Investigations into small-dose ingredients for human performance and recovery

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Capsules & Health Ingredients

Lonza Consumer Health Inc. is a leading global provider of integrated nutraceutical solutions, which seeks to drive the fields of health and human performance through advances in nutritional science, breakthroughs in performance, and the courage to raise the physical standards of humanity through the provision of small-dose supplements. Outlined here are Lonza's unique insights into recent breakthroughs in small-dose sports nutrition ingredients that show promising effects on endurance, recovery, and strength.

Over the past few decades, a variety of people have driven the boundaries of human performance forward at a rate that exceeds any ever witnessed. This revolution has occurred in a virtual blink of an eye, and it has redefined the limits of possibility. Though these advances have taken place in front of millions, it can still be difficult to comprehend. Beyond running faster and jumping higher, humans are riding 100-foot waves, jumping from 100-foot cliffs and performing unfathomable stunts. According to Jacob M. Wilson, CEO of the Applied Science and Performance Institute, a sports science facility in Tampa, Florida, "What appears to defy reality is actually gradual. Behind seemingly impossible feats is a series of small steps driven by elite physical training, nutrition, technology, innovation, grit, and fortitude."

KIMCHI, MICROBIOTA AND HUMAN PERFORMANCE

Perhaps the fastest expanding area in performance research is the human gut microbiota (the microscopic organisms that inhabit us) and microbiome (their genes). From our digestive tract to the skin, the human body abounds with microorganisms. Studies suggest that microbial cells outnumber human cells by nine trillion (39 trillion microbial cells to 30 trillion human cells) and contain a microbiome of as many as 20 million genes, greatly outnumbering the 20,000 human genes[1]. These complex ecosystems of microorganisms produce trillions of metabolites, known as the metabolome. Collectively, the microbiome and metabolome form an intricate and symbiotic relationship with humans. Scientists have only begun to unravel this interaction. Exploitation of these discoveries for human performance is in its infancy.

Several independent research groups have proposed a role for the gut microbiome on the optimization of whole-body lean mass, skeletal muscle mass and physical functioning (defined as the gut-muscle axis)[2]. Experiments have demonstrated that germ-free mice have a lower muscle mass to body weight ratio, decreased grip strength and endurance, and impaired fatigue resistance compared to control mice. Moreover, colonization with bacteria from control mice or the addition of the short-chain fatty acid butyrate to feed helped to restore deficits in germ-free mice[2]. These findings, collectively, suggest a link between metabolites, microbes and physical functioning.

The composition of gut microbiota can support the digestion of dietary nutrients and improve energy yield during movement, which may deliver metabolic benefits for high-performance individuals. Exploratory research has demonstrated that metabolic pathways that control carbohydrate, fat and protein metabolism are increased among the microbiomes of athletes compared with those in sedentary controls[3]. Research in large cohorts of volunteers, from children to seniors, found that exercise frequency was associated with gut microbiota enriched with the *Firmicutes* phylum[2]. The primary metabolic end product of *Firmicutes* is butyrate, which exerts multiple effects on human energy metabolism and performance. Similar outcomes have been found in elite endurance athletes and professional rugby players who both present with an elevated *Firmicutes* to *Bacteriodes* ratio[3]. Finally, experimental research demonstrates an increased abundance of these types of bacteria with increasing intensity of exercise[2].

Researchers at Synbio Tech Inc., a probiotics and microbiome research company based in Taiwan, have conducted extensive research identifying potential strains of probiotics that could help mimic the effects of exercise on gut health, while simultaneously improving endurance performance and body composition. One unique area of interest to researchers includes the advantageous effects of fermented foods. Fermented foods and beverages were among the first processed food products consumed by humans. It is increasingly clear that fermented foods can also have enhanced nutritional and functional properties. Many fermented foods contain living microorganisms, some of which are genetically similar to strains identified in healthy and athletic populations[4]. One highly studied fermented food is kimchi, a fermented cabbage-based dish. It is believed to include anti-oxidative, antimicrobial and immune-stimulatory effects[4]. Kimchi is rich in the *Lactobacillus* genus, part of the *Firmicutes* phylum. Numerous studies have found positive biological effects from the butyrate-producing *Lactobacillus*[4] that parallel the perceived benefits of kimchi. These findings are the catalyst for a number of investigations into a strain of *Lactobacillus plantarum* (*L. plantarum*) called TWK10, which was isolated from fermented Taiwanese kimchi.

The Taiwanese team began its exploratory research by administering zero, moderate (2.05×10^8 Colony Forming Units; CFU/kg/day), or higher

(1.03×10^9 CFU/kg/day) doses of *L. plantarum* TWK10 for six weeks to mice[5]. Results demonstrated that the probiotic was able to increase muscle mass relative to body weight. *L. plantarum* TWK10 appeared to increase grip strength and endurance performance, while decreasing serum markers of muscle damage in a dose-dependent fashion. These changes were associated with lower serum lactate levels. Changes in anaerobic metabolites are a critical measure. Decreased lactate and higher fat metabolism at higher relative intensities is the hallmark of elite athletes, including ultramarathon runners, and Tour de France competitors.

As described, athletes have higher butyrate-producing bacteria and greater fat metabolism than non-athletes. In light of this, a study, in mice, explored whether *L. plantarum* TWK10 influenced butyrate-producing bacteria and beta-oxidative genes that regulate fat oxidation[6]. Study results showed that *L. plantarum* TWK10 supplementation upregulated key enzymes involved in beta-oxidation, including long chain fatty acid acyl-CoA synthetase, promoting greater fat oxidation. Moreover, two proteins involved in fatty acid transportation were upregulated including the large subunit of the microsomal triglyceride transfer protein and protein disulfide-isomerase A4. Proteome data suggest that the fatty acid oxidation, which occurs in the liver, provides an energy source to other organs rather than to the liver itself. Finally, *L. plantarum* TWK10 induced a shifting of colonic microbiota towards the butyrate producing phylum. Building on these preliminary findings, researchers at Lonza and Synbio Tech continue to research the exact mechanism by which *L. plantarum* TWK10 exert its beneficial effects.

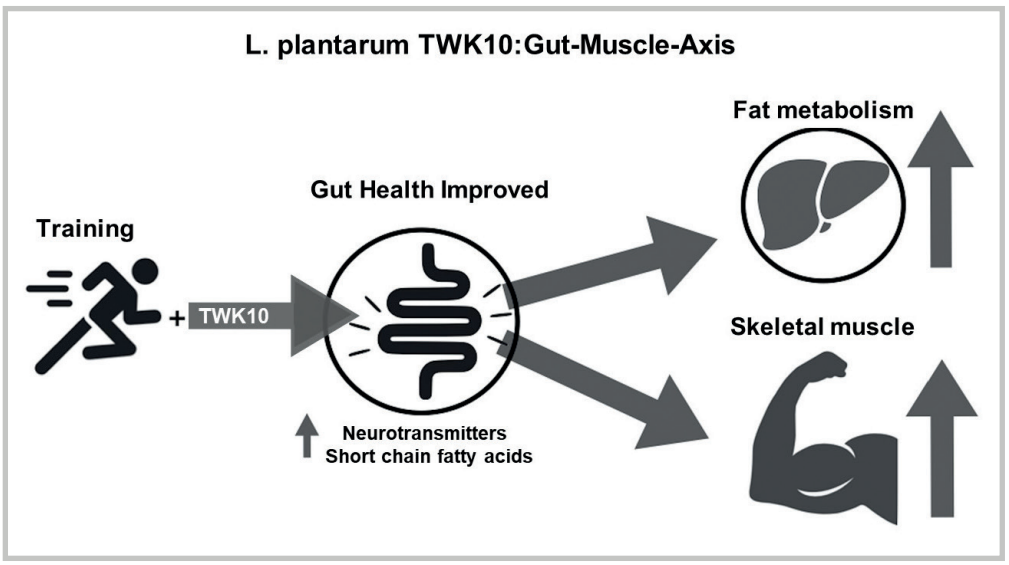

Figure 1. *L. plantarum* TWK10 interaction with gut health and performance.

Expanding on these proof-of-concept studies in mouse models, a 6-week, double-blind placebo-controlled clinical study was undertaken in young healthy participants supplemented with either a low (3×10^{10} CFU/day that translates to 100 mg) or high (9×10^{10} CFU/day) dose of *L. plantarum* TWK10[7]. Similar to mice, *L. plantarum* TWK10 induced a dose-dependent increase in exercise performance compared to the placebo. Moreover, during exercise, serum glucose concentrations were higher in TWK10-supplemented participants indicating an improved energy harvest during exercise. In addition, there were significantly lower levels of serum lactate and serum ammonia suggesting TWK10 reduces exercise-induced fatigue. Participants also experienced a greater relative increase in muscle mass and decline in fat mass. As summarized in Figure 1, these findings indicate that *L. plantarum* TWK10 supplementation is an effective and targeted probiotic, which may improve a variety of performance indicators, increase fat metabolism and improve body composition.

MARINE PHYTOPLANKTON AND MUSCLE RECOVERY

Phytoplankton is derived from the Greek phyton (plant) and planktos or (wanderer). It is often misconceived that phytoplankton is an aquatic plant or a seaweed, rather than a group of diverse eukaryotic, predominantly unicellular, microorganisms, also known as microalgae or marine algae. The marine phytoplankton exists on the aquatic surface in a drifting or wandering state, as it is too small or weak to travel against currents.

Marine phytoplankton obtains energy for growth and survival through photosynthesis, so must remain in the well-lit layers of aquatic environments. Marine phytoplankton comprises less than 1% of the photosynthetic biomass on Earth, yet it accounts for more than 45% of Earth's photosynthetic net primary production[8]. More than 50,000 species of marine microalgae exist, but only a limited number of species have been characterized. This group of microorganisms contains a number of important bioactive compounds, such as antioxidants, vitamins, fatty acids, sterols, amino acids, and carotenoids. Such composition could indicate that microalgae can operate as a functional food and perhaps even offer health benefits[9], but little progress has been made in the nutraceutical industry to fully utilize microalgae ingredients[10].

Fitoplancton Marino, SL, a company in Cadiz, Spain, that develops microalgae products and nutritional ingredients, has developed a freeze-dried preparation of microalgae, *Tetraselmis chuii (T. chuii) strain CCFM 03*, which is grown under controlled conditions to ensure high superoxide dismutase (SOD) activity (>30,000 U/g). Researchers at Lonza Consumer Health Inc. have taken this ingredient and developed it for sports nutrition. In their acute exercise trial using endurance-trained individuals, a small dose (25 mg) of *T. chuii* demonstrated the ability to sustain muscle performance and reduce muscle damage following completion of a vigorous cross-training protocol containing an array of strength (e.g., squat thrusters, inverted rows, and reverse lunges) and explosive-strength exercises (e.g., kettlebell swings, medicine ball slams, and drop jumps). Results indicate that creatine kinase, a biomarker of muscle damage, was significantly lower in people taking *T. chuii* (-14%) compared to

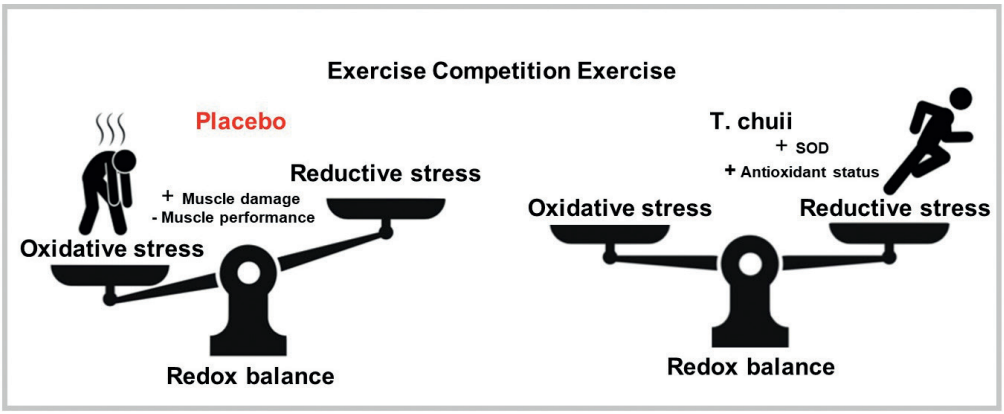

Figure 2. *T. chuii* supplementation improves redox balance.

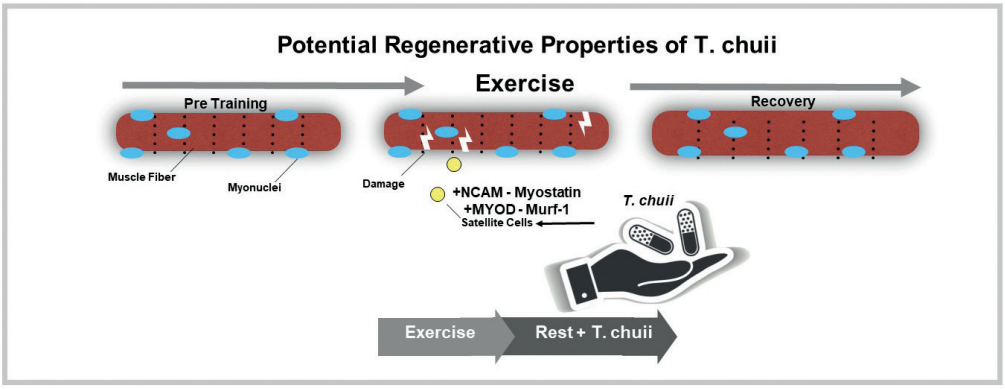

Figure 3. *Tetraselmis chuii* improves myogenic regulation of recovery.

placebo. Furthermore, measures of muscle power (+15%) and absolute muscle strength (+12%) were significantly higher in *T. chuii* group compared to placebo[11].

Researchers from Lonza conducted a follow-up study investigating the long-term effects of *T. chuii* supplementation in an exercise model. This study was five weeks, and the exercise program was designed to create non-functional overreaching on weeks two and five. Nonfunctional overreaching is a condition caused by excessive exercise training coupled with inadequate recovery. It leads to performance decrements from which it can take several days to weeks to fully recover[12]. The existence of overreaching was evident in the study as the *T. chuii* and placebo groups showed substantial decrements in explosive strength, as indicated by rate of force development, at the end of both overreaching weeks. During this period, participants were exercising five days a week and completing 124 sets of resistance training. That is an average of nearly 25 sets per exercise session. Notably, seven days after the final training session, explosive strength had returned to baseline levels in the *T. chuii* group, whereas the placebo group remained significantly lower. Also, the level of perceived recovery, measured by a validated perceived recovery scale, was greater in *T. chuii* supplemented participants than the placebo[13].

The acute and long-term study results suggest that *T. chuii* supplementation aids exercise recovery and reduces the muscle damage response to stressful training sessions. It is worth discovering how this unique preparation of *T. chuii* brings such outcomes. First, it is critical to understand the impact of reactive oxygen species (ROS) on exercise-induced muscle damage. During bouts of intense exercise, oxygen intake can rise by 20-fold over resting levels. This drastic demand for oxygen use can cause ROS to leak from the mitochondria. If not regulated, increases in ROS concentration will overtake the systemic antioxidant capacity and shift the physiological environment to a pro-oxidizing state (**Figure 2**). This is the fundamental mechanism that creates oxidative stress. Excessive exposure to oxidative stress can alter cellular integrity and function, promoting muscle damage and decrements in athletic performance. The *T. chuii* ingredient used in studies by Lonza carries a robust antioxidant profile. In muscle samples of rats supplementing with *T. chuii* for 6 weeks while subjected to treadmill exercise five days a week, Lonza researchers found a high prevalence of antioxidant enzymes, namely superoxide dismutase, catalase and glutathione peroxidase[11]. These enzymes are involved in accelerating the conversion of pro-oxidizing molecules to neutral molecules, thereby reducing oxidative stress.

A MOLECULAR VIEW ON MUSCLE RECOVERY

The deregulation of muscle recovery, from a molecular perspective, is dictated by a complex assortment of myogenic regulatory factors that drive satellite cell behavior. Satellite cells are paramount to remodeling damaged muscle tissue. Blunting the activation of satellite cells ultimately leads to the inability to counteract stressors stimulated during bouts of intense exercise. Positive regulators of satellite cell progression known to drive muscle recovery include myogenic differentiation factor (MyoD) and neural cell adhesion molecule (NCAM). Contrarily, myostatin, muscle atrophy F-box (MAFbx) and muscle RING-finger protein-1 (MuRF-1) impede muscle recovery by blunting satellite cell progression and augmenting protein breakdown. Research from Lonza in exercising rats, found that *T. chuii* enhanced the expression of MyoD and NCAM while mitigating the expression of myostatin, MAFbx, and MuRF-1 (**Figure 3**)[12]. This outcome further highlights the importance of nutrition on physiological recovery mechanisms. In a recent in vitro study, *T. chuii* treatment was also shown to increase the gene expression of nuclear factor erythroid 2-related factor 2 (NRF2) and heme oxygenase-1 in human skeletal muscle myoblasts[14]. This is a significant finding because NRF2 is a master regulator of antioxidant mechanisms by regulating the expression of about 200 cytoprotective genes.

Studies have shown that NRF2 mediates redox adaptation to exercise by counteracting the damaging effect of oxidative stress [5]. *T. chuii*, as previously mentioned, can decrease oxidative stress in the muscle, which provides a more favorable environment for satellite cell cycle progression. Additionally, essential fatty acids have been shown to have regulative effects on the myogenic factors of stem cells within skeletal muscle, giving potential to improving exercise recovery. *T. chuii* is high in essential fatty acids and provides ample antioxidants; providing a favorable nutritional profile to accelerate the repair of damaged muscle tissue.

Collectively, in a combination of acute and chronic exercise models, *T. chuii* appears to have demonstrated the ability to reduce muscle damage, improve perceived recovery and prevent declines in muscle strength, power and rapid force generation. It appears to promote these beneficial outcomes by increasing oxidative capacity and modulating certain myogenic cell cycle regulators in response to exercise in a small dose, despite what seems standard in sports nutrition.

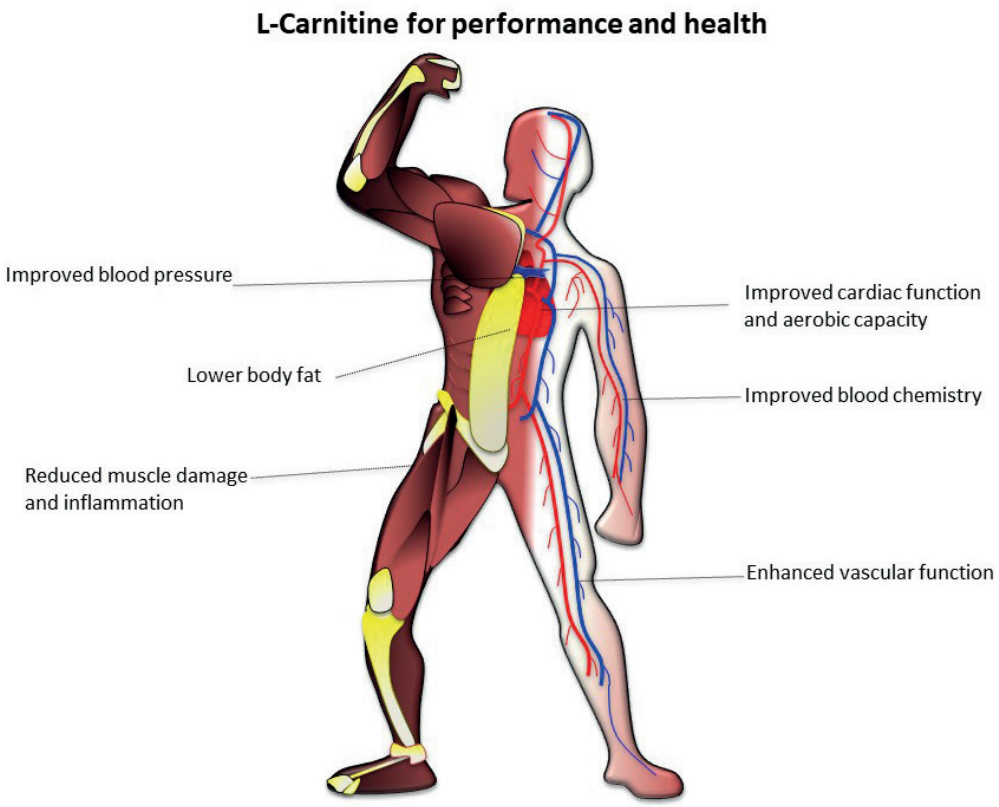

Figure 4. L-Carnitine's impact on health and performance.

NEW INSIGHTS INTO L-CARNITINE

The final area that Lonza Consumer Health Inc. has extensively researched is exogenous L-Carnitine supplementation. This ingredient has more than 30 years of clinical support in the areas of fat loss, healthy ageing, anti-inflammatory support, cardiovascular health, exercise performance and recovery (**Figure 4**). L-Carnitine is a compound that plays a critical role in energy production. The main metabolic function of L-Carnitine is to transport long-chain fatty acids into the mitochondrial matrix for beta-oxidation, making it essential to mitochondrial function.

L-Carnitine can be synthesized within the body from amino acids lysine and methionine, however, this process only accounts for about 25% of the daily requirement of this nutrient. The majority of the body's supply of L-Carnitine must come from diet.

Direct dietary supplementation has been shown to enhance L-Carnitine tissue uptake beyond an omnivorous diet. Lonza Consumer Health Inc. has produced L-Carnitine tartrate, which is a supplemental crystallized stable salt that yields the highest L-Carnitine concentration of any commercially available non-hygroscopic salt form. L-Carnitine is stored primarily in skeletal and cardiac muscle because these tissues have high mitochondrial density and energy demands. Due to its role in mitochondrial health, L-Carnitine may play a critical role in physical performance, body composition, inflammation and recovery from exercise. Supplementing with L-Carnitine during training has been shown to enhance maximal oxygen consumption, endurance, strength, power, training volume, and lower blood lactate across a variety of activities[16]. In addition, athletes in team sports that supplemented with L-Carnitine were able to improve efficiency of movement, as indicated by increased velocities and lower physiological strain[17]. The aforementioned benefits of L-Carnitine are observed at a daily dose of 1 to 2 grams.

Prolonged or high intensity exercise, such as running or resistance training, can damage muscle contractile proteins, increase muscle soreness, elevate inflammation and impair muscle function for several days or weeks. L-Carnitine supplementation has been shown to be effective in improving recovery as indicated by lower muscle damage, decreased oxidative stress, reduced muscle soreness/tenderness, and blunted declines in performance following multiple exercise modalities[18]. Evidence is mounting that L-Carnitine may improve recovery by increasing antioxidant status and preventing L-Carnitine depletion in the endothelium of blood vessels. The result is improved energy metabolism and blood flow, which in turn lowers metabolic, oxidative, and structural damage to contractile tissues[18].

Sex-based differences in the physiological responses to exercise have been studied extensively for the last four decades. Yet the study of sex-specific dietary supplement outcomes on recovery has only recently been a focus. Research has uncovered some specificity in females' physiological response to exercise and determined that biological sex is an important variable to control for in order to design robust research protocols. Research indicates the disparity between males and females when it comes to anaerobic power and strength is due to differences in

muscle and fat mass, anabolic hormone status and nutrition[19]. Despite these factors, trained females can greatly outperform non-trained males in many circumstances.

Within this context, a growing number of studies have turned their focus toward the effects of nutritional supplements on sex-specific recovery in sports, thereby contributing to a better comprehension of the similarities and disparities between males and females. Lonza Consumer Health Inc is addressing this issue through a trial in males and females in which the primary purpose was to investigate how L-Carnitine impacted recovery as measured by indices of muscle damage following high intensity exercise. L-Carnitine has been found to favorably effect biochemical markers of recovery from physical exertion, independent of sex[18]. Research suggests that daily L-Carnitine supplementation can help recreational and elite performance athletes improve health, body composition, performance, and recovery in a sex-independent fashion.

LOOKING FORWARD

Athletes are subjected to heavy loads of physiological stress, and performance can be compromised by the demands of competition, training and recovery. In an effort to combat inadequate recovery, athletes commonly turn to sports nutrition and supplementation to improve recovery and performance. *Lactobacillus plantarum* TWK10, *Tetraselmis chuii*, and L-Carnitine provide attractive nutritional strategies, delivered at low doses, that could be highly advantageous.

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