Comment on: “Creatine Monohydrate Supplementation, but not Creatyl-L-Leucine Increased Muscle Creatine Content in Healthy Young Adults: A Double-Blind Placebo-Controlled Trial”

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Recently, an article by Askow et al. (2022) was published ahead of print in the journal on August 25, 2022. Researchers in this study recruited 29 participants and had them consume 5 g/day of either creatine monohydrate (CM), creatyl-L-leucine (CLL), or a placebo for 14 days in a randomized, double-blind design. During this time, participants also performed three resistance training sessions per week. Muscle creatine content was quantified via muscle biopsies pre- and postintervention. The authors reported that CM supplementation significantly increased muscle creatine content within 14 days of supplementation, but no significant changes in muscle creatine content were observed for the placebo or CLL groups.

This letter will highlight a critical flaw in the design of the study, draw attention to other study limitations or inconsistencies, and point to a calculation error in the discussion. It will also question the reported initial and ending values of muscle creatine content as compared to other literature. Moreover, it will discuss the rather high increases in muscle creatine content for the CM group based on the dose used and the length of the study as compared to foundational work in this area. Finally, a concern for not fully disclosing a conflict of interest in the investigation will be emphasized, especially considering that major study limitations were not mentioned as part of their discussion, which is quite alarming.

The critical flaw of this investigation lies in the dosage of CM (C6H11N3O3) and CLL (C10H20N4O3) provided to the participants. Creatine, while not a traditional proteinogenic amino acid, is frequently referred to as an amino acid in the literature (Antonio et al., 2021; Bonilla et al., 2021; Fazio et al., 2022) because it can be considered a nonproteinogenic amino acid similar to ornithine, citrulline, and homoserine (Brosnan & Brosnan, 2007; Lu & Freeland, 2006; Ostojic, 2021). In a general sense, an amino acid can be any organic compound that contains both an amino group and a carboxyl group (Lu & Freeland, 2006). As such, CLL is a creatine dipeptide made of the amino acids creatine and L-leucine that are connected by a covalent bond (Reddeman et al., 2018) with a molecular weight of ~244 g/mole. On the other hand, CM has a molecular weight of ~149 g/mole and is made up of creatine and water molecule. This information is critical because 5 g of CM does not provide equimolar quantities of creatine when compared to 5 g of CLL. In particular, there is a simple relationship between grams and moles as demonstrated by this general chemistry formula: $M_s = (m_s + N_s)$ where $M_s$ is the molar mass of the substance in grams/mole, $m_s$ is the mass of the substance in grams, and $N_s$ is the quantity of the substance in moles. Using this equation, 5 g of CM provides ~0.03356 moles of creatine and 5 g of CLL provides ~0.02049 moles of creatine; thus, 5 g of CM yields ~0.01307 more moles of creatine than 5 g of CLL or ~64% more moles of creatine. By using Avogadro’s number to calculate the difference in creatine molecules from 5 g of CM versus 5 g of CLL to further elucidate this critical flaw, the CM group received $7.85 \times 10^{21}$ more creatine molecules than the CLL group. For obvious reasons, providing ~64% more molar creatine to the participants biases the study in favor of CM and against CLL. In order to provide equivalent molar quantities of creatine, the participants in the CLL group should have received 8.2 g of CLL as opposed to the underdosed 5 g of CLL.

Other major limitations with this investigation are the low doses and length of time used to load muscle creatine. For example, muscle creatine content can be rapidly increased via creatine supplementation by ingesting 20 g of CM per day for ~6 days; the higher muscle creatine content can then be maintained by ingesting ~2 g of CM per day (Hultman et al., 1996). Alternatively, ingesting 3 g of CM per day over a minimum of 4 weeks (28 days) can also be used to slowly increase muscle creatine content to similar levels as achieved by the creatine loading strategy (20 g of CM per day for 6 days; Hultman et al., 1996). Since no loading strategy was used and the duration of this study was only 14 days, this study is not ideally designed to increase muscle creatine content. More importantly, ~64% more molar creatine was provided to the CM group as compared to the CLL group and 14 days was not nearly long enough to load muscle creatine levels with an equivalent CM dosage of ~3 g/day. As previously stated, it takes a minimum of 28 days to increase muscle creatine content when ingesting only 3 g of CM per day (Antonio et al., 2021; Hultman et al., 1996), but this study design only supplemented participants for 14 days.

To highlight this limitation further, a recent meta-analysis examined the effects of different CM dosing strategies (lower: ≤5 g/ day and higher: >5 g/day), with and without a creatine loading phase (≥20 g/day for 5–7 days) and reported that when studies involving a creatine loading phase were excluded from the analyses, CM supplementation had no greater effect on strength as compared to a placebo (Forbes et al., 2021). Similarly, another study showed that when subjects consumed 6 g of CM per day for 6 days without a loading phase, no significant differences were observed in peak power, mean power, or total work (Hoffman et al., 2017).

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The results of these studies, which show no changes in training performance with relatively low doses of CM provided in the short term (i.e., <28 days), interestingly align with the results reported in Table 4 in this investigation. Hence, a loading phase appears to be critical to fully reap the ergogenic benefits of creatine supplementation when lower doses (i.e., 3–5 g) of creatine are used for <28 days (Antonio et al., 2021).

Although the authors of the investigation do refer to Hultman et al.’s (1996) study in the discussion of their manuscript, they inappropriately reference it to justify their methodology by stating the following:

... the typical use of CLL as a component of a beverage does not allow for a traditional loading phase. Thus, we elected to forgo the loading phase in our study. While this likely reduced the rate at which muscle creatine accumulated, foundational work in this area demonstrated that this strategy is a valid approach to saturate muscle creatine (Hultman et al., 1996). Along those lines, Hultman et al. (1996) report a ∼12% increase in muscle creatine following 14 days supplementation with CrM [creatine monohydrate] (3g/day). This is slightly lower than the ∼24% increase we observed in our study for the CM group, which is to be expected considering participants in the current investigation consumed a higher dose of CM (5 g/day) compared with Hultman et al. (1996).

CLL is indeed not sold as a stand-alone nutritional supplement as it is only found as one of several ingredients in a preworkout supplement or in a multi-ingredient energy drink. However, to test its effectiveness in increasing muscle creatine content as compared to the gold standard (CM), then equimolar quantities of creatine should be provided to each group and an appropriate dose should be used to increase muscle creatine content based on the length of the study. In this instance, the study should have been carried out for at least 28 days using equimolar quantities of creatine via lower doses of CM (5 g) and CLL (8.2 g). Alternatively, a creatine loading protocol for 5–7 days with equimolar quantities of creatine (20 g/day of CM vs. 32.8 g/day of CLL) could have been implemented.

Moreover, to state that a 12% increase in muscle creatine content is “slightly lower” than the 24% increase in their study is not only understated as that is a 100% difference, but it is inaccurate. The muscle creatine content in the CM group reported by the authors increased from 43.3 ± 6.2 mmol/kg of wet weight to 52.9 ± 7.8 mmol/kg of wet weight; this is a 22% increase and not a 24% increase as mistakenly reported by the authors. Regardless of this calculation error, this is still 83% higher than the 12% increase in muscle creatine content reported by Hultman et al. (1996) after ingesting 3 g of CM for 14 days. Most creatine loading studies using 20 g of CM per day only report average increases of muscle creatine content of ∼15%–25% in participants after 5–7 days of loading when carbohydrates are excluded (Harris et al., 1992; Hultman et al., 1996; Powers et al., 2003; Syrotuik & Bell, 2004). As stated previously, using lower quantities of creatine supplementation (i.e., 3 g of CM) raises muscle content creatine levels to only ∼12% (Hultman et al., 1996). Hence, for this investigation to report a (corrected) 22% increase in muscle creatine content with ingesting only 5 g of CM is rather remarkable since this is significantly higher than that reported by most CM loading studies where doses of CM are four times as much. Considering the impressive muscle creatine loading effects reported in the CM group, it is quite unexpected that no improvements in training performance, as previously stated, were reported. Indeed, one would expect concurrent increases in training performance with such large increases in muscle creatine content after CM supplementation as reported in other creatine literature (Kreider et al., 2017); however, the authors fail to address this in their discussion.

It should also be noted that the authors of this study reported muscle creatine content levels in millimole per kilogram of wet weight, but it is customary to report muscle creatine content level in millimole per kilogram of dry weight as reported in various creatine publications (Febbraio et al., 1995; Hultman et al., 1996; Kreider et al., 2017; Powers et al., 2003). Nevertheless, the content of water in muscle has been reported to range from ∼75% to 79% (Hargens et al., 1983; Mannion et al., 1993; Mitchell et al., 1945; Ward & Lieber, 2005) with a mean content of ∼77%; thus, data between muscle wet weight and muscle dry weight can be compared by applying the following conversion factor: 1 mmol/kg dry muscle mass = 0.23 mmol/kg wet muscle mass (Mannion et al., 1993). Importantly, this equation uses a constant of 77% for the water content of muscle, but this can vary depending on muscle glycogen content as ∼3–4 g of water is bound with each gram of glycogen (Olsson & Saltin, 1970). Despite the assumption of muscle being ∼77% water in the equation above, it is unlikely that the participants of this study had significantly depleted muscle glycogen levels to meaningfully decrease the content of water in muscle. In particular, the participants in this study were reported to abstain from exercise or other physical activities beyond activities of daily living for 72 days prior to testing and the habitual mean carbohydrate intake of the participants was reported to be ∼191 g/day. As such, it is highly unlikely that the muscle glycogen level in the participants of this study was depleted to the point that the content of water in muscle was much different than the ∼75%–79% reported in previous investigations. Table 1 demonstrates what the reported pre- and postintervention values of total muscle creatine content are in millimole per kilogram of wet weight and the converted values of muscle creatine content in millimole per kilogram of dry weight as per the equation above.

It is well known that the total creatine muscle averages ∼120 mmol/kg of dry muscle mass for a 70 kg individual and that the upper limit of creatine storage is ∼160 mmol/kg of dry muscle mass (Green et al., 1996; Hultman et al., 1996; Kreider et al., 2017). Considering these values, it is bewildering why the initial starting muscle creatine content levels in

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<th>Table 1</th>
<th>Pre- and Postintervention Muscle Creatine Content in Wet Weight Converted to Dry Weight</th>
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<tr>
<td></td>
<td>Pre: Wet weight (mmol/kg)</td>
</tr>
<tr>
<td>CM</td>
<td>43.3</td>
</tr>
<tr>
<td>CLL</td>
<td>39.1</td>
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<td>PLA</td>
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Note. CM = creatine monohydrate; CLL = creatyl-l-leucine; PLA = placebo.
millimole per kilogram of dry muscle were all significantly higher than the established upper limit of ~160 mmol/kg of dry muscle for all three groups. Moreover, the postintervention values of muscle creatine content in all three groups elevated to even higher levels, with the CM group reaching the highest value of 230.0 mmol/kg of dry muscle mass. It is concerning that the authors did not contextualize these muscle creatine values with those of the literature, given their unsupplemented values alone supersede the generally accepted upper limit in skeletal muscle. A rationale for reporting muscle creatine content in millimole per kilogram wet weight (as opposed to the standard millimole per kilogram dry weight) might resolve this concern and rectify their data in the context of the existing literature.

Finally, the authors of this study provide the following acknowledgments: “The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest. This study was funded by Monster Energy Company.” While they do disclose that Monster Energy, Inc. sponsored the study, they did not reveal that the findings of this study were being used as part of evidence in a $293 million dollar lawsuit involving Monster Energy, Inc. and one of its major competitors, Bang Energy, Inc. (Brittain, 2022). In fact, Bang Energy holds the patent on CLL, and Monster Energy filed a false-advertising lawsuit against Bang Energy over the CLL ingredient claiming it was not a form of creatine. Thus, since Monster Energy sponsored this study, there is an inherent conflict of interest that should have been disclosed since the results of this study likely impacted the outcome of the lawsuit. In light of this conflict of interest, the fact that the study was biased in favor of CM and against CLL by providing ~64% more moles of creatine to the CM group is quite alarming. Moreover, the publication states that “CLL was provided by the sponsor (lot no. 201907016)”; however, no independent laboratory certificate of analysis was reported verifying the purity and integrity of the CLL supplement despite the conflict of interest. Unfortunately, the critical flaws in the study, among the other study limitations mentioned, were not even mentioned in the discussion.

In conclusion, there are many concerns with the data reported and the conclusions drawn from this investigation. In light of the inherent conflict of interest of Monster Energy, Inc. funding this study, the flaws in the design of the study are unsettling. In particular, using unequal molar quantities of creatine between the groups and not using appropriate doses and/or length of time to load muscle creatine content brings about many questions. Beyond this, there are concerns related to the data presented in the initial and ending values of muscle creatine content that do not coincide with expected increases in training performance.

Given the concerns discussed above, I welcome the authors to comment on the following: (a) biased methodology, (b) significantly higher than normal starting and ending muscle creatine content levels relative to other literature, (c) reasons for reporting muscle creatine content in wet weight versus the commonly used dry weight, (d) lack of improvements in training performance in the CM group despite the reported high increases in muscle creatine content, (e) reasons for not addressing any of the major limitations of their study design in the discussion, (f) not fully disclosing the inherent conflict of interest, and (g) not reporting an independent laboratory certificate of analysis for the CLL supplement considering the conflict of interest of the sponsor of the study.

**Acknowledgments**

Escalante has served as an independent scientific consultant for Bang Energy, Inc. on various projects since 2018. Most recently, he was a creative expert witness for Bang Energy, Inc. for lawsuits involving Monster Energy, Inc. versus Bang Energy, Inc. He served on the Board of Directors for Bang Energy, Inc. in 2022. Additionally, he serves on the Scientific Advisory Board and as the lead Physique and Bodybuilding Program subject matter expert for the National Academy of Sports Medicine. St. Mart is an employee of the U.K. supplement brand Supplement Needs where he serves as the product formulator for the company. St. Mart does not own any formulas or intellectual property with any alternative forms of creatine, and the company only stocks a single source of CM.

**References**


