

Effect of avian immunoglobulin on post-exercise muscle damage and muscular soreness

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ABSTRACT

Delayed onset muscular soreness (DOMS) occurs following unaccustomed, strenuous activity and a variety of attempts have been used to reduce or prevent the discomfort and pain of DOMS. This study examined the effect of oral gamma globulin protein (IgY) on post-exercise creatine kinase (CK), C-reactive protein (CRP), and DOMS. In a randomized, double-blind, placebo controlled study, 25 healthy participants were assigned to either IgY or placebo (PL) followed by a blood draw. After 14 days of supplementation, muscle soreness was induced and participants continued to dose and rate DOMS for the next 48 hrs and followed by another blood draw. Analysis revealed that CK levels were significantly ($p < 0.05$) less for the IgY than for the PL group. For CRP there was no significant between group difference, however, the PL group registered a 54.4% increase in C-reactive protein while the IgY group gained 24.4%. The IgY group noted significantly less soreness than the PL at 36 and 48 hrs, suggesting that IgY provides a mechanism that mediates the DOMS inflammatory response. By mediating muscle soreness participants may continue to train and/or compete at a high level without compromising strength, power, or technique. **Keywords:** Muscle; Inflammation; Damage; Soreness; Exercise.

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INTRODUCTION

Delayed onset muscular soreness (DOMS) occurs approximately 24-72 hours after engaging in intense, unaccustomed resistance-like exercise and such strenuous overreaching yields substantial muscle damage and systemic inflammation (Brown, Child, Day & Donnelly, 1997; Chen, 2006; Fridén, 1983) and edema (Damas, et al., 2016). DOMS is the result of exercise-induced muscle damage (Brown, et al., 1997; Tofas, et al., 2008) and is thought to occur due to disruptions of the myofibrillar structure, myofiber necrosis, and subsequent inflammation (Braun & Dutto, 2003; Nieman, et al., 2014; Peake, et al., 2017). It is also believed that DOMS is associated with inflammation in the extracellular matrix (Tidball & Pierre, 1996) which includes the initiation of the inflammatory response, the resolution of inflammation, and tissue repair/regeneration to return to homeostasis (Chazaud, 2016). Additionally, DOMS damage theories have also included connective tissue damage and enzyme efflux (Cheung, Hume & Maxwell, 2003; Munhoz et al., 2014). Support for the connective tissue damage theory is based on the increase in urine excretion of hydroxyproline (HP) and hydroxylysine (HL) following exercise (Brown, et al., 1997), suggesting the occurrence of collagen degradation after intense exercise (Souglis, et al., 2015). The muscle damage theory is reinforced by the visual streaming, broadening and, at places, total disruption of the cross-striated band patterns originating from the myofibrillar z-lines (Fridén, et al., 1983; Mackey, et al., 2008). Little is known about how these factors affect intramuscular inflammation (Peake, et al., 2017), however, the wide-ranging agreement in the scientific community is that more than one theory may explain DOMS and the onset may be a sequence of the aforementioned events (Cheung, et al., 2003).

Reference values for CK for those 18 years and older range between 52 and 336 U/L (Johnsen, Wilsgaard, & Bekkelund, 2011). Serum enzyme CK is a reliable indicator of skeletal and cardiac muscle damage and, as such, is elevated following eccentric exercise. The magnitude of change in CK is influenced by the intensity and duration of the exercise (Chen, 2006; Nosaka & Sakamoto, 2001). Similarly, C-reactive protein (CRP) is a substance produced by the liver and a marker of systemic inflammation (Linzer, et al., 2015). Both CK and CRP are typical assessments following a cardiac event. Suggested normal CRP level is <0.1mg/L, while elevated levels may indicate systemic inflammation as well as risk for cardiovascular disease. It should also be noted that CRP levels also rise following strenuous exercise or sport competitions (Souglis, et al., 2017; DiLorenzo, Drager, & Rankin, 2014) thereby indicating a permeability of the muscle fiber membrane following z-line disruption.

Research efforts attempting to reduce DOMS following intense exercise have yielded mixed results. Most of the methods have focused on decreasing soreness and limiting edema (Nieman, et al., 2014). Several recent studies have attempted to curb the degree of muscle soreness due to exercise by ingestion of a variety of supplements (Manimmanakorn, et al., 2016; Rynders, et al., 2014; Svage & Clarkson, 2002) including, but not limited to black currant nectar Hutchinson, Flieller, Dillon & Leverett, 2002), ginger and cinnamon Mashhadi, et al., 2013, saffron (Meamarbashi, 2015), curcumin (Nicol, Rowlands, Fazakerly & Kellett, 2015, ginseng (Pumpa, Fallon, Bensoussan, & Papalia, 2013), taurine (Ra, et al., 2015, caffeine (Hurley, Hatfield, & Riebe, 2013), amino acids (Matsumoto, et al., 2009), selected fruit juices (Bell, Severson, Davison, Howatson, 2016), chocolate milk (Papacosta, Nassis, & Gleeson, 2015), and bee venom (Kim & Kim, 2014), all of which have met with varied results.

Immunoglobulins (Igs) or antibodies are substances produced in the immune system in to counteract a specific antigen. There are five major types of immunoglobulins in humans (IgA, IgD, IgE, IgG, and IgM), each contained in selected tissues or fluids and each charged with a specific immune fighting task. IgY, an avian immunoglobulin, has recently generated interest in human research (Müller, et al., 2015). IgY may be

transferred from the hen's acquired antibodies to the chicken egg yolk, thereby providing viral and bacterial immunity to the chick. A specifically immunized egg is the result of hens being inoculated with dead pathogens thereby building up immunity to the pathogens and passing their immunity (IgY) in a concentrated form into their eggs. IgY works via passive immunity, potentially decreasing the cytokine production via cytokine inhibitory factor (CIF) (Kato, Sakamoto, & Ito, 2007), thus decreasing the inflammatory response and promoting faster recovery.

The purpose of this study was to compare IgY Max Performance™, a polyvalent antigen specific IgY product from specifically immunized hen eggs to a placebo on serum levels of CK and CRP and perceived DOMS.

METHODS

Subjects

Subjects were healthy male ($n=11$) and female ($n=14$) college-aged students with no history of lower leg injury, high blood pressure, or any condition which could be of potential danger due to the proposed testing. Prior to initiating the study, IRB approval was sought and gained requiring all subjects to be briefed on the objectives of the study and to be told of the testing protocol. Additionally, the study followed published ethical standards (Harris & Atkinson, 2015). Following the preliminary information regarding the study, those wishing to volunteer read and signed an informed consent document and completed a brief health history questionnaire prior to the onset of the study. Subjects' demographic characteristics are illustrated in Table 1.

Table 1. Demographic characteristics of participants.

Variable	Mean	±SD
Male Age (yrs)	19.67	1.03
Female Age (yrs)	20.62	1.52
Male Wt (kg)	75.92	5.21
Female Wt (kg)	69.14	16.66
Male Ht (cm)	178.83	4.91
Female Ht (cm)	163.23	5.15

Procedure

Subjects reported to the lab for 24 h postprandial blood samples (5 ml) drawn by a trained phlebotomist. Blood was used to assay base-line levels of serum creatine kinase (CK) and C-reactive protein (CRP). Following the base-line blood draw, the subjects were instructed on how to use a visual analogue scale (VAS) to assess the degree of overall lower body muscle soreness and rated their resting muscle soreness which served as a baseline. VASs consists of a 100 mm line with polar extremes at each end (i.e., excruciating soreness to no detectable soreness) on which the participants places a mark denoting his/her perceived muscle soreness.

After blood draw and VAS instructions, subjects were randomly divided into a treatment group or a placebo group and given, on a double blind format, either the IgY supplement (MAX Performance™) or a placebo (PL). The IgY supplement claims to provide "targeted immune protection" and to "regulate the release of cytokines". The dosage regimen consisted of 14 consecutive days using the following dosage parameters: 2 days at 4.5g, 3 days at 9g, and 9days at 13.5 g (Table 2). Following 14 days of supplementation the subjects returned to the lab and muscle soreness was induced via a protocol which included performing four maximum sets of 10 eccentric leg extension repetitions at 120°/sec. on a Biodex Dynamometer (Biodex Medical

Systems, New York). Additionally, to ensure DOMS, each subject performed 3 sets of 10 repetitions of forward lunges with an approximate load of 50 - 65% BWT.

After inducing DOMS subjects were asked to continue with their supplement/placebo dosing for the next two days while rating their DOMS on VASs the following evening of day 1, the morning of day 2, the evening of day 2 and the following day when reporting to the lab for post-testing. After 48 hours DOMS inducement subjects returned to the lab for a post-test blood draw and to complete a final DOMS VAS (see Table 2).

Table 2. Research protocol timeline.

Day 1	Pre-test for subjective muscle soreness, serum CK and CRP via blood draws.	40-45 min
Day 1	Subjects were given a 14-day supply of either powdered IgY or a placebo (no intervention except for emails to assure compliance).	No contact – 10 days
Day 1-14	Subjects supplemented for 14 consecutive days (4.5 g for 2 days, 9 g for 3 days, 13.5 g for 9 days).	
Day 15	DOMS was induced by maximum eccentric quadriceps contractions using a Biodex dynamometer (4 sets of 10 at 120°/sec) and 3x10 dumbbell lunges at 50% - 65% BWT.	15-20 min
Day 16 -17	Subjects completed a daily visual analog scale to subjectively assess DOMS.	No contact 48 hrs
Day 18	48 hours after induced DOMS subjects return for retesting. Supplementation continued until completion of post-test blood draw, and DOMS assessment.	45-60 min

Data analysis

Statistical Package for Social Sciences (SPSS) 18.0 (Chicago, IL, USA) was employed to conduct analysis of gathered data. The design incorporated descriptive data and repeated measures ANOVA with Newman Keuls post hoc tests. Statistical significance was set at alpha (α) $p < 0.05$.

RESULTS

Of the initial 31 participants who volunteered for the study, three did not or could not attend the base-line blood draw and three failed to adhere to dosage protocol (Figure 1). One-way ANOVA statistical procedures yielded no significant ($p > 0.05$) baseline differences in CK levels between the groups. Significant ($p < 0.05$) within group differences were found for the IgY treatment, but not for the placebo treatment. Furthermore, significant between group post-test differences were found for CK serum levels with the IgY group showing significantly less CK serum. Proportionally, the PL group experienced a 130.5% pre- to post-test gain in serum CK (96.6 U/L vs. 222.7 U/L) while the experimental group increased only 53.1% from pre- to post-test (93.5 U/L vs. 146.3 U/L) (Figure 2). There were no significant pre- to post-test group differences in CRP. However, the PL group registered a 54.4% increase in CRP in contrast to the 24.4% increase for the IgY group (Figure 3).

With respect to subjective ratings of perceived DOMS based on the compared VAS ratings (1-100), there were not significant differences at the baseline measure, however, the IgY group noted less perceived soreness than the PL for each of the four post-exercise observations and demonstrated significantly ($p < 0.05$) lower DOMS than the placebo group for observations at 24, 36, and 48 hours (Figure 4). As is illustrated in Figure 4, the IgY group experienced less perceived soreness in all observations with the smallest difference

appearing in the initial observation and the largest and statistically different discrepancy in the last three observations where one might expect greater soreness.

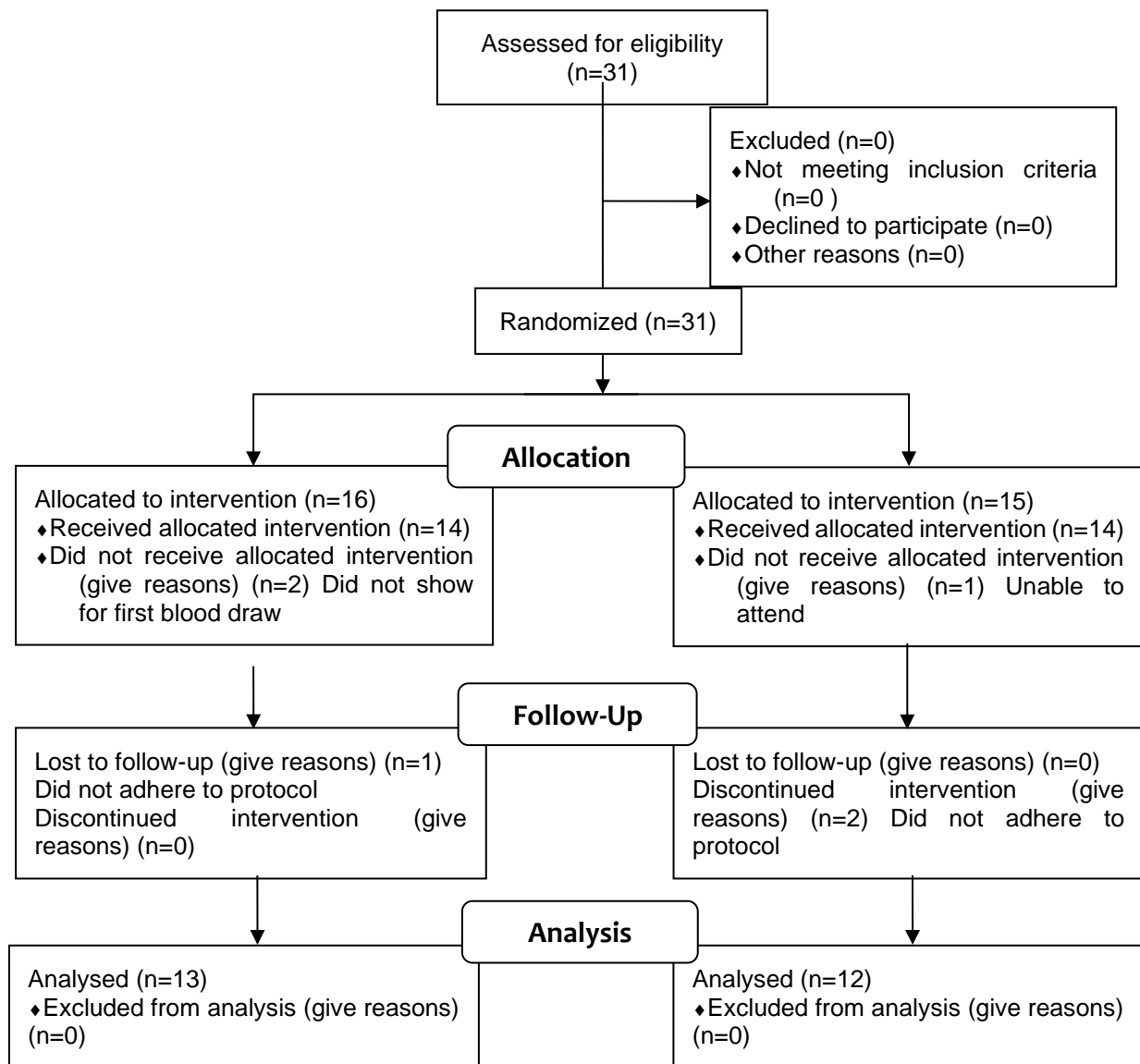


Figure 1. Flow diagram of participation.

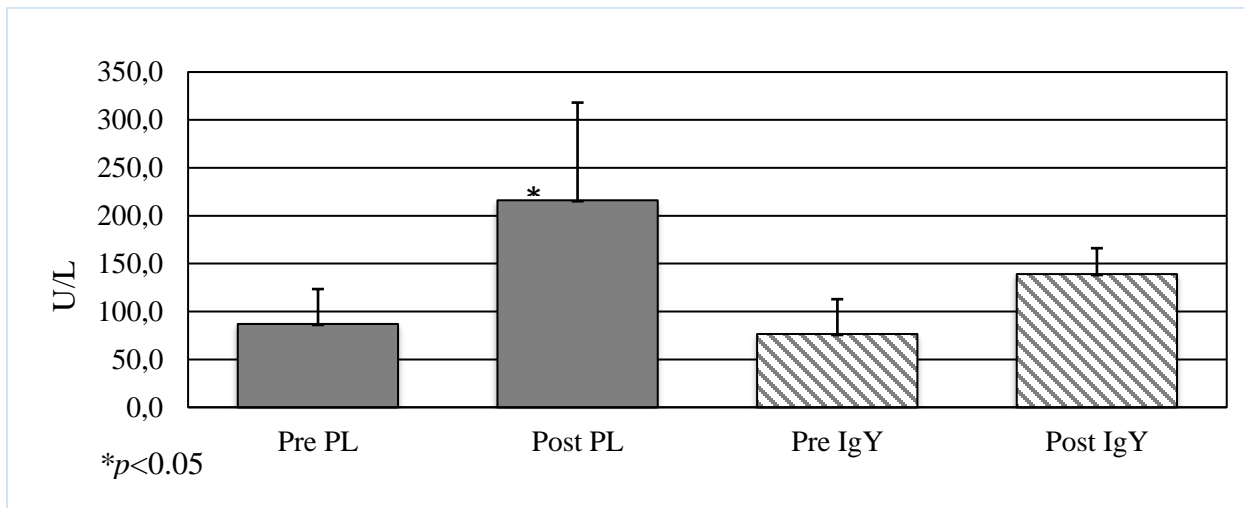


Figure 2. Pre- and post-test serum creatine kinase (mean \pm SD) by group following induced DOMS.

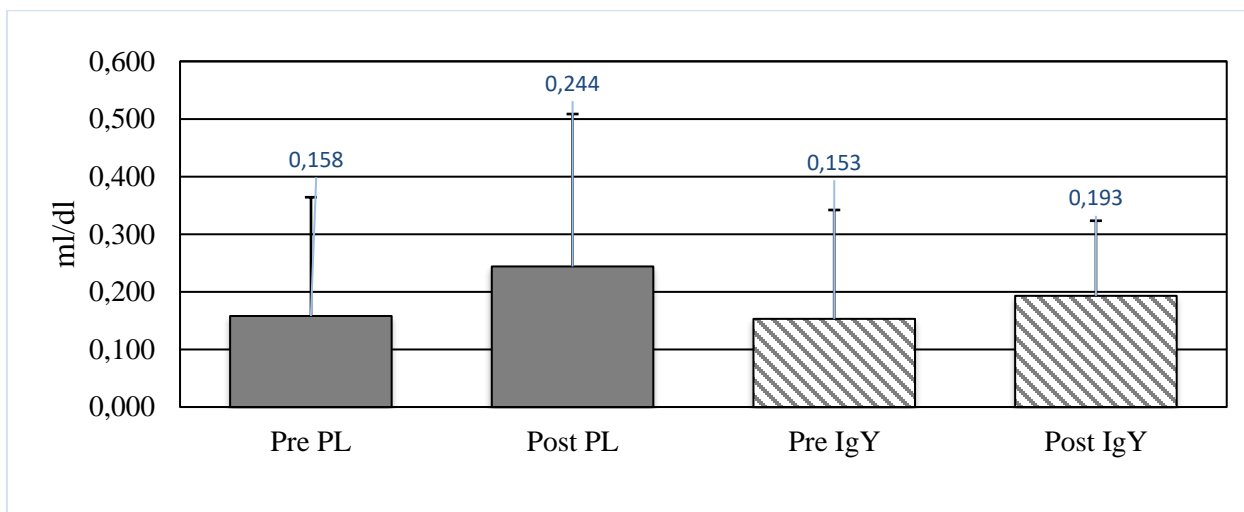


Figure 3. Pre- and post-test serum C-reactive protein (mean \pm SD) following induced DOMS.

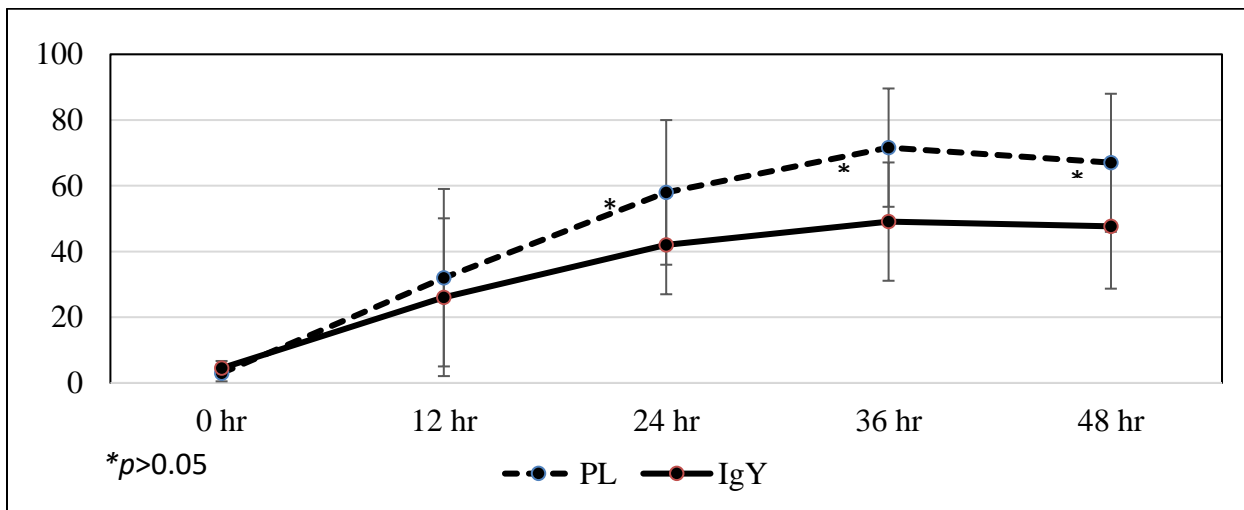


Figure 4. VAS ratings of muscle soreness (mean \pm SD) by group over time.

DISCUSSION

Muscle damage can be due to acute bouts of resistance exercises (Hasenoehrl, et al., 2017) or long distance running (Baum, Telford, & Cunningham, 2013; Tojima, Noma, & Torii, 2016). While the exact cause of muscle soreness is still debatable, the common assumption is that it is a multifaceted event at the cellular and subcellular levels. The focus of most studies, in the attempt to decrease the effects of DOMS has been to reduce muscle damage and inflammation. Such attempts have included, but are not limited to pre-ingesting selected anti-inflammatory supplements such as ginger (Mashhadi, et al., 2013; Matsamura, Zavorsky, & Smoliga, 2013), pomegranate juice (Ammar, et al., 2016), antioxidants (Luden. Saunders, & Todd, 2007), and ascorbic acid (Close, et al., 2006; Connolly, et al., 2006).

IgG, the most abundant antibody class in the human sera, has anti-inflammatory characteristics capable of treating disease (Lux, Aschermann, Biburger, & Nimmerjahn, 2010; Müller, et al., 2015). Avian immunoglobulin (IgY) found in the egg yolk laid by inoculated hens has a greater concentration of IgY than in blood of the hen and has been of recent research interest in treating various human conditions. For instance, IgY may be used to treat certain digestive diseases (Gujal, Suh, & Sunwoo, 2015), respiratory diseases, reduce allergic inflammation (Wei-Xu, et al., 2016), and modulate intestinal mucosal immune responses (Li, et al., 2016). IgY antibodies offer several advantages over mammalian IgG antibodies: they have 3 to 5 times higher immunogenicity, they do not increase inflammatory cytokines, and they have a 20 times higher immunoglobulin concentration per unit. Moreover, IgY antibodies have a highly specific rapid and local onset of action, are non-toxic, and they do not stimulate the human complement system, thus preventing non-specific inflammation (Matsumoto, et al., 2009; Rahman, et al., 2013).

The results of the current study suggests that oral consumption of IgY provides a mechanism to mediate the extent of the inflammatory response due to muscle damage following induced DOMS. While the pre- and post-levels of IgY remained within normal post-exercise values, the resistance exercises produced elevated serum levels in both groups. However, the post-exercise CK values were significantly higher in the PL group in comparison to the IgY group. Specifically, the CK values elevated 129.1 U/L in the PL group and only 52.6 U/L in the IgY group. Given that the female CK value range is generally lower than that of males, research separating gender may be warranted. Additionally, males typically carry greater muscle density than females which may warrant greater doses of IgY. Even more specific to muscle density, it may be of use to dose the IgY based on g/km body weight.

While the difference between pre- and post-CRP levels did not reach significance, the placebo levels increased 54.4% and the IgY only 26.1%. Thus, the beneficial effect of IgY was evident in both the serum assays as well as the subjects' perceived muscle discomfort/pain. Studies have determined that the onset of DOMS following exercise reduces the muscle's ability to generate maximum torque (Johnsen, et al., 2011; Nguyen, et al., 2009). Furthermore, muscle damage via DOMS leads to changes in stride mechanics such as stride length, running economy (Baum, et al., 2013; Calbet, Chavarren, & Dorado, 2001) and a greater reliance on anaerobic methods of energy production (Chung, et al., 2003). By mediating DOMS following an acute bout of exercise the participant may continue to train and/or compete at a high level without compromising strength, power, or technique.

DISCLAIMER

Citations of commercial products are not endorsed by the Army or the Department of Defense. The opinions or assertions contained herein are the private views of the author(s) and are not to be construed as official or reflecting the views of the Army or the Department of Defense.

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