placebo group or creatine (Cr) group, a Mg$^{2+}$-Cr chelate group would demonstrate improvements in the 1 repetition maximum (1RM) on the bench press and be able to perform more work at 70 percent of the 1RM for the bench press. Thirty-one weight-trained men were randomly assigned in a double-blind manner to a placebo group (multidextran), a Cr group (2.5 g of Cr daily), or a Mg$^{2+}$-Cr group (2.5 g of Cr daily). Baseline data were collected for the bench press 1RM and maximal work completed during a fatigue set at 70 percent of the 1RM. Following 10 days of Cr supplementation, follow-up tests were completed for the dependent variables. Groups were similar when the change in 1RM was evaluated either absolutely or relatively. Both the Cr and the Mg$^{2+}$-Cr groups had significantly larger increases in work, both absolutely and relatively, when compared with the placebo group. Partial support for the hypothesis suggests that low doses of Cr are effective at increasing fiber Cr content, and consequently, performance. Further, the Cr and Mg$^{2+}$-Cr groups were similar in both performance tests, suggesting that the proposed mechanism of entry is no better than the conventional method when 2.5 g of Cr is administered and performance is measured as work. This study raises the possibility that a low dose of Cr may be an effective means of enhancing performance after short-term ingestion [197].

**Combination of creatine with ribose and glutamine**

The purpose of one study was to examine the effects of a combination of effervescent creatine, ribose, and glutamine on muscular strength (MS), muscular endurance (ME) and body composition (BC) in resistance-trained men. Subjects were 28 men (age: 22) who had 2 or more years of resistance-training experience. A double blind, randomized trial was completed involving supplementation or placebo control and a progressive resistance-training program for 8 weeks. Dependent measures were assessed at baseline and after 8 weeks of resistance training. Both groups significantly improved MS and ME while the supplement group significantly increased body weight and fat-free mass. Control decreased body fat and increased fat-free mass. This study demonstrated that the supplement group did not enhance MS, ME, or BC significantly more than control after an 8-week resistance-training program [196].

**Combination of creatine with betaine**

It was aimed to investigate the role of betaine supplementation on muscle phosphoryl-creatine (PCr) content and strength performance in untrained subjects. Additionally, it was compared the ergogenic and physiological responses to betaine versus creatine supplementation. Finally, it was also tested the possible additive effects of creatine and betaine supplementation. This was a double-blind, randomized, placebo-controlled study. Subjects were assigned to receive betaine (BET; 2 g/day), creatine (CR; 20 g/day), betaine plus creatine (BET+CR; 2+20 g/day, respectively) or placebo (PL). At baseline and after 10 days of supplementation, it was assessed muscle strength and power, muscle PCr content, and body composition. The CR and BET+CR groups presented significantly greater increase in muscle PCr content than PL. PCr content was comparable between BET versus PL and
CR versus BET+CR. CR and BET+CR presented greater muscle power output than PL in the squat exercise following supplementation. Similarly, bench press average power was significantly greater for the CR-supplemented groups. CR and BET+CR groups also showed significant pre- to post-test increase in 1-RM squat and bench press. No significant differences for 1-RM strength and power were observed between BET versus PL and CR versus BET+CR. Body composition did not differ between the groups. In conclusion, it was reported that betaine supplementation does not augment muscle PCR content. Furthermore, it was shown that betaine supplementation combined or not with creatine supplementation does not affect strength and power performance in untrained subjects [198].

Combination of creatine and D-pinitol

Coingestion of D-pinitol with creatine (CR) has been reported to enhance creatine uptake. The purpose of one study was to evaluate whether adding D-pinitol to CR affects training adaptations, body composition, whole-body creatine retention, and/or blood safety markers when compared to CR ingestion alone after 4 weeks of resistance training. Twenty-four resistance trained males were randomly assigned in a double-blind manner to creatine + pinitol (CRP) or creatine monohydrate (CR) prior to beginning a supervised 4-week resistance training program. Subjects ingested a typical loading phase (i.e. 20 g/d-1 for 5 days) before ingesting 5 g/d-1 the remaining 23 days. Performance measures were assessed at baseline (T0), week 1 (T1), and week 4 (T2) and included 1 repetition maximum (1RM) bench press (BP), 1RM leg press (LP), isokinetic knee extension, and a 30-second Wingate anaerobic capacity test. Fasting blood and body composition using dual-energy x-ray absorptiometry (DEXA) were determined at T1 and T3. Data were analyzed by repeated measures analysis of variance (ANOVA). Creatine retention significantly increased in both groups as a result of supplementation but was not different between groups. Significant improvements in upper- and lower-body strength and body composition occurred in both groups. However, significantly greater increases in lean mass and fat-free mass occurred in the CR group when compared to CRP. Thus, adding D-pinitol to creatine monohydrate does not appear to facilitate further physiological adaptations while resistance training. Creatine monohydrate supplementation helps to improve strength and body composition while resistance training [199].

Combination of creatine and conjugated linoleic acid

Aging is associated with lower muscle mass and an increase in body fat. It was examined whether creatine monohydrate (CrM) and conjugated linoleic acid (CLA) could enhance strength gains and improve body composition (i.e. increase fat-free mass (FFM); decrease body fat) following resistance exercise training in older adults (>65 y). Men (n=19) and women (n=20) completed six months of resistance exercise training with CrM (5g/d)+CLA (6g/d) or placebo with randomized, double blind, allocation. Outcomes included: strength and muscular endurance, functional tasks, body composition (DEXA scan), blood tests (lipids, liver function, CK, glucose, systemic inflammation markers (IL-6, C-reactive protein)), urinary markers of compliance (creatinine/creatinine), oxidative stress (8-OH-2dG, 8-isoP) and bone resorption (Nu-telopeptides). Exercise training improved all measurements of functional capacity and strength, with greater improvement for the CrM+CLA group in most measurements of muscular endurance, isokinetic knee extension strength, FFM, and lower fat mass. Plasma creatinine, but not creatinine clearance, increased for CrM+CLA, with no changes in serum CK activity or liver function tests. Together, this data confirms that supervised resistance exercise training is safe and effective for increasing strength in older adults and that a combination of CrM and CLA can enhance some of the beneficial effects of training over a six-month period [200].
Combination of creatine and Russian tarragon

Extracts of Russian Tarragon (RT) have been reported to produce anti-hyperglycemic effects and influence plasma creatine (Cr) levels while supplementing with creatine monohydrate (CrM). The purpose of this preliminary study was to determine if short-term, low-dose aqueous RT extract ingestion prior to CrM supplementation influences whole body Cr retention, muscle Cr or measures of anaerobic sprint performance. In a double-blind, randomized, and crossover manner; 10 recreationally trained males (20 ± 2 years) ingested 500 mg of aqueous RT extract (Finzelberg, Andernach, Germany) or 500 mg placebo 30-minutes prior to ingesting 5 g of CrM (Creapure®, AlzChem AG, Germany) twice per day for 5-days then repeated after a 6-week wash-out period. Urine was collected at baseline and during each of the 5-days of supplementation to determine urine Cr content. Whole body Cr retention was estimated from urine samples. Muscle biopsies were obtained for determination of muscle free Cr content. Participants also performed two 30-second Wingate anaerobic capacity tests prior to and following supplementation for determination of peak power (PP), mean power (MP), and total work (TW). Whole body daily Cr retention increased in both groups following supplementation with no differences observed between groups. After 3 and 5-days of supplementation, respectively, both supplementation protocols demonstrated a significant increase in muscle free Cr content from baseline with no significant differences observed between groups. Absolute change in MP, percent change in MP, absolute change in TW, and percent change in TW increased over time in both groups with no differences observed between groups. Short-term CrM supplementation (10 g/d for 5-days) significantly increased whole body Cr retention and muscle free Cr content. However, ingesting 500 mg of RT 30-min prior to CrM supplementation did not affect whole body Cr retention, muscle free Cr content, or anaerobic sprint capacity in comparison to ingesting CrM with a placebo [201].
Cognitive effects of creatine

The effect of creatine supplementation and sleep deprivation, with intermittent moderate-intensity exercise, on cognitive and psychomotor performance, mood state, effort and salivary concentrations of cortisol and melatonin were examined. Subjects were divided into a creatine supplementation group and a placebo group. They took 5 g of creatine monohydrate or a placebo, dependent on their group, four times a day for 7 days immediately prior to the experiment. They undertook tests examining central executive functioning, short-term memory, choice reaction time, balance, mood state and effort at baseline and following 18-, 24- and 36-h sleep deprivation, with moderate intermittent exercise. Saliva samples were taken prior to each set of tests. A group x time analysis of covariance, with baseline performance the covariate, showed that the creatine group performed significantly better than the placebo group on the central executive task but only at 36 h. The creatine group demonstrated a significant linear improvement in performance of the central executive task throughout the experiment, while the placebo group showed no significant effects. There were no significant differences between the groups for any of the other variables. A significant main effect of time was found for the balance test with a linear improvement being registered. Cortisol concentrations on Day 1 were significantly higher than on Day 2. Mood significantly deteriorated up to 24 h with no change from 24 to 36 h. Effort at baseline was significantly lower than in the other conditions. It was concluded that, during sleep deprivation with moderate-intensity exercise, creatine supplementation only affects performance of complex central executive tasks [202].

Systemic creatine (Cr) supplementation increases brain phosphocreatine (PCr) and prevents hypoxic seizures in 15-day-old rabbits. Between 5 and 30 days of age during normal development, rabbit gray matter mitochondrial creatine kinase increases 400 percent while cytosolic CK increases 60 percent. In white matter, both isoenzymes show smaller, similar increases (40 %) during this period. The Cr transporter protein decreases 60 percent between 5 and 15 days in both regions. In vivo CK rate constants measured by $^{31}$P nuclear magnetic resonance increase 30 percent between 10 and 20 days, and then fall 50 percent between 20 and 30 days in predominantly gray matter slices. Similar maturational changes are seen in predominantly white matter slices. Injecting Cr at 15 days does not significantly change brain cytosolic creatine kinase or mitochondrial creatine kinase isoenzymes or the in vivo CK reaction rate constants. Thus, the largest change in the CK system associated with suppression of hypoxic seizures in Cr-treated rabbits is increased PCr in gray and white matter [203].

In one study the authors described an Italian child with guanidinoacetate methyltransferase deficiency, neurologic regression, movement disorders, and epilepsy during the first year of life. Brain MRI showed pallidal and periaqueductal alterations. In vivo 1H-MRS showed brain creatine depletion. The assessment of guanidinoacetic acid concentration in biologic fluids confirmed the diagnosis. Clinical, biochemical, and neuroradiologic improvement followed creatine supplementation [204].

Effect of creatine after sleep deprivation

The effect of creatine supplementation and sleep deprivation, with intermittent moderate-intensity exercise, on cognitive and psychomotor performance, mood state, effort and salivary concentrations of cortisol and melatonin were examined. Subjects were divided into a creatine supplementation group and a placebo group. They took 5 g of creatine monohydrate or a placebo, dependent on their group, four times a day for 7 days
immediately prior to the experiment. They undertook tests examining central executive functioning, short-term memory, choice reaction time, balance, mood state and effort at baseline and following 18-, 24- and 36-h sleep deprivation, with moderate intermittent exercise. Saliva samples were taken prior to each set of tests. A group x time analysis of covariance, with baseline performance the covariate, showed that the creatine group performed significantly better than the placebo group on the central executive task but only at 36 h. The creatine group demonstrated a significant linear improvement in performance of the central executive task throughout the experiment, while the placebo group showed no significant effects. There were no significant differences between the groups for any of the other variables. A significant main effect of time was found for the balance test with a linear improvement being registered. Cortisol concentrations on Day 1 were significantly higher than on Day 2. Mood significantly deteriorated up to 24 h with no change from 24 to 36 h. Effort at baseline was significantly lower than in the other conditions. It was concluded that, during sleep deprivation with moderate-intensity exercise, creatine supplementation only affects performance of complex central executive tasks [205].

**Effects on inflammatory markers**

The goal of one study was to evaluate the effects of creatine (Cr) supplementation on oxidative stress and inflammation markers after acute repeated-sprint exercise in humans. Twenty-five players under age 20 years were randomly assigned to two groups: Cr supplemented and placebo. Double-blind controlled supplementation was performed using Cr (0.3 g/kg) or placebo tablets for 7 d. Before and after 7 d of supplementation, the athletes performed two consecutive Running-based Anaerobic Sprint Tests (RAST). RAST consisted of six 35-m sprint runs at maximum speed with 10 sec rest between them. Blood samples were collected just prior to start of test (pre), just after the completion (0 h), and 1 h after completion. Average, maximum, and minimum power values were greater in the Cr-supplemented group compared with placebo. There were significant increases in plasma tumor necrosis factor alpha (TNF-alpha) and C-reactive protein (CRP) up to 1 h after acute sprint exercise in the placebo-supplemented group. Malondialdehyde, lactate dehydrogenase (LDH), catalase, and superoxide dismutase enzymes also were increased after exercise in both groups. Red blood cell glutathione was lower after exercise in both groups. Cr supplementation reversed the increase in TNF-alpha and CRP as well as LDH induced by acute exercise. Controversially, Cr supplementation did not inhibit the rise in oxidative stress markers. Also, antioxidant enzyme activity was not different between placebo and Cr-supplemented groups. It was concluded that creatine supplementation inhibited the increase of inflammation markers TNF-alpha and CRP, but not oxidative stress markers, due to acute exercise [206].

**Effect of creatine on plasma levels of pro-inflammatory cytokines**

The effect of creatine supplementation upon plasma levels of pro-inflammatory cytokines: interleukin (IL) 1 beta and IL-6, tumor necrosis factor alpha (TNFalpha), and Interferon alpha (INF alpha) and prostaglandin E2 (PGE2) after a half-ironman competition were investigated. Eleven triathletes, each with at least three years experience of participation in this sport were randomly divided between the control and experimental groups. During 5 days prior to competition, the control group (n=6) was supplemented with carbohydrate (20 g/day) whereas the experimental group (n=5) received creatine (20 g/day) in a double-blind trial. Blood samples were collected 48 h before and 24 and 48 h after competition and were used for the measurement of cytokines and PGE2. Forty-eight hours prior to competition there was no difference between groups in the plasma concentrations (pg/mL) of IL-6, TNFalpha, INF alpha, IL-1 beta, and PGE2. Twenty-four and 48 h after competition plasma levels of TNFalpha, INF alpha, IL-1 beta and PGE2 were significantly increased in both groups.
However, the increases in these were markedly reduced following creatine supplementation. An increase in plasma IL-6 was observed only after 24 h and, in this case, there was no difference between the two groups. It was concluded that creatine supplementation before a long distance triathlon competition may reduce the inflammatory response induced by this form of strenuous exercise [207].

**Effects of creatine on oxidative stress and inflammation markers after sprint**

The goal of one study was to evaluate the effects of creatine (Cr) supplementation on oxidative stress and inflammation markers after acute repeated-sprint exercise in humans. Twenty-five players under age 20 years were randomly assigned to two groups: Cr supplemented and placebo. Double-blind controlled supplementation was performed using Cr (0.3 g/kg) or placebo tablets for 7 d. Before and after 7 d of supplementation, the athletes performed two consecutive Running-based Anaerobic Sprint Tests (RAST). RAST consisted of six 35-m sprint runs at maximum speed with 10 sec rest between them. Blood samples were collected just prior to start of test (pre), just after the completion (0 h), and 1 h after completion. Average, maximum, and minimum power values were significantly greater in the Cr-supplemented group compared with placebo. There were significant increases in plasma tumor necrosis factor alpha (TNF-alpha) and C-reactive protein (CRP) up to 1 h after acute sprint exercise in the placebo-supplemented group. Malondialdehyde, lactate dehydrogenase (LDH), catalase, and superoxide dismutase enzymes also were increased after exercise in both groups. Red blood cell glutathione was lower after exercise in both groups. Cr supplementation reversed the increase in TNF-alpha and CRP as well as LDH induced by acute exercise. Controversially, Cr supplementation did not inhibit the rise in oxidative stress markers. Also, antioxidant enzyme activity was not different between placebo and Cr-supplemented groups. Thus, creatinin supplementation inhibited the increase of inflammation markers TNF-alpha and CRP, but not oxidative stress markers, due to acute exercise [208].

**Experimentally**

*Effect on inflammatory parameters*

Thirty-six male rats were used; divided into 6 groups (n=6): saline; creatine (Cr); eccentric exercise (EE) plus saline 24 h; eccentric exercise plus Cr 24 h; eccentric exercise plus Cr 48 h; and eccentric exercise plus Cr 48 h. Cr supplementation was administered as a solution of 300 mg/kg body weight/day in 1 mL water, for two weeks, before the eccentric exercise. The animals were submitted to one downhill run session at 1.0 km/h until exhaustion. Twenty-four and forty-eight hours after the exercise, the animals were killed, and the quadriceps were removed. Creatine kinase levels, superoxide production, thiobarbituric acid reactive substances (TBARS) level, carbonyl content, total thiol content, superoxide dismutase, catalase, glutathione peroxidase, interleukin-1beta (IL-1beta), nuclear factor kappa B (NF-kb), and tumour necrosis factor (TNF) were analysed. Cr supplementation neither decreases Cr kinase, superoxide production, lipoperoxidation, carbonylation, total thiol, IL-1beta, NF-kb, or TNF nor alters the enzyme activity of superoxide dismutase, catalase, and glutathione peroxides in relation to the saline group, respectively. There are positive correlations between Cr kinase and TBARS and TNF-alpha 48 hours after eccentric exercise. The present study suggests that Cr supplementation does not decrease oxidative stress and inflammation after eccentric contraction [209].

**Neuroprotective effect of creatine**

Impairment or interruption of oxygen supply compromises brain function and plays a role in neurological and neurodegenerative conditions. Creatine is a naturally occurring compound
involved in the buffering, transport, and regulation of cellular energy, with the potential to replenish cellular adenosine triphosphate without oxygen. Creatine is also neuroprotective in vitro against anoxic/hypoxic damage. Dietary creatine supplementation has been associated with improved symptoms in neurological disorders defined by impaired neural energy provision. Here we investigate, for the first time in humans, the utility of creatine as a dietary supplement to protect against energetic insult. The aim of this study was to assess the influence of oral creatine supplementation on the neurophysiological and neuropsychological function of healthy young adults during acute oxygen deprivation. Fifteen healthy adults were supplemented with creatine and placebo treatments for 7 d, which increased brain creatine on average by 9.2 percent. A hypoxic gas mixture (10 % oxygen) was administered for 90 min, causing global oxygen deficit and impairing a range of neuropsychological processes. Hypoxia-induced decrements in cognitive performance, specifically attentional capacity, were restored when participants were creatine supplemented, and corticomotor excitability increased. A neuromodulatory effect of creatine via increased energy availability is presumed to be a contributing factor of the restoration, perhaps by supporting the maintenance of appropriate neuronal membrane potentials. Dietary creatine monohydrate supplementation augments neural creatine, increases corticomotor excitability, and prevents the decline in attention that occurs during severe oxygen deficit. This is the first demonstration of creatine's utility as a neuroprotective supplement when cellular energy provision is compromised [210].

**Acute creatine loading increases fat-free mass**

Creatine monohydrate (CrM) administration may enhance high intensity exercise performance and increase body mass, yet few studies have examined for potential adverse effects, and no studies have directly considered potential gender differences. The purpose of one study was to examine the effect of acute creatine supplementation upon total and lean mass and to determine potential side effects in both men and women. The effect of acute CrM (20 g/d x 5 d) administration upon systolic, diastolic, and mean BP, plasma creatinine, plasma CK activity, and body composition was examined in 15 men and 15 women in a randomized, double-blind experiment. Additionally, ischemic isometric handgrip strength was measured before and after CrM or placebo (PL). CrM did not affect blood pressure, plasma creatinine, estimated creatinine clearance, plasma CK activity, or handgrip strength. In contrast, CrM significantly increased fat-free mass (FFM) and total body mass as compared with PL, with no changes in body fat. The observed mass changes were greater for men versus women. These findings suggest that acute CrM administration does not affect blood pressure, renal function, or plasma CK activity, but increases FFM. The effect of CrM upon FFM may be greater in men as compared with that in women [211].

**Acute creatine loading enhances human growth hormone secretion**

The main objective of one study was to explore the effect of acute creatine (Cr) ingestion on the secretion of human growth hormone (GH). In a comparative cross-sectional study, 6 healthy male subjects ingested in resting conditions a single dose of 20 g creatine (Cr-test) versus a control (c-test). During 6 hours the Cr, creatinine and GH concentrations in blood serum were measured after Cr ingestion (Cr-test). During the Cr-test, all subjects showed a significant stimulation of GH, but with a large interindividual variability in the GH response: the difference between Cr-test and c-test averaged 83 percent. For the majority of subjects the maximum GH concentration occurred between 2 hrs and 6 hrs after the acute Cr ingestion. In resting conditions and at high dosages Cr enhances GH secretion, mimicking the response of strong exercise which also stimulates GH secretion. Acute body weight gain and strength increase observed after Cr supplementation should consider the indirect anabolic property of Cr [212].
Creatine decreases plasma markers of adenine nucleotide degradation

It was investigated the effect of oral creatine supplementation (20 g/d for 7 days) on metabolism during a 1-h cycling performance trial. Twenty endurance-trained cyclists participated in this double-blind placebo controlled study. Five days after familiarization with the exercise test, the subjects underwent a baseline muscle biopsy. Thereafter, a cannula was inserted into a forearm vein before performing the baseline maximal 1-h cycle (test 1 (T1)). Blood samples were drawn at regular intervals during exercise and recovery. After creatine (Cr) loading, the muscle biopsy, 1-h cycling test (test 2 (T2)) and blood sampling were repeated. Resting muscle total creatine (TCr), measured by high performance liquid chromatography, was increased in the creatine group but was unchanged in the placebo group. The extent of Cr loading was unrelated to baseline Cr levels. Supplementation did not significantly improve exercise performance or change plasma lactate concentrations. Plasma concentrations of ammonia and hypoxanthine were lower in the Cr group from T1 to T2. The results indicate that Cr supplementation alters the metabolic response during sustained high-intensity submaximal exercise. Plasma data suggest that nett intramuscular adenine nucleotide degradation may be decreased in the presence of enhanced intramuscular TCr concentration even during submaximal exercise [213].

Acute creatine supplementation in older men

The hypothesis of this study was that short term creatine (Cr) ingestion in older individuals would increase body mass and exercise performance, as has been shown in younger subjects. Seventeen males 60-78 years old were randomly placed into two groups, Cr and placebo (P), and supplemented in double-blind fashion for 5 days. Subjects ingested either 5 g of Cr plus 1 g of sucrose 4x per day or 6 g of a sucrose placebo 4x per day. Isometric strength of the elbow flexors was assessed using a modified preacher bench attached to a strain gauge. Isokinetic exercise performance was assessed using an intermittent fatigue test of the knee extensors. Subjects performed 3 sets of 30 repetitions with 60 sec rest between sets. There was a small (0.5 kg) but statistically significant increase in body mass in the Cr group after supplementation. There was a significant overall interaction between groups in isokinetic performance from pre to post supplementation. However, analysis of the groups separately revealed that the subjects in the Cr group demonstrated a small non-significant increase in isokinetic performance while subjects in the P group demonstrated a small non-significant performance decrement. There was no significant difference in isometric strength between groups from pre to post supplementation. These data suggest that acute oral Cr supplementation does not increase isometric strength and only produces small increases in isokinetic performance and body mass in men over the age of 60 [214].

Effect of pre-exercise creatine ingestion on performance in healthy aging males

Pre-exercise creatine supplementation may have a beneficial effect on aging muscle performance. Using a double-blind, repeated measures, cross-over design, healthy males (n=9) were randomized to consume creatine (20 grams) and placebo (20 grams corn-starch maltodextrin), on 2 separate occasions (7 days apart), 3 hours prior to performing leg press and chest press repetitions to muscle fatigue (3 sets at 70 % 1-repetition maximum; 1 minute rest between sets). There was a set main effect for the leg press and chest press with the number of repetitions performed decreasing similarly for creatine and placebo. These results suggest that a bolus ingestion of creatine consumed 3 hours prior to resistance exercise has no effect on upper or lower body muscle performance in healthy aging males [215].
Positive effects in hot environments

Recent reports now suggest that creatine may enhance performance in hot and or humid conditions by maintaining hematocrit, aiding thermoregulation and reducing exercising heart rate and sweat rate. Creatine may also positively influence plasma volume during the onset of dehydration [013].

Considering these new published findings, little evidence exists that creatine supplementation in the heat presents additional risk and should be taken into consideration as position statements and other related documents are published [013].

Physiological responses to short-term exercise in the heat after creatine loading

One investigation was designed to examine the influence of creatine (Cr) supplementation on acute cardiovascular, renal, temperature, and fluid-regulatory hormonal responses to exercise for 35 min in the heat. Twenty healthy men were matched and then randomly assigned to consume 0.3 g/kg Cr monohydrate (n=10) or placebo (n=10) for 7 d in a double-blind fashion. Before and after supplementation, both groups cycled for 30 min at 60-70 percent VO$_{2\text{peak}}$ immediately followed by three 10-s sprints in an environmental chamber at 37 degrees C and 80 percent relative humidity. Body mass was significantly increased (0.75 kg) in Cr subjects. Heart rate, blood pressure, and sweat rate responses to exercise were not significantly different between groups. There were no differences in rectal temperature responses in either group. Sodium, potassium, and creatinine excretion rates obtained from 24-h and exercise urine collection periods were not significantly altered in either group. Serum creatinine was elevated in the Cr group but within normal ranges. There were significant exercise-induced increases in cortisol, aldosterone, renin, angiotensin I and II, atrial peptide, and arginine vasopressin. The aldosterone response was slightly greater in the Cr (263%) compared with placebo (224 %) group. Peak power was greater in the Cr group during all three 10-s sprints after supplementation and unchanged in the placebo group. There were no reports of adverse symptoms, including muscle cramping during supplementation or exercise. It was concluded that Cr supplementation augments repeated sprint cycle performance in the heat without altering thermoregulatory responses [216].

No effect on thermoregulation and isokinetic muscular performance

The purpose of this investigation was to determine the effects of 3 d of creatine supplementation on thermoregulation and isokinetic muscular performance. Fourteen males performed two exercise bouts following 3 day of creatine supplementation and placebo. Subjects exercised for 60 min at 60-65 percent of VO$_{2\text{max}}$ in the heat followed by isokinetic muscular performance at 60, 180, and 300 o/s. Dependent variables for pre- and post-exercise included nude body weight, urine specific gravity, and serum creatinine levels. Total body water, extracellular water and intracellular water were measured pre-exercise. Core temperature was assessed every 5 min during exercise. Peak torque and fatigue index were used to assess isokinetic muscular performance. Core temperature increased during the run for both conditions. Total body water and extracellular water were significantly greater following creatine supplementation. No significant difference was found between conditions for intracellular water, nude body weight, urine specific gravity, and serum creatinine. Pre-exercise scores for urine specific gravity and serum creatinine were significantly less versus post-exercise. No significant differences were found in peak torque values or fatigue index between conditions for each velocity. A significant overall velocity effect was found for both flexion and extension. As velocity increased, mean peak torque values decreased. It was concluded that 3 days of creatine supplementation does not affect thermoregulation during
submaximal exercise in the heat and is not enough to elicit an ergogenic effect for isokinetic muscle performance following endurance activity [217].

Role of creatine supplementation in exercise-induced muscle damage

Muscle damage is induced by both high-intensity resistance and endurance exercise. Creatine is a widely used dietary supplement to improve exercise performance by reducing exercise-induced muscle damage. Many researchers have suggested that taking creatine reduces muscle damage by decreasing the inflammatory response and oxidative stress, regulating calcium homeostasis, and activating satellite cells. However, the underlying mechanisms of creatine and muscle damage have not been clarified. Therefore, one review discusses the regulatory effects of creatine on muscle damage by compiling the information collected from basic science and sports science research. An initial study in 2000 verified the effect of creatine on exercise-induced muscle damage. In that study mice performed 150 eccentric muscle contractions in response to electric stimulation after ingesting 0.5 or 1 percent creatine for 14 days. However, creatine did not affect isometric strength after eccentric muscle contractions. Several studies have reported that creatine attenuates exercise-induced muscle damage. It was shown that healthy males ingesting creatine (loading period: 0.3 g/kg/day, four servings/day, 5 days; maintenance period: 0.1 g/kg/day, one serving/day, 14 days) beginning 5 days before exercise until 14 days after exercise improved maximal isometric strength and decreased CK compared with those who consumed a carbohydrate placebo only. It was also reported that ingesting 20 g/day creatine over 5 days decreases CK and LDH after a triathlon competition [218].

It was in 2009 reported the acute (20 g/day, 7 days) and chronic (6 g/day, after 7 days followed for 23 days) effects of creatine on exercise-induced muscle damage. One study demonstrated that chronic ingestion of creatine effectively increased maximal isometric strength after resistance exercise. It was suggested that taking 20 g/day creatine for 6 days between the first and the second exercise phase contributed to decreased muscle soreness, inhibited the elevation in CK, and enhanced range of motion. Two studies, suggested that taking creatine may increase the “repeated bout effect” after initial exercise-induced muscle damage. The repeated bout effect is protective against subsequent muscle damage through neural, mechanical, and cellular adaptations after exercise. However, several studies suggested that creatine had no benefit on exercise-induced muscle damage. It was demonstrated that creatine (20 g/day) taken for 5 days before and after exercise does not change the levels of muscle damage markers after exercise between subjects taking creatine and a placebo. Moreover, it was reported that creatine (loading period: 0.3 g/kg/day, three servings/day, 5 days; maintenance period: 0.03 g/kg/day, one serving/day, 5 days) does not change muscle damage marker levels after exercise. Similarly, it was reported that taking creatine (loading dose: 40 g, two servings/day, 5 days; maintenance period: 10 g, two servings/day, 5 days) had no effect on exercise-induced muscle damage. These conflicting results may be partly explained by differences in exercise protocols used in the studies [218].

Potential mechanisms of creatine on exercise-induced muscle damage

A number of potential mechanisms explain the effect of creatine on exercise-induced muscle damage, including the inflammatory response, oxidative stress, calcium homeostasis, and satellite cells activities in damaged muscle. The first potential mechanism of creatine is that it reduces the inflammatory response after exercise-induced muscle damage. It was in 2004 demonstrated that 20 g/day creatine for 5 days before 34 male marathon runners raced significant reduced LDH, prostaglandin E₂ (PGE₂), and tumor necrosis factor-alpha (TNF-alpha) after the 30 km race. These results agree with those of several studies. It was also reported that 11 male triathletes who ingested 20 g/day creatine for 5 days prior to a half-
Ironman competition had significant decreases in TNF-alpha, interferon-alpha (INF-alpha), interleukin-1beta (IL-1beta), and PGE₂ after the competition compared to those in the placebo group. It was also reported that ingesting 0.3 g/kg creatine for 7 days abolishes the increase in TNF-alpha after a repeated running-based anaerobic test. PGE₂ and TNF-alpha facilitate the inflammatory response and pain sensation after exercise-induced muscle damage [218].

Interestingly, all three studies showed a decrease in the inflammatory response. It was particularly shown a decrease in LDH and inflammation. The inflammatory response is associated with markers of sarcolemma damage and was reported a positive correlation between neutrophil migratory activity and myoglobin after exercise. These results indicate that the reduction of inflammatory response factors by creatine may decrease disruption of sarcolemma due to exercise-induced muscle damage. In addition, there was a report that taking creatine (5 g/kg/day, 5 days) significant decreases outflux of intracellular enzymes after continuous muscle contraction. In contrast, it was found that ingesting creatine (300 mg/kg/day, 15 days) does not significantly reduce inflammatory response markers, such as TNF-alpha, IL-1beta, and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kappaB) in mice [218].

The second potential creatine mechanism is diminished oxidative stress. It was in 2002 reported the first evidence for the antioxidant capacity of creatine. It was found that ingesting 2 percent creatine during the 28 days before acute exercise decreases thiobarbituric acid-reactive substances (TBARS) and lipid hydroperoxides but increases the glutathione (GSH) and glutathione disulfide (GSSG) ratio and total antioxidant capacity. However, these studies were limited to cultured cells models, and animals. According to a human study, taking 20 g/day creatine for 7 days decreases malonyldialdehyde (MDA) and 8-hydroxy-2-deoxyguanosine (8-OHdG) levels after resistance exercise compared to those taking a placebo. In contrast, several studies have reported that creatine does not decrease oxidative stress after exercise-induced muscle damage. Therefore, this mechanism remains unclear [218].

Another creatine mechanism is regulation of calcium homeostasis. Impaired sarcoplasmic reticulum due to muscle damage may increase calcium concentrations in the cytosol, causing secondary muscle damage. Creatine assists in maintaining the sarcoplasmic reticulum calcium pump function by phosphorylating ADP to ATP, which decreases cytosolic calcium levels. It was suggested that increasing muscle PCr accelerates ATP homeostasis, leading to reduced secondary damage due to an increase in calcium concentration. However, this hypothesis also needs further research [218].

Finally, creatine has been associated with satellite cells or so-called “muscle stem cells.” Satellite cells play a critical regenerating role after muscle damage. It was in 2006 demonstrated that ingesting creatine (loading period: 24 g/day, 6 g/serving, four servings/day, 7 days; maintenance period: 6 g/day, one serving/day, 15 weeks) and performing resistance exercise increases the number of satellite cells and myonuclei concentration in human muscle. In addition, it was reported that taking creatine (loading period: 20 g/day, 10 g/serving, two servings/day, 3 days; maintenance period: 5 g/day, one serving/day, 7 days) promotes proliferation and differentiation of satellite cells and activate cytoskeletal remodeling genes [218].

**Protein- and carbohydrate-induced augmentation of creatine retention**

One study investigated the effect of creatine supplementation in conjunction with protein and/or carbohydrate (CHO) ingestion on plasma creatine and serum insulin concentrations
and whole body creatine retention. Twelve men consumed 4 x 5 g of creatine on four occasions in combination with 1) 5 g of CHO, 2) 50 g of protein and 47 g of CHO, 3) 96 g of CHO, or 4) 50 g of CHO. The increase in serum insulin was no different when the protein-CHO and high-CHO treatments were compared, but both were greater than the response recorded for the low-CHO treatment. As a consequence, body creatine retention was augmented by approximately 25 percent for protein-CHO and high-CHO treatments compared with placebo treatment. The areas under creatine- and insulin-time curves were related during the first oral challenge but not after the fourth. It is concluded, first, that the ingestion of creatine in conjunction with approximately 50 g of protein and CHO is as effective at potentiating insulin release and creatine retention as ingesting creatine in combination with almost 100 g of CHO. Second, the stimulatory effect of insulin on creatine disposal was diminished within the initial 24 h of supplementation [219].

No effect of resistance training and creatine supplementation on blood lipids

In order to examine the effects of heavy resistance training and the influence of creatine supplementation on nonperformance measures of health status, 19 healthy resistance-trained men were matched and then randomly assigned in a double-blind fashion to either a creatine (n=10) or placebo (n=9) group. Periodized heavy resistance training was performed 3-4 times per week for 12 weeks. During the first week of training, creatine subjects consumed 25 g creatine monohydrate per day, while the placebo group ingested an equal number of placebo capsules. Five grams of supplement per day was consumed for the remainder of the study. Body composition, fasting serum creatinine, lipoproteins and triglycerides, and reported changes in body function were determined prior to and after 12 weeks of training and supplementation. After training, significant increases in body mass and fat-free mass were greater in creatine (5.2 and 4.3 kg, respectively) than placebo (3.0 and 2.1 kg, respectively) subjects. There was no change in percent body fat. Dietary energy and macronutrient distribution was not significantly different during Weeks 1 and 12. Serum creatinine was significantly elevated in creatine subjects after 1 (11.6 %) and 12 weeks (13.8 %); however, values were within normal limits for healthy men. There were no effects of training or supplementation on serum total cholesterol, HDL-cholesterol, LDL-cholesterol, or triglycerides. In healthy men, a 12-week heavy resistance training program, with or without creatine supplementation, did not significantly influence serum lipid profiles, subjective reports of body functioning, or serum creatinine concentrations [220].

Decreased range of motion

During high-intensity exercise, intracellular creatine phosphate (PCr) is rapidly broken down to maintain adenosine triphosphate turnover. This has lead to the widespread use of creatine monohydrate as a nutritional ergogenic aid. However, the increase in intracellular PCr and the concomitant increase in intracellular water have not been investigated with regard to their effect on active range of movement (ROM). Forty male subjects (age, 24+/−3.2 years) underwent restricted randomization into 2 equal groups, either an intervention group (CS) or a control group (C). The CS group ingested 25 g/day of creatine monohydrate for 5 days, followed by 5 g/day for a further 3 days. Before (24 h before starting supplementation (PRE) and after (on the 8th day of supplementation (POST)) this loading phase, both groups underwent goniometry measurement of the shoulder, elbow, hip, and ankle. Data indicated significant reductions in active ROM in 3 movements: shoulder extension, shoulder abduction, and ankle dorsiflexion. There was also a significant increase in body mass for the CS group. The results suggest that short-term supplementation with creatine monohydrate reduces the active ROM of shoulder extension and abduction and of ankle dorsiflexion. Although the mechanism for this is not fully understood, it may be related to the asymmetrical
distribution of muscle mass around those joints [221].

To investigate the effect of creatine (CR) supplementation on the acute interference induced by aerobic exercise on subsequent maximum dynamic strength (1RM) and strength endurance (SE, total number of repetitions) performance. Thirty-two recreationally strength-trained men were submitted to a graded exercise test to determine maximal oxygen consumption, and baseline performance (control) on the 1RM and SE (4 x 80 % 1RM to failure) tests. After the control tests, participants were randomly assigned to either a CR (20 g/day for 7 days followed by 5 g/day throughout the study) or a placebo (PL - dextrose) group, and then completed 4 experimental sessions, consisting of a 5-km run on a treadmill either continuously (90 % ATv) or intermittently (1:1 min at vVO$_{2max}$) followed by either a leg- or bench-press SE/1RM test. CR was able to maintain the leg-press SE performance after the intermittent aerobic exercise when compared with C. On the other hand, the PL group showed a significant decrease in leg-press SE. CR supplementation significantly increased bench-press SE after both aerobic exercise modes, while the bench-press SE was not affected by either mode of aerobic exercise in the PL group. Although small increases in 1RM were observed after either continuous (bench press and leg press) or intermittent (bench press) aerobic exercise in the CR group, they were within the range of variability of the measurement. The PL group only maintained their 1RM. In conclusion, the acute interference effect on strength performance observed in concurrent exercise may be counteracted by CR supplementation [222].

Effect on musculotendinous stiffness and performance

Anecdotal reports suggesting that creatine (Cr) supplementation may cause side effects, such as an increased incidence of muscle strains or tears, require scientific examination. In this study, it was hypothesized that the rapid fluid retention and "dry matter growth" evident after Cr supplementation may cause an increase in musculotendinous stiffness. Intuitively, an increase in musculotendinous stiffness would increase the chance of injury during exercise. Twenty men were randomly allocated to a control or an experimental group and were examined for musculotendinous stiffness of the triceps surae and for numerous performance indices before and after Cr ingestion. The Cr group achieved a significant increase in body mass counter movement jump height, and 20-cm drop jump height after supplementation. No increase was found for musculotendinous stiffness at any assessment load. There were no significant changes in any variables within the control group. These findings have both performance- and injury-related implications. Primarily, anecdotal evidence suggesting that Cr supplementation causes muscular strain injuries is not supported by this study. In addition, the increase in jump performance is indicative of performance enhancement in activities requiring maximal power output [223].

No positive effect of creatine on muscle wasting in cortisone treatment

It aimed to investigate the possible role of creatine (CR) supplementation in counteracting dexamethasone-induced muscle wasting and insulin resistance in rats. Also, it was examined whether CR intake would modulate molecular pathways involved in muscle remodeling and insulin signaling. Animals were randomly divided into four groups: (1) dexamethasone (DEX); (2) control pair-fed (CON-PF); (3) dexamethasone plus CR (DEX-CR); and (4) CR pair-fed (CR-PF). Dexamethasone (5 mg/kg/day) and CR (5 g/kg/day) were given via drinking water for 7 days. Plantaris and extensor digitorum longus (EDL) muscles were removed for analysis. Plantaris and EDL muscle mass were significantly reduced in the DEX-CR and DEX groups when compared with the CON-PF and CR-PF groups. Dexamethasone significantly decreased phospho-Ser(473)-Akt protein levels compared to the CON-PF group.
and CR supplementation aggravated this response. Serum glucose was significantly increased in the DEX group when compared with the CON-PF group. CR supplementation significantly exacerbated hyperglycemia in the dexamethasone-treated animals. Dexamethasone reduced GLUT-4 translocation when compared with the CON-PF and CR-PF groups and this response was aggravated by CR supplementation. In conclusion, supplementation with CR resulted in increased insulin resistance and did not attenuate muscle wasting in rats treated with dexamethasone. Given the contrast with the results of human studies that have shown benefits of CR supplementation on muscle atrophy and insulin sensitivity, it was suggested caution when extrapolating this animal data to human subjects [224].

**Creatine deficiency syndromes**

The cerebral creatine deficiency syndromes (CCDS), inborn errors of creatine metabolism, include the two creatine biosynthesis disorders, guanidinoacetate methyltransferase (GAMT) deficiency and L-arginine:glycine amidinotransferase (AGAT) deficiency, and the creatine transporter (CRTR) deficiency. Intellectual disability and seizures are common to all three CCDS. The majority of individuals with GAMT deficiency have a behavior disorder that can include autistic behaviors and self-mutilation; about 40 percent have movement disorder. Onset is between ages three months and three years. Only 14 individuals with AGAT deficiency have been reported. The phenotype of CRTR deficiency in affected males ranges from mild intellectual disability and speech delay to severe intellectual disability, seizures, movement disorder and behavior disorder; age at diagnosis ranges from two to 66 years. Clinical phenotype of females heterozygous for CRTR deficiency ranges from asymptomatic to severe phenotype resembling male phenotype. Cerebral creatine deficiency in brain MR spectroscopy (1H-MRS) is the characteristic hallmark of all CCDS. Diagnosis of CCDS relies on: measurement of guanidinoacetate (GAA), creatine, and creatinine in urine and plasma; and molecular genetic testing of the three genes involved, GAMT, GATM, and SLC6A8. If molecular genetic test results are inconclusive, GAMT enzyme activity (in cultured fibroblast or lymphoblasts), GATM enzyme activity (in lymphoblasts), or creatine uptake in cultured fibroblasts can be assessed. GAMT deficiency and AGAT deficiency are treated with oral creatine monohydrate to replenish cerebral creatine levels. Treatment of GAMT deficiency requires supplementation of ornithine and dietary restriction of arginine or protein. In males with CRTR deficiency creatine supplementation alone does not improve clinical outcome and does not result in replenished cerebral creatine levels; likewise, high-dose L-arginine and L-glycine supplementation so far has not consistently improve clinical or biochemical outcome in males although some have been reported to have increased muscle mass and improved motor and personal social IQ skills. One female with intractable epilepsy responded to high-dose L-arginine and L-glycine supplementation with cessation of seizures. Early treatment at the asymptomatic stage of the disease in individuals with GAMT and AGAT deficiencies appears to be beneficial: treatment in newborn sibs of individuals with AGAT or GAMT deficiency prevented disease manifestations. In those treated with creatine monohydrate, routine measurement of renal function to detect possible creatine-associated nephropathy is warranted. Evaluation of relatives at risk: Early diagnosis of neonates at risk for GAMT deficiency, AGAT deficiency, and CRTR deficiency by biochemical or molecular genetic testing allows for early diagnosis and treatment of the defects in creatine metabolism. GAMT deficiency and AGAT deficiency are inherited in an autosomal recessive manner. At conception, each sib of an individual with GAMT deficiency or AGAT deficiency has a 25% chance of being affected, a 50% chance of being an asymptomatic carrier, and a 25% chance of being unaffected and not a carrier. CRTR deficiency is inherited in an X-linked manner. Mothers who are carriers have a 50% chance of transmitting the pathogenic variant in each pregnancy; sons who inherit the pathogenic variant will be affected; daughters who inherit the pathogenic variant will be heterozygous and may have learning and behavior problems. Carrier testing for at-risk relatives and prenatal testing for pregnancies at
increased risk are possible for all three defects in creatine metabolism if the pathogenic variants in the family are known [225].

Experimental

Energetic driving forces are maintained in resting rat skeletal muscle after creatine

The total creatine (TCr) pool of skeletal muscle is composed of creatine (Cr) and phosphocreatine (PCr). In resting skeletal muscle, the ratio of PCr to TCr (PCr/TCr; PCr energy charge) is approximately 0.6-0.8, depending on the fiber type. PCr/TCr is linked to the cellular free energy of ATP hydrolysis by the Cr kinase equilib.

Dietary Cr supplementation increases TCr in skeletal muscle. However, many previous studies have reported data indicating that PCr/TCr falls after supplementation, which would suggest that Cr supplementation alters the resting energetic state of myocytes. One study investigated the effect of Cr supplementation on the energy phosphates of resting skeletal muscle. Male rats were fed either rodent chow (control) or chow supplemented with 2 percent (wt/wt) Cr. After 2 wk on the diet, the gastrocnemius and soleus muscles were freeze clamped and removed from anesthetized animals. Cr supplementation increased TCr, PCr, and Cr levels in the gastrocnemius by 20, 22, and 17 percent, respectively. A numerical 6 percent higher mean soleus TCr in Cr-supplemented rats was not statistically significant. All other energy phosphate concentrations, free energy of ATP hydrolysis, and PCr/TCr were not different between the two groups in either muscle. It was concluded that Cr supplementation simply increased TCr in fast-twitch rat skeletal muscle but did not otherwise alter resting cellular energetic state [226].

Effects of dietary creatine supplements on the contractile properties in the rat

Daily creatine supplements (0.258 g/kg) were administered to adult male Wistar rats (n=7) in the drinking water. Age matched rats (n=6) acted as controls. After 5-6 days, contractile properties were examined in soleus and extensor digitorum longus (EDL) muscle strips in vitro at 30 degrees C. In soleus muscles, creatine supplements decreased the half-relaxation time of the isometric twitch from 53.6 + 4.3 ms in control muscles to 48.4 + 5.5 ms but had no effect on twitch or tetanic tension or on twitch contraction time. In EDL muscles twitch tension, tetanic tension, twitch contraction and half-relaxation times were all unaffected by creatine supplements. Creatine supplements increased the fatigue resistance of the soleus muscles but had no effect on that of the EDL muscles. After a 5 min low-frequency fatigue test, tension (expressed as a percentage of initial tension) was 56 + 3 percent in control soleus muscles, whereas that in the creatine-supplemented muscles was 78 + 6 percent. In the EDL muscles, the corresponding values were 40 + 2 percent and 41 + 9 percent, respectively. The force potentiation which occurred in the EDL muscles during the initial 20-30 s of the fatigue test was 170 + 10 percent of initial tension in the control muscles 24 s after the initial stimulus train but was reduced to 130 + 20 percent in the creatine-supplemented muscles. In conclusion, soleus muscle endurance was increased by creatine supplements. EDL endurance was unaffected but force potentiation during repetitive stimulation was decreased [227].

Creatine loading and depletion on rat skeletal muscle contraction

In humans, the effects of dietary creatine supplementation are controversial, with some studies showing increased muscle force and fatigue resistance and others reporting no effect on exercise performance. Little is known about the effects of creatine on muscle contractile properties. Rats were fed a standard diet, creatine for 10 days or beta-guanidinopropionate, which depletes muscle creatine, for 7 days. Contractile properties were measured in isolated
extensor digitorum longus and sternohyoid muscle as representative limb and upper airway dilator muscles, respectively. Creatine had no effect on specific twitch and tetanic tension, contractile kinetics, twitch/tetanus tension ratio, the tension-frequency relationship or fatigue in both muscles. beta-Guanidinopropionate had no effect on the twitch and tetanic tension, contractile kinetics, twitch/tetanus tension ratio or tension-frequency relationship, but significantly increased fatigue in both muscles. Therefore, although creatine depletion increases fatigue, creatine loading has no effects on extensor digitorum longus and sternohyoid muscle contractile properties [228].

Effect of creatine supplementation on cardiac muscle in rats

The role of creatine supplementation in altering the physiological parameters regulating cardiac muscle's functional capacity through the initiation of cardiac hypertrophy and altered contractile protein expression has not been determined. The purpose of this study was to determine the effect of creatine supplementation, with and without exercise stress, on physiological parameters regulating functional capacity through alterations in rat cardiac mass and contractile-protein expression. Thirty male Sprague-Dawley rats were subjected to 30 min of exercise stress 5 days/week for 3 weeks with 2 percent of total body mass attached to the tail. Animals were randomly assigned to one of four treatment groups: group 1 (Con) received (1 mL/day) sucrose water by intubation tube (n=8); group 2 (Cr) received (1 mL/day) sucrose/creatine solution (n=6); group 3 (EX) received 1 ml/day sucrose water and the exercise stimulus (n=8), and group 4 (Cr/EX) received (1 mL/day) sucrose/creatine solution and the exercise stimulus (n=8). At the conclusion of the 21-day exercise-training period, the heart was collected and weighed for determination of wet weight, total protein, total RNA, and myosin heavy chain protein expression. RNA concentration decreased significantly (13 %) in the EX group, but not in the CR/EX group, indicating an interactive effect of creatine and exercise. Total RNA significantly decreased (15 %) in the EX group. Protein concentration significantly increased (9 %) in the exercising treatments, while total protein did not change. Cardiac myosin heavy chain expression significantly shifted towards a predominant expression of the beta-isoform in the Cr/EX group. These results indicate an interaction of creatine supplementation and swimming exercise stress that potentially alters cardiac protein synthesis and demonstrates a possible mechanism through which the combination of creatine supplementation and swimming stress stimuli act to alter the physiological parameters regulating cardiac functional capacity [229].

Decreased plasma lipid peroxidation and enhanced anaerobic performance in rats

One study was to investigate the effects of creatine (Cr) supplementation on oxidative stress markers and anaerobic performance in rats. Sixty-four rats (Wistar) were divided into two groups: C, anaerobic exercised group (n=32) and Cr, anaerobic exercised group supplemented with creatine (n=32). Cr supplementation consisted of the addition of 2 percent Cr monohydrate to the diet. After 28 days, the rats performed acute exercise (6 × 30 seconds of vertical jumps in the water with 30 seconds rest and 50 % of total body weight load attached in the back). The animals were euthanized before (pre) and at 0, 2, and 6 hours (n=8) after acute exercise. Acute exercise induced an increase in plasma malondialdehyde (MDA) and advanced oxidation protein products (AOPP), as well as increased total lipid hydroperoxides and AOPP in gastrocnemius muscle. Cr supplementation inhibited the formation of MDA and lipid hydroperoxides in plasma. However, the antioxidant action of Cr was observed only against AOPP in gastrocnemius muscle. Cr supplementation also increased (P < 0.05) anaerobic performance compared to the C group. It was concluded that creatine supplementation is able to inhibit the increase in plasma lipid peroxidation markers induced by high-intensity and short-duration exercise in rats; equivalent actions, however, were not observed fully in muscle tissue [230].
**Guanidinoacetic acid (GAA), a precursor of creatine**

Guanidinoacetic acid (GAA), a precursor of creatine and an innovative dietary agent, activates gamma-aminobutyric acid (GABA) receptors yet clinical effects of dietary GAA on GABA metabolism are currently unknown. The main aim of this pilot research was to investigate whether GAA loading affected peripheral GABA homeostasis in healthy humans. Eight healthy male volunteers aged 22-25 years were randomized in a double-blind design to receive either GAA (three grams daily) or placebo by oral administration for 3 weeks. At baseline and after 3 weeks participants provided fasting blood samples for free plasma levels of GABA, GAA, creatine and glutamine. Following 3 weeks of intervention, plasma GABA level dropped significantly in participants receiving 3 g of GAA per day as compared to the placebo. GAA loading significantly decreased plasma GABA by 88.8 nmol/L (95% confidence interval; 5.4 to 172.1) after 3 weeks of intervention as compared to the baseline. GAA intervention positively affected both plasma GAA and creatine, while no effects of intervention were reported for plasma glutamine. Results indicate that supplemental GAA affects peripheral GABA metabolism, and potentially down-regulates GABA synthesis in peripheral tissues. Possible GABAergic action of dietary GAA adds to the safety profile of this novel dietary supplement [231].
REFERENCES


