

WHITE PAPER

Muscle Marinade™ (PURUS LABS™)

Richard J. Bloomer, PhD

INTRODUCTION

Dietary supplements are big business in the United States. According to *Nutrition Business Journal*, consumer sales of dietary supplements grew almost 7% to \$25 billion in 2008 and are expected to continue growing despite current poor economic conditions. While this number represents sales across all channels, it is evident that sport/exercise performance-enhancing supplements represent a large portion of these sales. This assertion is well-supported by the widespread use of dietary supplements (~90%) amongst athletes (Erdman et al., 2007; Froiland et al., 2004). While several classes of sport supplements are currently available and extensively used, those that appear to be most commonly used include pre, intra, and post workout drinks.

PRE WORKOUT DIETARY SUPPLEMENTS

Overview

Although other supplement classes may have some merit in their own regard, it is generally well accepted that the pre workout supplement/drink is an absolute “must have” for all serious athletes, bodybuilders in particular. Such drinks are typically purchased in powder form, mixed with water to taste, and consumed 20-30 minutes prior to strenuous exercise. Most products contain a mixture of stimulants (caffeine being most common), certain amino acids (such as arginine—as discussed in more detail below as related to nitric oxide), performance-enhancing agents (such as creatine), some so-called “novel” ingredients usually included at insanely low dosages and hidden within a “proprietary blend” to beef up the label panel, and a cheap carbohydrate filler (usually maltodextrin). The interesting thing to consider about this class of dietary supplement (and many supplements for that matter), is that there *does* exist scientific evidence to support the use of *some* ingredients found within the pre workout products currently available. However, because so many companies are more concerned about including a long list of ingredients on their label panel in favor of a few scientifically supported ingredients that actually do something beneficial (in human subjects for that matter) and making a greater profit rather than producing a quality product (with some exceptions), most products eventually turn into a container of maltodextrin and caffeine with 50 other ingredients provided at such a low dosage that they could not possibly provide any benefit to the consumer (even when used at the 2-3 scoop dosing level—an absolute necessity for most products despite claiming a serving size of 1 scoop). Take a careful look at many such labels and see for yourself. The unfortunate reality is that if certain ingredients were actually provided within each product at dosages that were proven to be efficacious (based on clinical studies in human subjects), pre workout products would likely be an extremely valuable tool in the dietary supplement arsenal, rather than simply a stimulant-loaded placebo. The section below discusses this in some detail.

What to Include

The most crucial decision in developing a new dietary supplement is what to include within the actual finished product. In a well thought out plan, *hundreds* of hours can be spent reviewing the available scientific literature in an attempt to identify ingredients of interest for inclusion within the finished product. The process of product formulation should ideally involve reviewing scientific abstracts, attending presentations at scientific and industry-focused meetings, retrieving and evaluating *full text* scientific manuscripts, discussing data with chemists and technical support staff working for companies selling ingredients of interest, conversing with investigators actually conducting the research, self-using of and experimenting with ingredients of interest, and/or designing studies and actually conducting clinical trials with yourself as the principal

investigator (assuming you have the expertise and resources to do this). All of the above steps were thoroughly exhausted by PURUS LABS™ and colleagues over the course of a six month period in formulating Muscle Marinade™.

Ingredients Tested in Human Subjects

In regards to the above, it is imperative that the majority of chosen ingredients have been investigated within human subjects who received the ingredient via *oral ingestion* and at a dosage similar to that provided within the finished product of sale. Although some ingredients may meet these criteria, a decision not to include them may still be rendered for other reasons (as discussed in some detail below). The two most common reasons for this decision are: 1) the overall effects of the ingredient may be physiologically negligible, albeit of statistical significance; and/or 2) the subject population in the research study may not be representative of the product's target market (e.g., elderly heart failure patients used in research study; product marketed to young, healthy bodybuilders). Under such circumstances, it is important that the formulation team make an educated and informed decision regarding the use of such ingredients. Alternatively, if a novel ingredient is identified and has yet to receive a great deal of attention from the scientific community (e.g., perhaps one obscure study has been done) but appears to have excellent potential based on anecdotal reports, this may be considered for inclusion after careful review. However, use of such ingredients should clearly not be the norm, as objective and independent scientific data should ultimately drive the development of any new dietary supplement.

If the above plan is stringently adhered to, it is certainly possible to develop a product that is scientifically sound, will provide for the desired effects, and will likely yield results for *most* individuals who choose to use it (see the section on Muscle Marinade™ below for ingredients that meet these criteria for inclusion within a pre workout product). Unfortunately, the more commonly used alternative to this multi-component plan is to simply look at your competitor's label and use what they use. Muscle Marinade™ exclusively uses *only* ingredients with proven effects noted in human clinical research studies coupled with strong anecdotal evidence within human subjects.

What not to Include

While the list of *what to include* in a pre workout product is far from extensive, the list of *what not to include* is indeed much longer. At PURUS LABS™, the rationale for not including certain ingredients is based largely on one simple fact: *There exists absolutely no scientific studies or anecdotal reports obtained from human subjects pertaining to said ingredient of interest.* The fact that PURUS LABS™ only considers information gleaned from human subjects is important, as many ingredients contained within the majority of pre workout products have only been investigated in cell culture with the ingredient simply being added to the incubation medium. The research ends there without even the inclusion of animal studies indicating a benefit. This being the case, how can a company feel confident they have any idea of the correct dosing of such an ingredient (something that *can* carefully be calculated if animal data are available) or that the ingredient would do anything remotely similar to what they are claiming? The answer is simple—these companies are merely guessing! Of course, it is possible (but not probable) that some of these novel ingredients *may* actually provide a benefit, but the studies ideally need to be done *before* people should be making such ridiculous claims. At the very least, the ingredient

needs to be provided to human subjects in oral form at the recommended dosage for an assessment in a “non-scientific environment” (e.g., field testing in the gym). Considering the above and having spent countless hours reviewing the scientific findings and anecdotal reports (or lack thereof) related to the ingredients contained within several pre workout supplements currently being sold on the market today, I can say with confidence that not only are there no human data to support the inclusion of many of these ingredients, but there exists little to no biochemical rationale as to why many of these ingredients would be included within the product in the first place. It all goes back to companies just wanting to beef up their label panel for marketing purposes regardless of an ingredient’s efficacy. This is especially true when considering the ridiculously low dosage used of most ingredients, coupled with the fact that oral intake will likely render much of the ingredient inactive once it reaches the gut.

Ingredients with Little Scientific Rationale

Regarding the above, a great example of this industry foolishness over the past 5 years is the hype surrounding ingredients touted to increase the gaseous molecule known as nitric oxide (NO). While NO is indeed an important signaling molecule promoting vasodilation by acting on vascular smooth muscle (Maiorana et al., 2003) and mediating increased blood flow at rest (Hickner et al., 1997) and during exercise (Gilligan et al., 1994), there exists *no* direct evidence that increasing NO is associated with improved exercise performance (Bloomer et al., 2009; In press). Companies claiming insane exercise intensity and muscle pumps with use of their pre workout products due to the supposed NO increase have no evidence to back their claims: I base this assertion on the fact that if companies actually had such evidence, they would certainly feature it in their marketing pieces. All of the advertising, marketing, testimonials, and endorsements are mere hype. Granted, they may mention a few references pertaining to a certain NO precursor such as L-arginine, but such studies often have absolutely nothing to do with the product of sale or to the claim being made.

For example, although L-arginine is indeed the precursor to NO biosynthesis and has been associated with enhanced vasodilatation (Bode-Böger et al., 1994; Giugliano et al., 1997), the rationale for inclusion of L-arginine within pre workout powders is based largely on research using *intravenous* L-arginine, often at dosages of *20-30 grams*, not oral intake of L-arginine at the usually included *2-3 grams*. This is obviously a major concern considering most products currently on the market only use 1-3 grams at most. In fact, studies directly comparing intravenous and oral L-arginine indicate *no effect of oral L-arginine on vasodilatation*, possibly attributed to variance in oral L-arginine bioavailability (Bode-Böger et al., 1998). Additionally, studies involving oral intake of L-arginine at dosages from 10-20 grams indicate *no benefit* with regards to increasing circulating NO or enhancing blood flow (Adams et al., 1995; Chin-Dusting et al., 1996; Robinson et al., 2003). Logic dictates that if 10-20 grams of oral L-arginine fails to provide a favorable effect for blood NO and vasodilation, 3 grams is not going to get the job done! Further substantiating my point, a recent study using 3 grams per day of L-arginine found no increase in NO availability, and actually noted a reduction in exercise time to fatigue in a sample of patients with peripheral arterial disease (Wilson et al., 2007). A final consideration is that L-arginine itself may not be the rate limiting component to NO biosynthesis; but NO synthase enzymes may be most important (Kurz and Harrison, 1997). Therefore, adding L-arginine to a pre workout powder for purposes of NO elevation makes little scientific sense.

In the same vein, companies often like to egregiously claim percent increases for their pre workout NO supplement, with some being close to 1000%. This is ridiculous, bearing in mind that NO itself can react with superoxide to form peroxynitrite, a very harmful chemical (Beckman et al., 1996) involved in nitrosative stress (Wink et al., 2001). A 1000% increase in NO is not good. It's very bad. Companies making such claims should first consider consultation with a chemist instead of haphazardly misleading consumers.

Ingredients with Little Physiological Effect (despite a statistically significant effect) or No Direct Data Related to the Outcome Variable of Interest

Aside from human evidence, it is important to consider what *overall benefit* an ingredient will lend to the product's desired effect. For example, some ingredients may have been studied in both humans and animals and may have been reported to increase or decrease a certain variable *thought to be linked to* improved physical performance (e.g., increased catecholamine release, decreased cortisol, etc.). Unfortunately, many of those same studies *have failed to actually measure exercise performance variables* and merely speculate that because one specific variable was altered, exercise performance would then also be improved. Such speculation is rampant within the sport supplement field and is not grounded in firm scientific process. Unless more work is done with the particular ingredient of interest that includes exercise performance as the chief outcome variable, companies should either not include the ingredient within the finished product or should temper their outlandish claims for that ingredient. Of course, doing so would limit the company's ability to develop their misleading marketing pieces.

In addition to the above, studies on a given ingredient have often ended after one or two trials, and other studies related to the role of the particular measured variable have been published demonstrating that the alteration in the variable is clearly *not* associated with improved physical performance. Therefore, when collectively considering the data, one would easily deduce that the ingredient, despite altering a particular variable, would not favorably affect exercise performance. In such cases, companies often choose to *ignore* the studies failing to support the use of said ingredient and only cite those studies that support their position. A great example of this is found in the use of the amino acid glutamine within many pre workout supplements. While glutamine has clinical application in conditions of trauma and burn, and has been reported to decrease the self-reported incidence of illness in endurance athletes (Castill, 2003), the majority of exercise studies involving human subjects receiving glutamine supplementation in an attempt to combat post exercise immunosuppression have failed to note significant benefits despite daily dosages of glutamine typically ranging from 10-20 grams. For a detailed overview of such work, the reader is referred to the following review articles on this topic (Gleeson, 2008; Hiscock and Pedersen, 2002).

Furthermore, and in specific relation to pre workout dietary supplements, while certain ingredients have been reported in a few isolated studies to favorably impact *one variable* that is believed to influence exercise performance, there remains *many* other variables that contribute to exercise performance that were either not influenced by these ingredients or were not assessed within the study design. Hence, it would be premature to conclude that exercise performance will be improved because one variable was positively affected by these ingredients. While a favorable impact on one variable may be interesting to note and may possibly be associated with improved performance, additional research would then be necessary to provide such evidence. If this

association were proved to be accurate, the ingredient could then be considered for inclusion within the finished product.

Ingredients which are Cost Prohibitive

It should be stated up front that PURUS LABS™ is unaware of any single ingredient, outside of those already included within Muscle Marinade™ that has been reported in the scientific literature to result in such a significant impact on physical performance or recovery that it absolutely must be included within a pre workout dietary supplement independent of cost. With that understanding, a final consideration of what *not* to include within a finished product is the actual price of a particular ingredient. That is, some ingredients may have shown promise in human clinical trials, but the reality is no company can realistically afford to include it within a finished product due to industry pre-established end consumer pricing parameters. The way around this is to hide the ingredient within a proprietary blend, use it at a dosage that is so insignificant that it might as well not be included at all, and then market it as though it delivers “drug like” effects. Make no mistake about it; this happens all the time in the dietary supplement industry.

The scenario goes something like this: Companies know that consumers may be familiar with a certain ingredient name. Therefore, they use trace amounts of the ingredient, plaster it all over the product label and advertisements, and cite the clinical studies pertaining to the ingredient—all the while using the ingredient at a dosage equal to *a minute fraction* of the dosage used in the clinical studies they are referencing. Although extremely misleading, that *is* the business. If the company used the full clinically effective dosage, they might have to charge the consumer \$119.95 for their pre workout powder rather than \$39.95. That’s the absolute reality, and everyone in the industry knows it. The consumer needs to understand this and demand the best product possible for the price being paid. This amounts to the inclusion of only high-quality ingredients that have been shown to provide a favorable effect, included at the clinically supported dosages with no fillers and no “window dressing”.

Pertaining to the cost issue, the company also needs to consider that despite the fact that the ingredient has been shown to favorably impact a given variable, it may not be worth spending the additional money needed to include the ingredient within the finished product. For example, suppose a given ingredient had been shown to improve high intensity exercise performance by an average of 4.5% in a sample of resistance-trained men (with 60% of subjects responding to treatment and 40% of subjects not responding) but would cost an additional \$20 per container to include within the finished product. Given the small likelihood of the ingredient eliciting a significant improvement in performance, it is likely not worth the cost of inclusion.

Considering all of the above sections, the focus of product development at PURUS LABS™ is quite simple: Include those ingredients *supported* by peer-reviewed and published scientific data obtained from *human* subjects reporting a significant and meaningful affect on a given outcome variable at the *dosage used* in the clinical research studies. Using this approach, PURUS LABS™ formulated Muscle Marinade™ for consumers who expect the best, including us. No hype. No outlandish claims. No pixie dust proprietary blend. Muscle Marinade™ is simply a quality product containing the specific dosages of performance-enhancing/recovery ingredients supported by human clinical research to provide the stated benefit.

INTRODUCING MUSCLE MARINADE™

Muscle Marinade™ represents a true breakthrough in the supplement industry with a specific focus on pre workout nutrition. As outlined above, Muscle Marinade™ was engineered using a detailed, systematic, and scientifically sound approach *including only those ingredients supported by peer-reviewed and published scientific data in human subjects (in addition to strong anecdotal evidence) and included at the dosage used in the clinical research studies*. The ingredient matrix comprising Muscle Marinade™ addresses all components related to both exercise performance and exercise recovery. Performance-related factors include mind/ muscle stimulation and energy production, hydrogen ion buffering, electrolyte balancing and hydration, and enhancement of muscle power and endurance. Recovery-related factors include insulin release and nutrient shuttling, cortisol reduction, protein anabolism, enhancement of cellular immunity, and improved health and antioxidant defense. Collectively, the ingredients provided within Muscle Marinade™ serve a dual purpose: 1) to improve acute exercise performance and 2) to facilitate post-exercise recovery.

What Makes Muscle Marinade™ Different?

As stated above, unlike other products within the pre workout category, Muscle Marinade™ contains only those ingredients supported by peer-reviewed and published scientific data in human subjects, in addition to anecdotal evidence for effect. Additionally, all ingredients included are at dosages used in the clinical research studies and are fully disclosed on the nutrition panel either by individual ingredient or by the specific ingredient class. While the *actual number* of ingredients contained within Muscle Marinade™ is lower than most other pre workout products on the market, it is important to note that the *gram amount* of *active* ingredients is much higher than most other products in this class. After all, as established throughout this paper, “*quality and then quantity*” of effective ingredients is much more important than sheer quantity of random individual ingredients. What good does it do to add 50 mg of a proven effective ingredient when the clinical studies report an effect only when used at 1000 mg? For many companies it increases the number of ingredients on the nutrition panel and allows them to hype the ingredient based on the original research using *the effective dosage*. Likewise, what good does it do to add 2000 mg of an absolutely useless ingredient that has been shown time and time again to have no impact on exercise performance or recovery, or that has never been tested at all for that purpose? For many companies it not only increases the number of ingredients on the nutrition panel but it also increases the gram weight of the serving, and hence the weight of the container. These companies bank on the fact that the consumers will do no real research of their own and instead be naively overwhelmed by a lengthy and confusing nutrition panel, a “heavy jug”, and fancy marketing within the major magazines. Such practices are commonplace within the sport supplement industry and, for these aforementioned reasons, give sport nutrition supplements a bad name.

This practice is akin to a bodybuilder going to a new gym and seeing that they have 12 different machines to train legs, all of which are shiny, appear effective, and have fancy pictures posted on them for instruction. Rather than waste time using each of the 12 machines for one set over the course of the workout, the bodybuilder decides to stick with what they knows works well and simply knocks out 10 sets of barbell squats and stiff leg deadlifts. Using this approach, the bodybuilder recognizes that it’s not about the *total number of ineffective* exercises that matters; it’s about selecting a *small number of effective exercises* and applying the correct “dosage” of

effort. In straightforward terms: Quality exercise done at the correct volume = effectiveness. The same principle applies with nutritional supplements: Quality ingredients provided at the correct dosage = effectiveness. It's really that simple, yet most companies don't have the initiative or integrity to toil over stacks of research literature to identify just what these quality ingredients actually are.

Also, many products contain large amounts of maltodextrin, which essentially comprises one-third to one-half of the entire gram weight. This is an inexpensive way for companies to increase the serving size and container weight while minimally increasing their costs. This is a *trick* to make consumers think they are getting more for their money. Another trick most companies selling pre workout products frequently use lies within their "supposed servings" per container. Many products claim to have 40 or 50 servings/scoops on the front of their product label, but when the consumer reads the directions for use they are instructed to take 2 or even 3 servings/scoops as opposed to the advertised 1 scoop (look on your current product's label). This scam is used to convince consumers that they are getting more for their money. *Muscle Marinade™ contains 30 "true" servings*, and each serving is designed to include the maximum clinically-supported efficacious dose of each performance/ recovery-impacting component and is devoid of inactive and useless ingredients and fillers. Therefore, only one serving/scoop is needed at any one time. No guessing. No experimentation. No false advertising. In fact, one serving is so substantial in strength that additional servings are strongly discouraged.

The text below provides specifics related to the Muscle Marinade™ formula. It is in no way meant to represent a detailed discussion of *all* available evidence for the highlighted ingredients. Readers are encouraged to review the reference data provided at the end of this paper for additional information. While dosages of individual ingredients vary considerably across studies and not every study using a particular ingredient has been met with positive effects, consumers should feel confident that a great deal of unbiased attention has been put into the decision to include the below-discussed ingredients (as well as the exclusion of other commonly used ingredients). All those mentioned and included within the formula have been proven to be effective in human subjects with oral consumption based on peer-reviewed scientific reports and anecdotal (in the gym) evidence. Moreover, as consistently mentioned, the dosage of each ingredient within Muscle Marinade™ matches the dosage used in these clinical studies. This is indeed a novel concept in the field of sport nutrition supplements. Because the referenced studies often provide ingredients to subjects on a daily basis over the course of days or weeks (e.g., creatine, beta alanine), it should be understood that the below-discussed effects for a given ingredient may only be observed after continued use of that ingredient. It is assumed that individuals will use Muscle Marinade™ on a daily basis along with their normal nutrition and exercise training program in order to reap the product's full benefits.

Muscle Marinade™: Energy Production, Stimulation, and Exercise Performance

Creatine—Overview

Creatine is a naturally occurring nitrogenous organic compound produced in the human body from the amino acids arginine, glycine, and methionine. While production occurs primarily in the kidney and liver, creatine is transported in the blood and taken up by other tissues (skeletal muscle primarily). To date, aside from carbohydrate, creatine is likely the most well-researched

sport supplement in history. In fact, a PubMed search performed on 11/12/09 using the term “creatine and exercise” returned 3217 articles, while the term “creatine and exercise performance” returned 588 articles. Clearly, this is a well-researched ingredient and is thought to pose no adverse effects to healthy individuals (Poortmans and Francaux, 2000).

While the effects of creatine supplementation are multiple, including antioxidant activity, maintenance of neuronal health, and improved cardiac muscle performance, the effect of most interest related to exercise performance in otherwise healthy individuals is improved performance during high intensity anaerobic exercise (Hespel and Derave, 2007). Creatine aids in adenosine triphosphate (ATP) resynthesis and can lead to high intensity performance improvements as demonstrated in literally hundreds of scientific studies. Creatine supplementation has also been associated with enhanced muscle hypertrophy, which may relate to satellite cell proliferation, as well as myogenic transcription factors and insulin-like growth factor-1 signaling. Other evidence indicates that creatine could enhance muscle glycogen accumulation and glucose transporter (GLUT4) expression. Positive findings for creatine are noted for both healthy and diseased populations. Although noted in animals and not human subjects, oral creatine supplementation has been shown to significantly increase carnosine (+88%) and anserine (+40%) content in skeletal muscle, which coincides with improved resistance to contractile fatigue (Derave et al., 2008). The physiological effect of carnosine is discussed below in the section on beta alanine.

Creatine—Form

Much discussion exists related to the optimal form of creatine to be used. While *creatine monohydrate* is certainly the most well-researched and most common form available, other forms such as creatine ethyl ester (CEE), di-creatine malate, tri-creatine citrate, creatine gluconate, creatine taurinate, creatine pyruvate, creatine l-pyroglutamate, and “pH balanced” creatine among others are currently marketed throughout the sport supplement industry. In addition, some companies are now using an agent known as creatinol-O-phosphate within their products. Although this agent is not technically creatine, some companies proceed to falsely market it as a super-creatine. Most reports for COP were published in the late 1970s in the journal entitled *Arzneimittelforschung*, and most studies focus on cardiac function with COP. A PubMed search indicates that there is only one study dealing with physical performance (Nicaise, 1975) and included 50 *female in-patients ranging in age from 58-96 years*. Patients were treated *intramuscularly and intravenously* (not orally) with 2 ampoules of 500 mg each of COP. Muscular strength was then measured by having women squeeze a bulb in each hand 5 times. Results were of statistical significance but were rather meaningless from a physiological perspective (e.g., sum of 85.86 vs. 90.40 (kg/cm²)10⁻¹ for placebo and intravenous COP, respectively; sum of 82.00 vs. 88.60 (kg/cm²)10⁻¹ for placebo and intramuscular COP, respectively). Perhaps companies have other data to support their use of COP in their products (although they must be quite obscure, because these are not readily available via PubMed). If companies are basing their use of COP on the particular study described above and assuming that because *intramuscular or intravenous* COP increased hand strength to a minor extent in *elderly in-patient women* that oral intake of COP will lead to increased strength in young healthy men and women, they really need to reevaluate their formulation guidelines or do some *real* applied research using this ingredient. Inclusion of COP within a formula designed for young healthy

men and women based on the data presented above is an absolute joke. Unfortunately, this is no exception in this industry.

While the more modern creatine versions are often heavily advertised so as to appear superior, there exists very little evidence that any of these creatine forms are better than creatine monohydrate, despite their substantial costs. For example, a study presented at the National Strength and Conditioning Association meeting in 2007 indicated that CEE was actually *less stable* than creatine monohydrate and experienced an accelerated breakdown to the byproduct known as creatinine (Child and Tallon, 2007). Other work agrees with this finding (Spillane et al., 2009). Authors comparing CEE with creatine monohydrate have concluded “when compared to creatine monohydrate, CEE was not as effective at increasing serum and muscle creatine levels or in improving body composition, muscle mass, strength, and power.” Investigators from another recent study concluded that “the half-life of CEE in blood is on the order of one minute, suggesting that CEE may hydrolyze too quickly to reach muscle cells in its ester form (Katseres et al., 2009). Collectively, these findings indicate that CEE is not a desired form of creatine to be used as a nutritional supplement.

Similar negative findings have been noted for the supposed “pH balanced” creatine known as Kre-Alkalyn® (Tallon and Child, 2007). Marketers claim that this product, unlike creatine monohydrate, is stabilized and will not undergo conversion into creatinine. To the contrary, investigators noted that the rate of creatinine formation for creatine monohydrate was <1% of the initial dose, indicating that creatine monohydrate is actually very stable under acidic conditions. Additionally, the Kre-Alkalyn® resulted in 35% *greater conversion* to creatinine than creatine monohydrate.

As for other creatine forms, very little research has been conducted to determine differences in either absorption or effectiveness as compared to creatine monohydrate. Therefore, at the present time, there is little to no evidence to support the use of forms other than creatine monohydrate. One recent study determined the plasma creatine appearance in men and women assigned to ingest a single dose of isomolar amounts of creatine (4.4 grams) as creatine monohydrate, tri-creatine citrate, or creatine pyruvate (Jäger et al., 2007). The investigators noted that while peak concentration and area under the curve of plasma creatine was highest for creatine pyruvate, there was no difference between the estimated velocity constants of absorption or elimination between the three creatine forms. These investigators concluded that “differences in bioavailability are thought to be unlikely *since absorption of creatine monohydrate is already close to 100%*. The small differences in kinetics are unlikely to have any effect on muscle creatine elevation during periods of creatine loading.” This is especially true considering that oral creatine monohydrate is rapidly and efficiently absorbed, a fact established over 10 years ago (Vanakoski et al., 1998).

Despite these solid findings related to the *already excellent absorption of creatine monohydrate*, new products continue to be developed in an attempt to further improve creatine absorption. One such product (BIOCREAT) was recently studied and reported to yield similar adaptations in both muscle strength and lean mass as compared to a creatine+carbohydrate supplement, with *no significant differences* noted between the two creatine conditions (Lewing et al., 2009). Another form recently studied is polyethylene glycosylated creatine (creatine bound to polyethylene

glycosylate [PEG]), hypothesized to result in increased creatine absorption and uptake into muscle cells (Herda et al., 2009). Subjects were assigned to a placebo condition, 1.80 or 3.60 grams of PEG (providing 1.25 and 2.50 grams of creatine, respectively), or 5 grams of creatine monohydrate per day for 30 days. Although the dosage of actual creatine was less in the PEG conditions, the results indicated that the *creatine monohydrate condition yielded similar or better results* in terms of lean body mass and performance improvement as compared to the PEG. These data reinforce the fact that creatine monohydrate, despite being considered “old school”, yields favorable results comparable or better than those observed with “new school” creatine forms.

Another new product, CON-CRĒT (Creatine HCL) marketed by ProMera health, is also being heavily advertised as a superior form of creatine in terms of solubility and absorption in the bloodstream. However, regardless of whether or not this is true (see below for more info), the company makes no claim related to the variable of *real* importance—*skeletal muscle creatine uptake*. While the product website indicates two university studies were conducted demonstrating this enhanced absorption, no reference data are provided, and no such studies are readily available via PubMed. Therefore, it is unknown whether or not the findings being claimed by ProMera health can stand up to the scrutiny of peer review. While it is possible that we may someday see published scientific evidence in support of CON-CRĒT (to date we simply have testimonials—which are alone, next to worthless in the scientific world), the question remains as to how much greater benefit a consumer *could* experience using this product (or any other novel creatine product for that matter) in order to justify the significant increase in cost as compared to creatine monohydrate. Aside from this important consideration, the fact that ProMera health *boldly and deceptively* states on their website, “CON-CRĒT is 59-times more potent than creatine monohydrate,” and “CON-CRĒT offers steroid-like results in strength, endurance and muscle recovery” is concerning to say the least. If the first claim were true, one serving (1500 mg for a 200 pound man) of CON-CRĒT would be equal to 88,500mg of creatine monohydrate. It is also stated on the website that one serving has potency equal to 5-10 grams of creatine monohydrate. There is clearly a discrepancy here within ProMera’s *own* claims. Such ridiculous and contradicting statements lead me to believe that this is yet another product fueled by pure marketing and hype, not hard scientific evidence.

As alluded to above, it should be understood that even if small differences in absorption time or concentration were noted between a novel form of creatine and creatine monohydrate, the question a consumer should have is “Who cares?” What real difference does this make considering creatine monohydrate already has absorption of close to 100% (Jäger et al., 2007)? Is it really worth paying more in order to use one of these hyped up novel creatine forms only to *maybe* experience a 5-10% increased plasma appearance rate? The rate of appearance of creatine is irrelevant anyway considering it is *intramuscular* and not plasma creatine that is important. Consumers also need to keep in mind that it is not the creatine taken immediately prior to each workout that is assisting in that particular workout; rather, it is the creatine that has been taken *repeatedly over time* that is now *built up within the muscle* that can provide for a benefit. Taking the daily dosage of creatine prior to (or immediately following) exercise makes good sense simply based on the fact that creatine transport into muscle may be enhanced due to the increased blood flow (Candow and Chilibeck, 2008) and possibly the increased activity of creatine transport proteins associated with acute exercise.

Aside from acute exercise, intake of creatine along with carbohydrate (usually simple sugars at high dosage; the basis of many creatine+carbohydrate products) has been shown to enhance creatine absorption in skeletal muscle (Green et al., 1996) and may enhance the effectiveness of creatine supplementation. Therefore, if adding extra carbohydrate to the diet does not interfere with daily caloric requirements, combining creatine and carbohydrate supplementation may be something to consider. That being said, PURUS LABS™ has chosen not to include carbohydrate within Muscle Marinade™, as more emphasis is placed on actual *active ingredients* rather than on inexpensive fillers. After all, carbohydrates are an inexpensive and readily-available addition if one chooses to include them.

Creatine—Dosage

Oral supplementation with creatine has been reported to substantially elevate the creatine content of human skeletal muscle. The most common dosage schedule in research studies has included a “loading” phase of 20 grams per day taken in 4 dosages of 5 grams each for a period of 5-7 days. Following this, creatine saturation in skeletal muscle can be maintained at a daily dosage as low as 2-5 grams for most individuals (Preen et al., 2003), although the International Society of Sport Nutrition (ISSN) has recommended a daily intake as high as 0.1 gram/kg body mass/day (Kerksick et al., 2008). As with all dietary supplements, individual needs may vary. As mentioned above, it has been suggested that creatine ingestion proximate to resistance exercise may be more beneficial for increasing muscle mass and strength than ingestion at times distant to the exercise session, possibly due to increased blood flow and therefore increased transport of creatine to skeletal muscle (Candow and Chilibeck, 2008). Hence, inclusion of creatine within a pre workout supplement appears logical, and this is why creatine is contained within Muscle Marinade™.

Beta alanine

Beta alanine, also referred to as 3-aminopropanoic acid, is a non-proteinogenic amino acid. Although initially discovered over 100 years ago, research with beta alanine pertaining to exercise performance in human subjects is relatively new, with the first scientific paper published just a few years ago. The plasma concentration of beta alanine is significantly and rapidly elevated following oral intake of beta alanine ranging from 20-40 mg/kg body mass (Harris et al., 2006). Moreover, the muscle carnosine (beta-alanyl-l-histidine) concentration, comprised of both beta alanine and histidine, is significantly increased when beta alanine is provided at a dosage of 3-6 grams per day (Harris et al., 2006). Carnosine helps to stabilize muscular pH by acting as a buffer for hydrogen ions that are released as a result of high intensity exercise. While not all studies have reported positive findings, the majority of work involving beta alanine supplementation indicates a significant performance-enhancing effect with regards to high intensity exercise.

One concern expressed in relation to beta alanine is the mild “prickling/tingling” sensation often felt soon after ingestion (e.g., as soon as 15 minutes and often lasting up to 60 minutes). This is referred to as paresthesia, and is thought to be caused by beta-alanine binding to nerve receptors and causing them to fire. While this is well-tolerated by some users, others would prefer not to feel this prickling/tingling. In a study involving acute ingestion of beta alanine at dosages of 10, 20, and 40 mg/kg body mass, extreme tingling was noted with the 40 mg/kg body mass dosage,

while only moderate tingling was experienced with the 20 mg/kg body mass dosage (Harris et al., 2006). Moreover, the increase in plasma beta alanine from the 10 to 20 mg/kg body mass dose was 6-8 fold, while the increase from 20-40 mg/kg body mass was only 2.2 fold. Peak plasma concentration of beta alanine occurred within 30-40 minutes following acute ingestion, and a subsequent study indicates that chronic supplementation (e.g., 15 days) does not affect this. Additionally, less beta alanine is lost in the urine following a 20 vs. 40 mg/kg body mass dosage. Therefore, based on the relatively small further increase in plasma beta alanine following ingestion of a single dosage from 20 to 40 mg/kg body mass, the fact that dosages as low as 2 grams per day have been found to be efficacious in scientific investigations (Van Thienen et al., 2009), and the fact that higher dosages of beta alanine lead to greater paresthesia, Muscle Marinade™ contains a dosage of beta alanine equivalent to 25 mg/kg body mass for an 80 kg man. This dosage should minimize profound paresthesia and is close to the dosage previously reported to increase muscle carnosine content by ~40% following four weeks of ingestion (Harris et al., 2006).

As discussed above in the section on creatine, although noted in animals and not human subjects, it has been reported that *creatine intake alone* results in enhanced muscle carnosine content (Derave et al., 2008). Considering this evidence, using an adequate dosage of creatine along with beta alanine may justify using a slightly lower dosage of beta alanine. As with creatine, it has been suggested that beta alanine uptake into skeletal muscle to form carnosine may be enhanced by carbohydrate intake due to the insulin response from such feeding. Again, users may add carbohydrate as they see fit.

Betaine

Betaine (chemically known as 2-(Trimethylammonio) ethanoic acid, hydroxide, inner salt) is an osmolyte (i.e., protects the cells against dehydration), an antioxidant agent, as well as a methyl group donor serving a chief purpose of lowering homocysteine (Olthof and Verhoef, 2005), a known risk factor for cardiovascular disease (Boushey et al., 1995). The B-vitamins folic acid (B₉), B₁₂, and B₆ are often used for this same purpose of lowering homocysteine. As a methyl group donor, betaine has a potential effect on creatine biosynthesis by providing a methyl group to guanidinoacetate via methionine that can synthesize creatine in skeletal muscle (du Vigneaud et al., 1946).

In regards to exercise performance, a few studies have been conducted over the past few years using betaine (*anhydrous form*). The dosage of betaine in these studies has been 2.5 grams per day. Muscle endurance (Hoffman et al., 2009) as well as muscular power and force (Maresh et al., 2007) have been reported to increase following 14 days of betaine supplementation. Mechanistically, betaine may improve exercise performance by providing antioxidant activity, maintaining cellular hydration, and increasing blood flow, the latter possibly mediated by the effect betaine has on increasing NO (unpublished data). Although betaine is relatively new to the sport nutrition market, PURUS LABS™ believes that this ingredient has promise as a sport supplement. For this reason it is included within Muscle Marinade™ at the proper, research-supported dosage.

1,3,7-trimethylxanthine

Commonly referred to as caffeine, 1,3,7-trimethylxanthine is very well studied in relation to exercise. Findings for improved aerobic (Ganio et al., 2009) and anaerobic (Davis and Green, 2009) exercise performance are common with acute ingestion of caffeine prior to exercise (typically 30-60 minutes prior). Multiple mechanisms are associated with caffeine's ergogenic effects including improved cognitive performance, increased catecholamine secretion and lipolysis, enhanced calcium mobilization and phosphodiesterase inhibition, enhanced Na⁺/K⁺ pump activity to enhance excitation contraction coupling, and adenosine receptor antagonism. While individual response to caffeine varies, dosages in the literature have generally ranged from 3-6 mg/kg body mass, and individuals who do not frequently use caffeine appear to respond to the greatest extent (Ganio et al., 2009).

2-amino-4-methylhexane

The ingredient 2-amino-4-methylhexane is a component of geranium oil and appears to provide a sympathomimetic effect in human subjects. That is, it mimics the effects of the sympathetic nervous system such as the chemicals epinephrine, norepinephrine, and dopamine. In this way it may stimulate energy release and provide a feeling of euphoria. Very little is known about this ingredient, but anecdotal reports are impressive. It should be noted that this is the one ingredient contained within Muscle Marinade™ that is not yet supported by peer-reviewed *published* clinical data. However, a controlled laboratory study investigating the effects of 2-amino-4-methylhexane combined with caffeine on resistance exercise performance in a sample of resistance trained men was recently completed (unpublished data). The results indicate that the simple combination of 2-amino-4-methylhexane and caffeine is as effective as the top selling pre workout powders currently being sold on the sport nutrition market in terms of enhancing upper-body muscular power and endurance (using bench press throws and bench press exercise to fatigue, respectively). These findings reinforce the position of PURUS LABS™ that the *correct ingredients* provided at the *correct dosages* are much more effective than the sheer number of ingredients. That is, 2-amino-4-methylhexane and caffeine (mixed into 16 grams of maltodextrin in an attempt to match the carbohydrate content of other pre workout powders used for comparison) was similar in effectiveness as the other products which contained *35-65 individual ingredients*! This is a great example of the “window dressing” hype within the sport supplement industry. It is truly a shame that most companies are more concerned with beefing up their product label with worthless ingredients used at ridiculously low dosages, rather than providing a solid dosage of *real* ingredients that actually have been shown in human subjects to yield an effect.

In addition to the laboratory study mentioned above, the dosage of 2-amino-4-methylhexane contained within Muscle Marinade™ is based on pilot testing in a variety of healthy men and women using this ingredient either alone at varying dosages, as well as in combination with caffeine. Subjective reports related to subjects' “perceived feeling of energy and focus” as well as subjects' actual exercise performances have guided the dosing of this ingredient. Indeed, further research is warranted in relation to 2-amino-4-methylhexane and exercise performance alone and in combination with other performance aids—to scientifically validate the inclusion of this ingredient.

Electrolytes

Electrolytes are ionized salts (dissociated into positive and negative ions) found within body fluids. Electrolytes serve the function of maintaining concentration and charge differences across cell membranes and are involved in neural and muscle cell functioning. In relation to dietary supplements, electrolytes are most commonly contained within sport drinks primarily for rehydration purposes and maintenance of blood flow. The chief electrolytes contained within such products appearing to have an effect on hydration status following strenuous physical exercise are sodium, chloride, potassium, and magnesium. Coupled with adequate fluid intake before, during, and following an acute exercise bout, the electrolyte mix contained within Muscle Marinade™ aids in maintaining optimal hydration. This effect may be assisted by the addition of the osmolyte betaine (as discussed above).

One other consideration, once again, is the ingestion of a dilute (6-10%) carbohydrate solution during the exercise bout. This will not only improve hydration status (Evan et al., 2009; von Duvillard et al., 2008) but will also serve the purpose of enhancing cellular immunity (Braun and Von Duvillard, 2004; Nieman et al., 2001) possibly working in conjunction with other immune-supporting agents within Muscle Marinade™ such as vitamin C and zinc. It should be understood that hypohydration (loss of fluid) is associated with increased cortisol and possibly increased protein catabolism/tissue breakdown (Judelson et al., 2008). Therefore, efforts to maintain hydration status during and following exercise should be undertaken by all serious trainees. Muscle Marinade™ imparts a precise blend of potassium, magnesium, sodium, and chloride to further compliment its performance-enhancement properties.

Muscle Marinade™: Exercise Recovery

Essential Amino Acids

Amino acids are critical to physiological function and have multiple roles within biological systems. There are both essential and non-essential amino acids; the former meaning that the body cannot synthesize these from other compounds at the level needed for normal growth; they must be supplied in the diet. Perhaps the most notable function of amino acids is to act as building blocks for proteins. Proteins are required for muscular growth and repair and are the dietary focus of most bodybuilders and fitness enthusiasts. While it is well accepted that active individuals require significantly more protein than their sedentary counterparts (Rodriguez et al., 2009), an often overlooked component related to protein intake is the specific *timing* of amino acid ingestion in the precise combination with relation to an acute bout of resistance exercise. Specifically, several studies support the use of a precise *essential amino acid* mixture prior to resistance exercise. These investigations have included an oral dosage of essential amino acids equal to 6 grams, taken both with (Tipton et al., 2001) and without (Bird et al., 2006a; 2006b; 2006c) carbohydrate. Findings from such studies indicate enhanced protein synthesis with ingestion of essential amino acids before resistance exercise to a greater extent than compared to essential amino acid ingestion post-exercise (Tipton et al., 2001; Wolfe, 2001). Post-exercise insulin has also been noted to be higher following intake of an essential amino acid mixture (Bird et al., 2006b) while both 3-methylhistidine (a marker of protein breakdown) (Bird et al., 2006b) and cortisol have been noted to be lower (Bird et al., 2006c). Taken together, these results suggest an “anti-catabolic effect” of essential amino acid ingestion.

Aside from acute intake, other work indicates that chronic (i.e., daily) intake of a precise 6 gram essential amino acid mixture attenuates 3-methylhistidine excretion during the days following strenuous exercise (Bird et al., 2006a). As with many aforementioned ingredients, it should be noted that carbohydrate added to the essential amino acid mixture provides additional anti-catabolic activity (Bird et al., 2006b). Therefore, if a diluted (6-10%) carbohydrate solution is tolerable from a caloric standpoint, Muscle Marinade™ could be mixed into a carbohydrate drink (e.g., juice) as opposed to water. An alternative would be to add carbohydrate powder (30-40 grams) to one scoop of Muscle Marinade™ and mix in water.

Vitamins and Minerals—Overview

According to the Center for Disease Control and Prevention, “vitamins are organic substances made by plants or animals, while minerals are inorganic elements that come from the earth. Soil and water are absorbed by plants and animals, and humans absorb minerals from the plants they eat.” While moderate levels of vitamins and minerals are necessary for normal growth, development, and metabolic processes, higher amounts of certain vitamins and minerals have been shown to provide protection against various stressors. One such stressor is heavy physical exercise. In an attempt to combat the stress caused by intense physical exercise, the use of antioxidant vitamins (C and E) and minerals (zinc and selenium) as well as complementary B-vitamins (B₆, B₉, and B₁₂) may be considered.

Vitamins and Minerals—Ascorbic Acid

Ascorbic acid, commonly known as vitamin C, is a water soluble vitamin with multiple physiological properties. It is one of the most well-researched antioxidants, particularly related to exercise-induced free radical production. When free radical production exceeds the body’s antioxidant defense mechanisms (by way of both endogenous antioxidant enzymes/thiols and exogenous antioxidant vitamins/minerals consumed through dietary sources) a condition referred to as *oxidative stress* may occur. Oxidative stress ultimately has the potential to damage cellular structures including phospholipid membranes, protein, mitochondria, and DNA (Valko et al., 2007). While a low level of free radical production is actually beneficial and necessary for normal physiological function, excessive radical production, which is common with strenuous physical exercise (Bloomer, 2008), can directly impair muscle contractile function and force. This may occur via defects in excitation-contraction coupling (Goldhaber and Qayyum, 2000) and lead to greater fatigue rates in skeletal muscle (Juel, 2006). An attempt to curtail this impairment is generally the rationale for inclusion of supplemental antioxidant vitamins/minerals for athletes.

While vitamin C has multiple physiological properties beyond the scope of this discussion (Deruelle and Baron, 2008), when considering the above, several studies have included vitamin C (typically at a dosage of 1000 mg/day and often in combination with alpha tocopherol [vitamin E]) in an attempt to lessen the oxidative stress and associated loss in muscle function. Although not all studies have noted effects for vitamin C in this regard, several have been met with positive findings for at least some biochemical or functional measures, as reviewed in detail by Fisher-Wellman and Bloomer (2009). In addition to acting as an antioxidant agent in the attenuation of exercise-induced oxidative stress, vitamin C has been reported to reduce the rise in circulating cortisol following exercise (Carrillo et al., 2008), as well as function as an immune-boosting nutrient (Wintergerst et al., 2005) with effects on reducing the incidence of the common

cold (Hemilä, 2004) and post-exercise upper respiratory tract infections (Peters et al., 1993). For these reasons, after careful review of the available literature, vitamin C intake is suggested at a daily dosage of 1000 mg (Deruelle and Baron, 2008) and is included within Muscle Marinade™ at this exact dosage. Intake of vitamin C at this dosage is well-absorbed and has been shown to significantly elevate plasma vitamin C concentration (Bloomer et al., 2006).

Vitamins and Minerals—d-alpha-tocopherol (natural vitamin E)

The lipid soluble vitamin referred to as alpha tocopherol works in conjunction with vitamin C (as well as other antioxidants such as selenium, zinc, and glutathione) in a process known as redox cycling. These antioxidants maintain each other in their reduced and active form. For this reason, inclusion of an antioxidant “complex” within a dietary supplement is most appropriate. *Alpha tocopherol* is typically used to denote vitamin E due to the fact that alpha tocopherol is the only form of vitamin E that is actively maintained in the body. However, it should be mentioned that a more correct depiction of vitamin E is the inclusion of alpha, beta, gamma, and delta tocopherols *and* tocotrienols (mixed tocopherols/tocotrienols). While results are mixed, some evidence indicates that the combination of all may best produce an antioxidant effect. Regardless, one finding related to vitamin E is common: Natural vitamin E (d-alpha tocopherol) is better absorbed and shows higher bio-potency (1.5-2 fold) than synthetic vitamin E (dl-alpha-tocopherol) (Hoppe and Krennrich, 2000). This may be due to the fact that the natural form consists of one isomer; in contrast, the synthetic form contains eight different isomers of which only one is the same as the natural form. The other seven isomers have been noted to range in potency from ~20 percent to 90 percent of natural vitamin E (d-alpha-tocopherol). Considering this, effective dosages of natural vitamin E can be lower than synthetic and have ranged from just slightly higher than the Recommended Daily Allowance (RDA) of 15 mg/day to several hundred mg/day. Due to the concern over high intake of lipid soluble vitamins, in particular vitamin E (Greenberg, 2005), the fact that vitamin E as low as 60 mg/day has been reported to provide effects (Meydani et al., 1997), and that many scientists believe that a dosage of 100IU (67 mg) to 200IU (134 mg) of natural d-alpha tocopherol is adequate (although this is refuted by some reports—see Roberts et al., 2007), Muscle Marinade™ contains a judicious dosage of vitamin E thought to be both safe and clinically effective when combined with the other antioxidants within the formula.

Aside from working in conjunction with vitamin C and other antioxidants, vitamin E independently functions as an immune-boosting agent (Meydani et al., 1997), a potent chain-breaking antioxidant to inhibit the oxidation of cellular lipids, and serves to stabilize cell membranes (e.g., sarcolemma) in conjunction with zinc (Chien et al., 2006). This often results in less leakage of intracellular components such as creatine kinase following muscle-damaging exercise (Fisher-Wellman and Bloomer, 2009).

Vitamins and Minerals—Selenium

Selenium is a trace element essential in small amounts, but like all essential elements, it is toxic at high levels. Humans and animals require selenium for the function of a number of selenium-dependent enzymes including glutathione peroxidase (GPx). Glutathione peroxidase is a collective term for a family of enzymes with antioxidant activity serving to reduce potentially damaging agents such as lipid hydroperoxides into alcohol and hydrogen peroxide into water. In this process, the important antioxidant glutathione (GSH) is “used up” and oxidized to GSSG.

The enzyme known as glutathione reductase then serves to reduce glutathione back to the active form (GSH). All of this occurs along with vitamin C and vitamin E in a process known as redox cycling. In this way, these antioxidants complement one another. In conjunction with vitamin C and vitamin E, Muscle Marinade™ contains a decisive dosage of selenium, the same dosage successfully used to combat exercise-induced oxidative stress (Goldfarb et al., 2005) and muscle damage/soreness (Bloomer et al., 2004).

Vitamins and Minerals—Zinc

Zinc is an essential trace element for all life forms playing roles in numerous aspects of cellular metabolism such as growth and development, the immune response, and neurological function. In conjunction with both vitamin C and vitamin E, zinc provides antioxidant and immune support with combination therapy commonplace in the literature (Huang et al., 2006; Wintergerst et al., 2006). Studies have repeatedly shown the beneficial properties of zinc as related to both antioxidant (Mocchegiani, 2008) and immune-stimulating function (Haase and Rink, 2009; Prasad, 2008). The dosage of zinc used in many studies has ranged from 15-50 mg/day. While zinc is used in many different forms (e.g., gluconate, picolinate, methionine), zinc methionine has been reported to have the greatest antioxidant activity (Bagchi et al., 1997) and superior bioavailability (Chien et al., 2006). This is, of course, the form of zinc utilized in Muscle Marinade™.

Vitamins and Minerals—B-vitamins (B₆, B₉, B₁₂)

B-vitamins are water soluble and important for a number of processes within the body involved in energy production (Woolf and Manore, 2006). Many common foods are fortified with B-vitamins, and a daily supplement is often recommended for individuals who do not eat adequate amounts of B-vitamin rich foods. The RDA for these vitamins is relatively low and can typically be met by consuming a good quality multivitamin. However, the requirements for B-vitamins may be increased by strenuous activity (Woolf and Manore, 2006). It has been recommended that pyridoxine HCL (vitamin B₆) intake be calculated based on the protein intake (0.02 mg per gram of protein) while cyanocobalamin (vitamin B₁₂) is usually recommended at 6 to 30 µg/day. Folic acid (vitamin B₉) is recommended at 400 µg/day and often higher for women who are pregnant or who are of child bearing years. As with betaine (discussed earlier in this paper) the B-vitamins act to reduce levels of the sulfur-containing amino acid homocysteine, an intermediate of methionine noted to be linked to an increased risk of cardiovascular disease (Boushey et al., 1995). Therefore, B-vitamins serve the dual role in athletes of enhancing energy production and potentially decreasing the risk for cardiovascular disease. Considering the above, Muscle Marinade™ contains adequate amounts of B-vitamins necessary for measureable effects.

SUMMARY

The pre workout dietary supplement Muscle Marinade™ was specifically engineered to meet the performance and recovery needs of hard-training men and women. Using a detailed, systematic, and scientifically-sound approach to product development, Muscle Marinade™ utilizes only research-supported ingredients at the precise dosages used in the clinical research studies. Collectively, the ingredients provided within Muscle Marinade™ serve the dual purpose of improving acute exercise performance and supporting post-exercise recovery. Muscle Marinade™ is clearly an avant-garde product at the pinnacle of its class and should be considered for inclusion within any athlete's arsenal. As with all nutritional supplements,

potential users should consult their personal physician prior to using Muscle Marinade™. In addition, potential users should review the product nutrition panel and label for information regarding ingredients, dosing, and precautions for use. For more information on PURUS LABS™ and its other performance/physique-enhancing products, please visit www.puruslabs.net.

REFERENCES

Adams, MR, Forsyth, CJ, Jessup, W, Robinson, J, and Celermajer, DS. Oral arginine inhibits platelet aggregation but does not enhance endothelium-dependent dilation in healthy young men. *J. Amer. Col. Cardiology*. 1995; 26(4):1054-1061.

Bagchi D, Bagchi M, Stohs SJ. Comparative in vitro oxygen radical scavenging ability of zinc methionine and selected zinc salts and antioxidants. *Gen Pharmacol*. 1997 Jan;28(1):85-91.

Beckman, JS, and Koppenol, WH. Nitric oxide, superoxide, and peroxynitrite: the good, the bad, and ugly. *Am. J. Physiol*. 1996; 271(5 Pt 1):C1424-1437.

Bird SP, Tarpenning KM, Marino FE. Independent and combined effects of liquid carbohydrate/essential amino acid ingestion on hormonal and muscular adaptations following resistance training in untrained men. *Eur J Appl Physiol*. 2006a May;97(2):225-38.

Bird SP, Tarpenning KM, Marino FE. Liquid carbohydrate/essential amino acid ingestion during a short-term bout of resistance exercise suppresses myofibrillar protein degradation. *Metabolism*. 2006b May;55(5):570-7.

Bird SP, Tarpenning KM, Marino FE. Effects of liquid carbohydrate/essential amino acid ingestion on acute hormonal response during a single bout of resistance exercise in untrained men. *Nutrition*. 2006 Apr;22(4):367-75.

Bloomer RJ. Nitric Oxide Stimulating Dietary Supplements: Where is the Evidence? *AgroFOOD industry hi-tech*. 2009; 20(1): 40-42.

Bloomer RJ. Nitric oxide supplements for sports. *Strength Cond J*. In Press.

Bloomer RJ, Goldfarb AH, McKenzie MJ. Oxidative stress response to aerobic exercise: comparison of antioxidant supplements. *Med Sci Sports Exerc*. 2006 Jun;38(6):1098-105.

Bloomer RJ, Goldfarb AH, McKenzie MJ, You T, Nguyen L. Effects of antioxidant therapy in women exposed to eccentric exercise. *Int J Sport Nutr Exerc Metab*. 2004 Aug;14(4):377-88.

Bode-Böger, SM, Boger, RH, Galland, A, Tsikas, D, and Frolich, J. L-arginine-induced vasodilatation in healthy humans: pharmacokinetic-pharmacodynamic relationship. *Br. J. Clin. Pharmacol*. 1998; 46(5):489-497.

Bode-Böger, SM, Böger, RH, Creutzig, A, Tsikas, D, Gutzki, FM, Alexander, K, and Frölich, JC. L-arginine infusion decreases peripheral arterial resistance and inhibits platelet aggregation in healthy subjects. *Clin. Sci. (Lond)*. 1994; 87(3):303-310.

Boushey CJ, Beresford SA, Omenn GS, Motulsky AG. A quantitative assessment of plasma homocysteine as a risk factor for vascular disease. Probable benefits of increasing folic acid intakes. *JAMA*. 1995;274(13):1049-1057.

- Braun WA, Von Duvillard SP. Influence of carbohydrate delivery on the immune response during exercise and recovery from exercise. *Nutrition*. 2004 Jul-Aug;20(7-8):645-50.
- Candow DG, Chilibeck PD. Timing of creatine or protein supplementation and resistance training in the elderly. *Appl Physiol Nutr Metab*. 2008 Feb;33(1):184-90.
- Carrillo AE, Murphy RJ, Cheung SS. Vitamin C supplementation and salivary immune function following exercise-heat stress. *Int J Sports Physiol Perform*. 2008 Dec;3(4):516-30.
- Chien XX, Zafra-Stone S, Bagchi M, Bagchi D. Bioavailability, antioxidant and immune-enhancing properties of zinc methionine. *Biofactors*. 2006;27(1-4):231-44.
- Child R, and Tallon MJ. Creatine ethyl ester rapidly degrades to creatinine in stomach acid. Presented at the National Strength and Conditioning Association Annual Meeting, 2007.
- Chin-Dusting, JP, Alexander, CT, Arnold, PJ, Hodgson, WC, Lux, AS, and Jennings, GL. Effects of in vivo and in vitro L-arginine supplementation on healthy human vessels. *J. Cardiovasc. Pharmacol*. 1996; 28(1):158-166.
- Davis JK, Green JM. Caffeine and anaerobic performance: ergogenic value and mechanisms of action. *Sports Med*. 2009;39(10):813-32.
- Derave W, Jones G, Hespel P, Harris RC. Creatine supplementation augments skeletal muscle carnosine content in senescence-accelerated mice (SAMP8). *Rejuvenation Res*. 2008 Jun;11(3):641-7.
- Deruelle F, Baron B. Vitamin C: is supplementation necessary for optimal health? *J Altern Complement Med*. 2008 Dec;14(10):1291-8.
- du Vigneaud V, Simonds S, Chandler JP, Cohn M: A further investigation of the role of betaine in transmethylation reactions in vivo. *J Biol Chem* 1946, 165:639-648.
- Erdman KA, Fung TS, Doyle-Baker PK, Verhoef MJ, Reimer RA. Dietary supplementation of high-performance Canadian athletes by age and gender. *Clin J Sport Med*. 2007 Nov;17(6):458-64.
- Evans GH, Shirreffs SM, Maughan RJ. Postexercise rehydration in man: the effects of carbohydrate content and osmolality of drinks ingested ad libitum. *Appl Physiol Nutr Metab*. 2009 Aug;34(4):785-93.
- Fisher-Wellman KH, & Bloomer RJ. Impact of vitamin C on exercise-induced oxidative stress and tissue injury. *Inter J Med Biol Front*. 2009: 16(5/6). pp.
- Froiland K, Koszewski W, Hingst J, Kopecky L. Nutritional supplement use among college athletes and their sources of information. *Int J Sport Nutr Exerc Metab*. 2004 Feb;14(1):104-20.

- Ganio MS, Klau JF, Casa DJ, Armstrong LE, Maresh CM. Effect of caffeine on sport-specific endurance performance: a systematic review. *J Strength Cond Res.* 2009 Jan;23(1):315-24.
- Gilligan DM, Panza JA, Kilcoyne CM, Waclawiw MA, Casino PR, and Quyyumi AA. Contribution of endothelium-derived nitric oxide to exercise-induced vasodilation. *Circulation* 1994; 90: 2853-2858.
- Giugliano, D, Marfella, R, Verrazzo, G, Acampora, R, Coppola, L, Cozzolino, D, and D'Onofrio, F. The vascular effects of L-Arginine in humans. The role of endogenous insulin. *J. Clin. Invest.* 1997; 99(3): 433-438.
- Gleeson M. Dosing and efficacy of glutamine supplementation in human exercise and sport training. *J Nutr.* 2008 Oct;138(10):2045S-2049S.
- Goldfarb AH, Bloomer RJ, McKenzie MJ. Combined antioxidant treatment effects on blood oxidative stress after eccentric exercise. *Med Sci Sports Exerc.* 2005 Feb;37(2):234-9.
- Goldhaber JJ, Qayyum MS. Oxygen free radicals and excitation-contraction coupling. *Antiox Redox Signal.* 2000;2(1):55-64.
- Green AL, Hultman E, Macdonald IA, Sewell DA, Greenhaff PL. Carbohydrate ingestion augments skeletal muscle creatine accumulation during creatine supplementation in humans. *Am J Physiol.* 1996 Nov;271(5 Pt 1):E821-6.
- Greenberg ER. Vitamin E supplements: good in theory, but is the theory good? *Ann Intern Med.* 2005 Jan 4;142(1):75-6.
- Haase H, Rink L. The immune system and the impact of zinc during aging. *Immun Ageing.* 2009 Jun 12;6:9.
- Harris RC, Tallon MJ, Dunnett M, Boobis L, Coakley J, Kim HJ, Fallowfield JL, Hill CA, Sale C, Wise JA. The absorption of orally supplied beta-alanine and its effect on muscle carnosine synthesis in human vastus lateralis. *Amino Acids.* 2006 May;30(3):279-89. Epub 2006 Mar 24.
- Hemilä H. Vitamin C supplementation and respiratory infections: a systematic review. *Mil Med.* 2004 Nov;169(11):920-5.
- Herda TJ, Beck TW, Ryan ED, Smith AE, Walter AA, Hartman MJ, Stout JR, Cramer JT. Effects of creatine monohydrate and polyethylene glycosylated creatine supplementation on muscular strength, endurance, and power output. *J Strength Cond Res.* 2009 May;23(3):818-26.
- Hespel P, Derave W. Ergogenic effects of creatine in sports and rehabilitation. *Subcell Biochem.* 2007;46:245-59.
- Hickner RC, Fisher JS, Ehsani AA, and Kohrt WM. Role of nitric oxide in skeletal muscle blood flow at rest and during dynamic exercise in humans. *Am. J. Physiol.* 273: H405-10, 1997.

Hiscock N, Pedersen BK. Exercise-induced immunodepression- plasma glutamine is not the link. *J Appl Physiol*. 2002 Sep;93(3):813-22.

Hoffman JR, Ratamess NA, Kang J, Rashti SL, Faigenbaum AD. Effect of betaine supplementation on power performance and fatigue. *J Int Soc Sports Nutr*. 2009 Feb 27;6:7.

Hoppe PP, Krennrich G. Bioavailability and potency of natural-source and all-racemic α -tocopherol in the human: a dispute. *Euro J of Nutr* 2000;39:183-93.

Huang HY, Caballero B, Chang S, Alberg A, Semba R, Schneyer C, Wilson RF, Cheng TY, Prokopowicz G, Barnes GJ 2nd, Vassy J, Bass EB. Multivitamin/mineral supplements and prevention of chronic disease. *Evid Rep Technol Assess (Full Rep)*. 2006 May;(139):1-117.

Jäger R, Harris RC, Purpura M, Francaux M. Comparison of new forms of creatine in raising plasma creatine levels. *J Int Soc Sports Nutr*. 2007 Nov 12;4:17.

Judelson DA, Maresh CM, Yamamoto LM, Farrell MJ, Armstrong LE, Kraemer WJ, Volek JS, Spiering BA, Casa DJ, Anderson JM. Effect of hydration state on resistance exercise-induced endocrine markers of anabolism, catabolism, and metabolism. *J Appl Physiol*. 2008 Sep;105(3):816-24.

Juel C. Muscle fatigue and reactive oxygen species. *J Physiol (Lond)*. 2006; 576(Pt 1).

Katseres NS, Reading DW, Shayya L, Dicesare JC, Purser GH. Non-enzymatic hydrolysis of creatine ethyl ester. *Biochem Biophys Res Commun*. 2009 Jun 12. [Epub ahead of print]

Kerksick C, Harvey T, Stout J, Campbell B, Wilborn C, Kreider R, Kalman D, Ziegenfuss T, Lopez H, Landis J, Ivy JL, Antonio J. International Society of Sports Nutrition position stand: nutrient timing. *J Int Soc Sports Nutr*. 2008 Oct 3;5:17.

Kurz, S, and Harrison, DG. Insulin and the arginine paradox. *J. Clin. Invest*. 1997; 99:369-370.

Lewing M, Pena E, Poole C, Dufour F, Consancio E, Jacobson H, Dugan K, Jones T, Ervin N, Foster C, Kreider R, Taylor L, Wilborn C. Effects of BIOCREAT supplementation on strength and body composition during an 8-week resistance training program. Presented at the International Society of Sport Nutrition Annual Meeting, 2009.

Maiorana A, O'Driscoll G, Taylor R, and Green D. Exercise and the nitric oxide vasodilator system. *Sports Med*. 2003; 33: 1013-1035.

Maresh CM, Farrell MJ, Kraemer WJ, Yamamoto LM, Lee EC, Armstrong LE, Hatfield DL, Sokmen B, Dias JC, Spiering BA, Anderson JA, Volek JS. The effects of betaine supplementation on strength and power performance. Presented at the American College of Sports Medicine Annual Meeting, 2007.

Meydani SN, Meydani M, Blumberg JB, Leka LS, Siber G, Loszewski R, Thompson C, Pedrosa MC, Diamond RD, Stollar BD. Vitamin E supplementation and in vivo immune response in healthy elderly subjects. A randomized controlled trial. *JAMA*. 1997 May 7;277(17):1380-6.

Mocchegiani E; Zincage Consortium. Zinc, metallothioneins, longevity: effect of zinc supplementation on antioxidant response: a Zincage study. *Rejuvenation Res*. 2008 Apr;11(2):419-23.

Nicaise J. Creatinol O-phosphate (COP) and muscular performance: a controlled clinical trial. *Curr Ther Res Clin Exp*. 1975 Jun;17(6):531-4.

Nieman DC. Exercise immunology: nutritional countermeasures. *Can J Appl Physiol*. 2001;26 Suppl:S45-55.

Olthof MR, Verhoef P. Effects of betaine intake on plasma homocysteine concentrations and consequences for health. *Curr Drug Metab*. 2005 Feb;6(1):15-22.

Peters EM, Goetzsche JM, Grobbelaar B, Noakes TD. Vitamin C supplementation reduces the incidence of postrace symptoms of upper-respiratory-tract infection in ultramarathon runners. *Am J Clin Nutr*. 1993 Feb;57(2):170-4.

Poortmans JR, Francaux M. Adverse effects of creatine supplementation: fact or fiction? *Sports Med*. 2000 Sep;30(3):155-70.

Prasad AS. Zinc in human health: effect of zinc on immune cells. *Mol Med*. 2008 May-Jun;14(5-6):353-7.

Preen D, Dawson B, Goodman C, Beilby J, Ching S. Creatine supplementation: a comparison of loading and maintenance protocols on creatine uptake by human skeletal muscle. *Int J Sport Nutr Exerc Metab*. 2003 Mar;13(1):97-111.

Roberts LJ 2nd, Oates JA, Linton MF, Fazio S, Meador BP, Gross MD, Shyr Y, Morrow JD. The relationship between dose of vitamin E and suppression of oxidative stress in humans. *Free Radic Biol Med*. 2007 Nov 15;43(10):1388-93.

Robinson, TM, Sewell, DA, and Greenhaff, PL. L-arginine ingestion after rest and exercise: effects on glucose disposal. *Med. Sci. Sports Exerc*. 2003; 35:1309-1315.

American Dietetic Association; Dietitians of Canada; American College of Sports Medicine, Rodriguez NR, Di Marco NM, Langley S. American College of Sports Medicine position stand. Nutrition and athletic performance. *Med Sci Sports Exerc*. 2009 Mar;41(3):709-31.

Spillane M, Schoch R, Cooke M, Harvey T, Greenwood M, Kreider R, Willoughby DS. The effects of creatine ethyl ester supplementation combined with heavy resistance training on body

composition, muscle performance, and serum and muscle creatine levels. *J Int Soc Sports Nutr.* 2009 Feb 19;6:6.

Tallon MJ, Child R. Kre-alkalyn® supplementation has no beneficial effect on creatine-to-creatinine conversion rates. Presented at the National Strength and Conditioning Association Annual Meeting, 2007.

Tipton KD, Rasmussen BB, Miller SL, Wolf SE, Owens-Stovall SK, Petrini BE, Wolfe RR. Timing of amino acid-carbohydrate ingestion alters anabolic response of muscle to resistance exercise. *Am J Physiol Endocrinol Metab.* 2001 Aug;281(2):E197-206.

Valko M, Leibfritz D, Moncol J, Cronin MT, Mazur M, Telser J. Free radicals and antioxidants in normal physiological functions and human disease. *Int J Biochem Cell Biol.* 2007;39(1):44-84.

Vanakoski J, Kosunen V, Meririnne E, Seppälä T. Creatine and caffeine in anaerobic and aerobic exercise: effects on physical performance and pharmacokinetic considerations. *Int J Clin Pharmacol Ther.* 1998 May;36(5):258-62.

Van Thienen R, Van Proeyen K, Vanden Eynde B, Puype J, Lefere T, Hespel P. Beta-alanine improves sprint performance in endurance cycling. *Med Sci Sports Exerc.* 2009 Apr;41(4):898-903.

von Duvillard SP, Arciero PJ, Tietjen-Smith T, Alford K. Sports drinks, exercise training, and competition. *Curr Sports Med Rep.* 2008 Jul-Aug;7(4):202-8.

Wilson, AM, Harada, R, Nair, N, Balasubramanian, N, and Cooke, JP. L-arginine supplementation in peripheral arterial disease: no benefit and possible harm. *Circulation.* 2007; 116(2):188-195.

Wintergerst ES, Maggini S, Hornig DH. Immune-enhancing role of vitamin C and zinc and effect on clinical conditions. *Ann Nutr Metab.* 2006;50(2):85-94. Epub 2005 Dec 21.

Wink DA, Miranda KM, Espey MG. Cytotoxicity related to oxidative and nitrosative stress by nitric oxide. *Exp Biol Med (Maywood).* 2001 Jul;226(7):621-3.

Wolfe RR. Effects of amino acid intake on anabolic processes. *Can J Appl Physiol.* 2001;26 Suppl:S220-7.

Woolf K, Manore MM. B-vitamins and exercise: does exercise alter requirements? *Int J Sport Nutr Exerc Metab.* 2006 Oct;16(5):453-84.