

Functional dairy protein supplements for elite athletes

Introduction

Elite athletes have nutritional requirements that differ quantitatively from normally active people (Campbell *et al.* 2007). These requirements extend from the simple need to replace electrolytes lost in sweat through to higher energy and protein requirements to replenish glycogen stores and promote muscle repair and accretion (Burke 2001; Sawka *et al.* 2007; Kerksick *et al.* 2008). For athletes, nutrition is not only important for normal physiologic maintenance, but can also impact directly on athletic performance (Burke and Deakin 2006). While the physiological demands associated with elite performance differ between sports, the building or maintenance of skeletal muscle mass and muscular function is critical for success in almost all sporting endeavours.

A substantial body of research demonstrates that individuals engaged in regular exercise training require more dietary protein than sedentary people (Campbell *et al.* 2007). Numerous studies have investigated the effects of protein supplementation in training individuals at the functional, tissue, cellular and molecular level. It is clear that dietary protein not only provides the essential building blocks for muscle proteins, but that amino acids are directly required to activate the anabolic pathways leading to muscle hypertrophy when combined with an exercise stimulus (Willoughby *et al.* 2007; Koopman *et al.* 2007). It has also become apparent that the timing of nutritional protein supply adjacent to exercise is pivotal in maximizing muscle growth, minimizing muscle damage, and promoting strength recovery (Kerksick *et al.* 2008). While it is possible for athletes to obtain their daily protein requirements through a varied, regular diet, sports protein supplements provide a convenient means of delivering the extra protein that athletes need at the time when it is most effective.

A plethora of protein supplement formats and compositions are available to athletes. These include ready-to-mix powders, bars and beverages with formulations that may also supply rehydration, electrolytes and carbohydrates. Numerous different protein types and sources are used in these supplements, with soy and dairy protein isolates being the most common. The benefits of different dietary proteins for all of the specific aspects of athletic performance have been only scanty explored. However, enough research has been performed in well controlled human studies to show that not all protein isolates have equivalent effects on athletic performance (Cribb *et al.* 2006; Hartman *et al.* 2007). Recently, it has also been demonstrated that modifying the structure of proteins through manufacturing processes such as protein hydrolysis can alter the effects of ingested proteins on muscles (Cribb *et al.* 2006; Buckley *et al.* 2008). Thus, there is scope to identify and/or develop novel protein ingredients for improving athletic performance which have benefits that target specific aspects of muscle function.

This paper reviews the evidence for the contention that specific protein isolates can provide improvements to sports performance beyond basic supply of amino acids. It outlines an approach of using cell models to identify and evaluate the efficacy of functional

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Abstract

Elite athletes require a greater dietary protein intake than recreationally active people to maintain optimal muscular function. The timing of protein ingestion relative to exercise is critical to maximizing its physiological impact on skeletal muscles. Sports protein supplements provide a convenient means of supplying athletes with an adequate and timely source of quality dietary protein. There is now strong evidence that not all dietary proteins are equipotent in their effects on various aspects of athletic performance and specific protein isolates can provide benefits to athletes beyond simple supply of nutritional amino acids. Thus, there is an opportunity to develop new functional protein supplements to maximize athletic performance. This paper outlines the clinical evidence for the benefits of dairy proteins in sports performance and describes the development of new dairy protein supplements to build muscle strength, and to expedite recovery of strength following muscle-damaging eccentric exercise.

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dairy protein supplements for improving specific facets of muscle function. This approach has been used successfully to develop new sports protein supplements that are backed by sound science and with proven efficacy in human clinical trials.

Dairy proteins in sports performance

Dairy proteins have a long, safe, and importantly, legal history of use for improving sports performance. The benefits of dairy proteins for increasing muscle mass were first exploited by body builders aiming to increase their size. Their largely anecdotal reports of benefits provided the impetus for an increasing interest in the benefits of protein supplementation from athletes and coaches aiming to improve sports performance as well as sports scientists trying to identify the physiological mechanisms underpinning the effects of these proteins.

Casein and whey protein isolates

Both casein and whey protein supplements are widely available and have been shown to behave differently once ingested, with

implications for athletic performance. Studies using intrinsically labelled milk protein fractions have shown that casein is digested much more slowly than whey proteins, resulting in differing rates of appearance of amino acids in the circulation following their ingestion. This has led to the concept of 'slow' (casein) and 'fast' (whey) proteins that differently modulate postprandial amino acid profiles and muscle protein accretion (Borie *et al.* 1997). Ingestion of whey protein induces a dramatic, but transient increase in plasma amino acid levels that stimulates anabolic muscle protein synthesis. Recently, the mechanisms for these anabolic actions have been identified, with transient hyperaminoacidaemia activating the mTOR (mammalian Target Of Rapamycin) pathway of intracellular kinases. mTOR and its associated downstream members are a family of kinases necessary for the stimulation of protein synthesis, via the activation of translation initiation (Proud, 2007). Interestingly, the mTOR pathway is also responsive to resistance exercise, such that strength exercise, with amino acid ingestion at the time of exercise facilitates both greater mTOR activation and muscular protein synthesis (Baar *et al.* 2006; Blomstrand *et al.* 2006).

In contrast to whey proteins, casein induces a prolonged plateau of moderate hyperaminoacidemia that may act to attenuate muscle protein catabolism (Frühbeck 1998). This difference theoretically allows the tailoring of whey and casein protein supplement combinations to favour muscle building, and/or muscle maintenance and repair. However, further research is needed to determine the optimal protein combinations to achieve specific effects on muscle tissue, and then to confirm the impact of these effects on the various aspects of sports performance.

Evidence that consumption of whey protein can elicit an anabolic stimulus beyond that provided simply by its nutritional supply of amino acids was recently demonstrated in a human trial involving elderly volunteers (Katsanos *et al.* 2008). Ingestion of a whey protein induced a significant increase in an index of protein accretion and a significantly greater insulin response (essential endocrine response for muscle anabolism) compared to treatment groups receiving equivalent amounts of essential and non-essential amino acids. The study design used does have its limitations and possible confounders. Nevertheless, the effect of the whey protein was greater than the combined effects of the individual amino acid treatments, suggesting that the whey protein increases skeletal muscle protein accretion to a greater extent than can be attributed to its amino acid content alone. Thus, the ingestion of whey protein may mediate skeletal muscle anabolism via regulation of the kinetics of digestion and subsequent effects on postprandial hyperaminoacidemia or through the action of functional proteins or peptides that have anabolic effects that are independent of direct nutrition. Either way, this study suggests that it may be possible to use whey protein to design sports protein ingredients which elicit anabolic effects on skeletal muscle which are the result of effects beyond simple nutrition. Indeed, there is already evidence that this is the case from studies which have investigated the effects of a dairy food that is rich in anabolic growth factors, and that now has accumulating evidence of benefits in sports performance: colostrum.

Colostrum

Colostrum has both a nutrient profile and immunological composition that differs substantially from mature milk. It is highly enriched in antimicrobial factors, particularly antibodies,

and is rich in growth factors, cytokines and other immune modulating components (Pakkanen and Aalto 1997). It has long been favoured as a safe and legal supplement by athletes aiming to improve performance and to boost innate immunity to compensate for deteriorations in immune function associated with the stresses of intense training and competition.

A growing body of evidence now supports the benefits of colostrum supplements for improving athletic performance, recovery and well-being (Table 1). The observed effects include improved body composition, improved speed, muscular power and recovery, as well as maintenance of immune function. The studies have been well designed, randomised, placebo controlled (mostly with isocaloric, nitrogen balanced whey or casein controls), double blind trials with doses of colostrum ranging between 10 and 60 g per day. They show that the benefits of colostrum are only apparent after 4 to 8 weeks of supplementation, with studies investigating supplementation over shorter timeframes generally failing to show statistically significant effects (Mero *et al.* 2005).

The improvements in sports performance provided by colostrum were initially postulated to be due to the well-known anabolic hormone insulin-like growth factor-1 (IGF-1). Colostrum contains IGF-1 at 20 to 100-times that of mature milk, with the bovine protein highly homologous to human IGF-1 (Schams 1994; Francis *et al.* 1988). However, numerous trials in athletes have now conclusively demonstrated that consumption of even high daily doses of colostrum for several weeks does not alter circulating levels of IGF-1 (Kuipers *et al.* 2002; Coombes *et al.* 2002; Buckley *et al.* 2002, 2003). A study in Finland using radiolabelled IGF-1 fed to athletes during training showed it was completely digested and did not pass intact into the blood stream (Mero *et al.* 2002). Therefore, it seems unlikely that IGF-1 is the active component responsible for colostrum's beneficial effects on athletic performance, and the mechanisms by which colostrum improves athletic performance remain to be elucidated.

Developing new dairy protein ingredients for elite athletes

In addition to providing casein, whey protein and colostrum for sports supplements, MG Nutritionals has embarked on a biodiscovery program to identify new dairy protein isolates and hydrolysates with functional benefits for elite athletes. Since bovine milk contains a multitude of different bioactive proteins there is an opportunity to develop new isolates that are tailored to maximizing athletic performance. Additionally, encrypted in milk proteins are numerous peptides with biological activities that differ from the parent protein and that only elicit effects once liberated by hydrolysis (Korhonen and Pihlanto 2003). However, designing targeted sports performance protein isolates requires some understanding of the properties of different milk proteins/peptides and their possible influence on muscle physiology. We have been using cell models as a means of screening milk protein fractions and hydrolysates to identify new ingredients that may benefit athletic performance, and then testing these in double blind, randomised, controlled trials. The two areas of sports performance that have been the initial focus of research using these technologies have been increased muscle strength and improved recovery following intense, muscle damaging eccentric exercise.

Increasing strength

Skeletal muscle contains a high proportion of branched chain amino acids (BCAA) including leucine, isoleucine and valine (Riazi *et al.* 2003). There is evidence that BCAA supplementation stimulates muscle anabolism via the mTOR pathway, inhibits exercise-induced muscle protein breakdown, and aids in muscle repair after intense exercise (Busquets *et al.* 2000; Campbell *et al.* 2007; Greer *et al.* 2007; Matsumoto *et al.* 2007). Of the major milk proteins suitable for use as a bulk protein supplement β -lactoglobulin is relatively rich in branched chain amino acids (>26% (w/w) vs ~20% (w/w) in milk protein isolate). Using chromatographic processes it has been possible to isolate a whey protein isolate (WPI; NatraBoost® BLG) containing a high concentration of β -lactoglobulin (>85% of protein) which is considerably enriched in BCAA relative to other sports protein supplements. In healthy, non-strength training young male subjects, supplementation immediately following ingestion was found to significantly enhance leg strength gains. Relative to the placebo control and WPI groups, those young male subjects ingesting NatraBoost® BLG demonstrated approximately a 25% greater gain in eccentric leg strength. Muscle biopsies were used to examine effects on gene expression and showed that the functional changes in strength were accompanied by an increase in the expression of genes involved in muscle hypertrophy and

a decrease in the expression of genes regulating inflammatory pathways (Carey *et al.* 2006).

Based on proteomic analysis of whey proteins, a separate isolation process was used to develop another protein fraction rich in growth factors. When blended with NatraBoost® BLG the new extract (called "Catalyst") further enhanced the effect of the BCAA-rich WPI on leg press strength over a 12-week training period (Figure 1).

Improving recovery

Intense exercise, and particularly eccentric exercise, causes muscle damage that results in muscle soreness and loss of strength that can last several days (Buckley *et al.* 2008). The muscle damage is characterised by mechanical disruption of myofibril sarcomeres that induces an inflammatory response (Peake *et al.* 2005). Accelerating muscle recovery is important to athletic performance. It allows athletes a more rapid resumption of optimal training, resulting in greater adaptation and improved long-term performance, and facilitates improved performance during frequent bouts of competition.

There is evidence that protein hydrolysates can speed tissue repair following damage and may therefore be useful for accelerating recovery from exercise-induced muscle damage. With the aim of developing a new, bioactive hydrolysate to facilitate

Table 1: Clinical studies demonstrating benefits of colostrum supplements for athletes.

Effect	Study design and outcome	Reference
Improved lean body mass	Double blind, placebo (milk protein) controlled trial. N = 49 athletes. 60 g per day for 12 weeks with resistance training. Significant improvement in fat-free mass.	Kerksick <i>et al.</i> 2007
	Double blind, placebo (whey protein) controlled trial. N = 34 young men. 60 g per day for 8 weeks with resistance training. Significant increase in limb circumference and cross-sectional area in trained limb.	Brinkworth <i>et al.</i> 2004
	Double blind, placebo (whey protein) controlled trial. N = 20 young, active men and women. 20 g per day for 8 weeks with resistance and anaerobic training. Significant increase in lean body mass.	Antonio <i>et al.</i> 2001
Increased power	Double blind, placebo (whey protein) controlled trial. N = 51 athletes. 60 g per day for 8 weeks with resistance and plyometric training. Significantly increased peak anaerobic power (vertical jump and cycle power).	Buckley <i>et al.</i> 2003
Increased speed	Double blind, placebo (whey protein) controlled trial. N = 35 elite field hockey players. 60 g per day for 8 weeks with training. Significantly improved sprint performance.	Hofman <i>et al.</i> 2002
Improved endurance	Double blind, placebo (whey protein) controlled trial. N = 42 competitive cyclists. 20 or 60 g/day for 8 weeks with training. Significant improvement in time trial performance.	Coombes <i>et al.</i> 2002
Improved recovery	Double blind, placebo (whey protein) controlled trial. N = 30 active males. 60 g/day. 8 weeks + endurance running training. Significant improvement in maintenance of peak running speed during 2nd bout of running to exhaustion after 20 min passive recovery.	Buckley <i>et al.</i> 2002
Stronger immunity	Double blind, placebo (whey protein) controlled trial. N = 29 highly trained male road cyclists. 10 weeks + 5 days high intensity training (HIT). 10g per day. Significant protection against drop in immune function (T cell numbers and antibody levels) following HIT. Trend towards reduced upper respiratory illness.	Shing <i>et al.</i> 2006
	Double blind, placebo controlled (protein adjusted skim milk) trial. N = 35 distance runners. 26 g/day. 12 weeks. Significant increase in salivary IgA (mucosal antibody) levels.	Crooks <i>et al.</i> 2006
	Double blind, placebo (maltodextrin) controlled trial. N = 30 adult male and female athletes. 20 g/day. 2 weeks + training. Significant increase in salivary IgA (mucosal antibody) levels	Mero <i>et al.</i> 2002
	Double blind, placebo (whey protein) controlled trial. N = 174 adult males. 60 g/day. 8 weeks + training. Significant reduction in incidence of upper respiratory tract infection.	Brinkworth and Buckley 2003

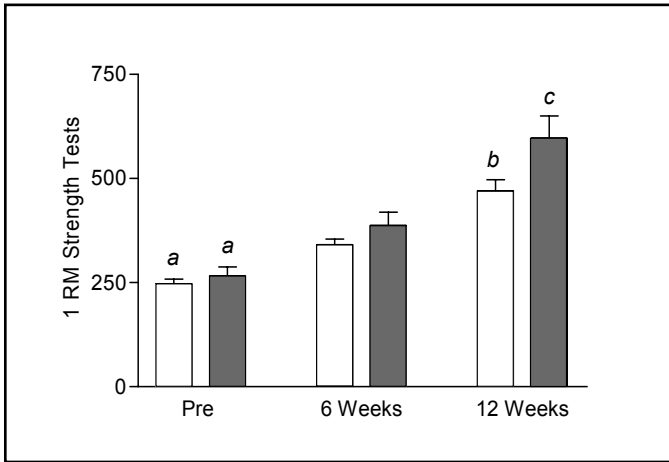


Figure 1: Gains in maximal leg press strength following 12 weeks (three times per week) of incremental strength training in young males. Supplements ingested immediately post-exercise in a randomized, double-blinded fashion. Placebo (white bars) (n=8) and NatraBoost®BLG + "Catalyst" (grey bars) (n=7). a, b, c: Bars with different letter designations were statistically different ($p < 0.05$).

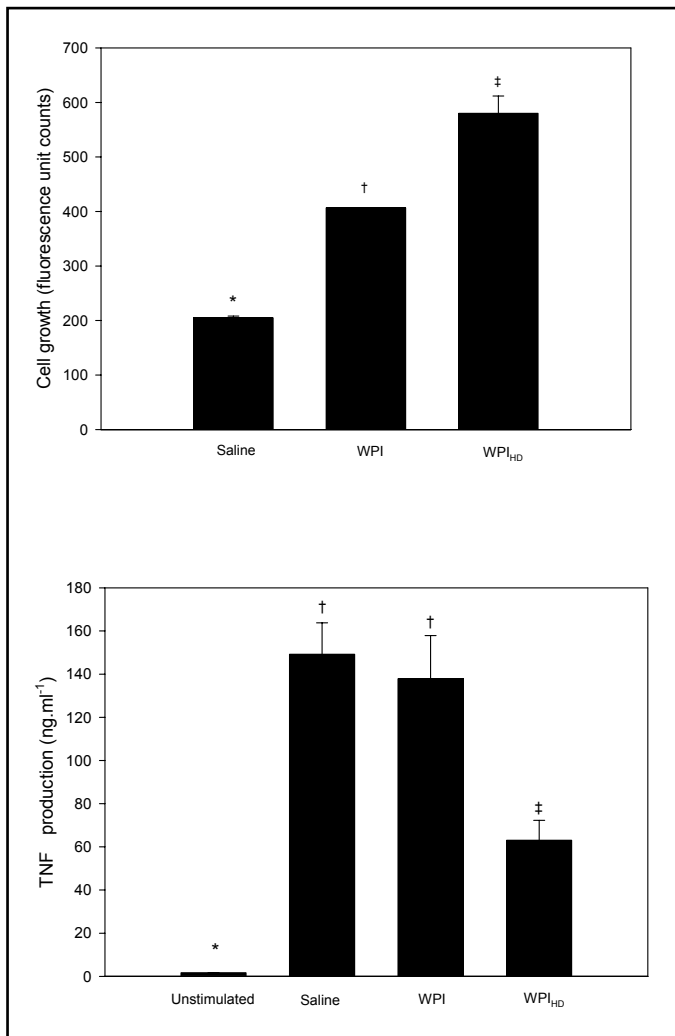


Figure 2: (Top panel) Growth of BalbC3T3 fibroblasts cultured, in duplicate, in the presence of normal saline, whey protein isolate (WPI) or NatraBoost® XR (WPI_{HD}). (Bottom panel) TNF α production by RAW264.7 murine macrophages when unstimulated, or stimulated by lipopolysaccharide in the presence of normal saline, whey protein isolate (WPI) or NatraBoost® X (WPI_{HD}). Values not sharing the same symbol are significantly different ($p < 0.05$). Values are mean \pm SEM.

rapid muscle recovery MG Nutritional produced more than 600 different milk protein hydrolysates using different source protein isolates, proteases and reaction conditions. These were screened using cell models, with one hydrolysate (NatraBoost® XR) demonstrating both substantially higher stimulation of cell growth and anti-inflammatory activity in comparison to unhydrolysed protein or saline control (Figure 2).

The efficacy of NatraBoost® XR was then tested in a randomised, double blind, placebo controlled clinical study (Buckley *et al.* 2008). Participants had muscle damage induced in the quadriceps of their dominant leg through the performance of maximal eccentric exercise and were then supplemented with water, casein, whey protein isolate or NatraBoost® XR. Muscle strength was reduced by approximately 23% as a result of performing the eccentric exercise, but strength had recovered completely within 6 hours in participants supplemented with NatraBoost® XR, whereas in all other participants, strength was only partially restored after 24 hours (Figure 3). Preliminary data from a subsequent pilot study using the same design has confirmed that the process to produce the hydrolysate could be repeated, and that a different hydrolysate with a similar degree of hydrolysis produced from the same starting protein isolate did not improve recovery (unpublished data). This demonstrated the specificity of NatraBoost® XR for improving recovery of muscle strength, and suggests that the recovery effect may be due to a specific peptide(s) produced in the hydrolysis reaction. Research is ongoing to determine the mechanism of action and to identify the active peptide(s).

Commercialisation

The novel functional protein ingredients developed during this research program have now been commercialised by MG Nutritional under the ASCEND brand (www.ascendsport.com.au) with the mission to supply sports supplements that have

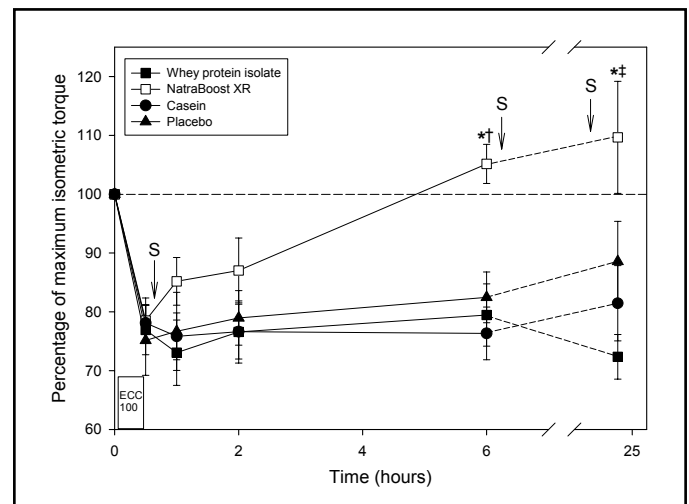


Figure 3: Percentage of peak isometric torque prior to and following the performance of 100 maximal eccentric contractions and then supplementation with various dairy peptide and protein supplements over a 24 hr period. S – dose of supplement consumed. ECC100 – 100 maximal eccentric contractions of the quadriceps muscles.

* significantly different from immediately post-eccentric exercise ($p < 0.001$). † significantly different from all other treatments ($p < 0.01$). ‡ significantly different from all other treatments ($p < 0.001$). Adapted from Buckley *et al. J. Sci Med. Sport* with permission from Elsevier.

been scientifically substantiated. Extensive protein separation knowledge and capacity, using both membrane and continuous chromatography, has been used to produce the protein ingredients. An important element in the commercialisation has been the use of in vitro cellular screens as a quality assurance tool during process development and to ensure supply of bioactive products. This involves testing the supplements for cell growth stimulation using BalbC3T3 fibroblasts, and testing anti-inflammatory action by incubating with RAW264.7 murine macrophages stimulated with lipopolysaccharide. These novel active proteins are now supplied in a range of product formats including powders, bars and beverages.

Conclusions

There is now substantial evidence to demonstrate that measured and well timed protein supplementation can benefit muscle mass and function, and hence, the performance of elite athletes. The biodiscovery research and clinical studies undertaken to develop NatraBoost® BLG and NatraBoost® XR add further weight to the contention that different protein sources and hydrolysates have different impacts on physiology relevant to sporting performance. These impacts do not appear to be limited to simple supply of nutritional requirements for amino acids, but instead are related to functional benefits of specific proteins and hydrolysates. Bovine milk contains an array of proteins and bioactive peptides that we are only beginning to characterize in terms of their potential to influence human physiology. A significant opportunity remains to discover new bioactive dairy protein ingredients that can aid sports performance by stimulating muscle accretion, preventing muscle catabolism, or facilitating recovery of muscle following exercise-induced damage.

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