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# Human skeletal muscle has large capacity to increase carnosine content in response to beta-alanine supplementation: a systematic review with Bayesian individual and aggregate data E-Max model and meta-analysis.

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2019

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1 **Human Skeletal Muscle has Large Capacity to Increase Carnosine Content in Response to Beta-Alanine Supplementation.**

2 ***A Systematic Review with Bayesian Individual and Aggregate Data E-Max Model and Meta-Analysis***

3  
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34 **ABSTRACT:**

35 Beta-alanine (BA) supplementation increases muscle carnosine content (MCarn), and is ergogenic in many situations. Currently, many questions on the  
36 nature of the Mcarn response to supplementation are open, and the response to these has considerable potential to enhance the efficacy and applications  
37 of this supplementation strategy. **Objective:** To conduct a Bayesian analysis of available data on the Mcarn response to BA supplementation. **Methods:** A  
38 systematic review with meta-analysis of individual and published aggregate data using a dose response (Emax) model was conducted. The protocol was  
39 designed according to PRISMA guidelines. A three-step screening strategy was undertaken to identify studies that measured the Mcarn response to BA  
40 supplementation. In addition, individual data from 5 separate studies conducted in the authors' laboratory were analysed. Data were extracted from all  
41 controlled and uncontrolled supplementation studies conducted on healthy humans. Meta-regression was used to consider the influence of potential  
42 moderators (including dose, sex, age, baseline Mcarn and analysis method used) on the primary outcome. **Results and Conclusion:** The Emax model  
43 indicated that human skeletal muscle has large capacity for non-linear Mcarn accumulation, and that commonly used BA supplementation protocols may  
44 not come close to saturating muscle carnosine content. Neither baseline values, nor sex, appear to influence subsequent response to supplementation.  
45 Analysis of individual data indicated that Mcarn is relatively stable in the absence of intervention, and effectually all participants respond to BA  
46 supplementation (99.3% response [95%CrI: 96.2 – 100]).

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48 **Key-Words:** Beta-alanine; histidine containing dipeptides; dose; supplement; performance; metabolism.

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68 **INTRODUCTION**

69 Carnosine is a dipeptide formed from the amino acids  $\beta$ -alanine and L-histidine, and is present in high concentrations in human skeletal muscle  
70 (approximately 20 - 30 mmol·kg<sup>-1</sup> dry muscle). Its purported roles include: proton buffering [1]; anti-oxidation [2]; anti-glycation; metal chelation [3] and  
71 influencing calcium sensitivity [4,5], and hence muscle contractility. Although *in vitro* evidence supports carnosine's capacity to contribute to each of these  
72 processes, the strongest line of *in vivo* and *in vitro* evidence supports an important role for carnosine in intracellular skeletal muscle buffering [3,6]. The  
73 pKa of carnosine's imidazole ring (6.83 [7]) renders it ideally placed to aid in pH regulation within the physiological range of skeletal muscle (which  
74 decreases from approximately 7.1 at rest to 6.5 after exhaustive exercise [8]). This mechanistic action is particularly relevant in a sporting context, given  
75 that sustained high-intensity efforts are largely fuelled by anaerobic bioenergetic pathways, which lead to hydrogen ion accumulation and an acidic  
76 environment. Acidosis is known to contribute to fatigue and limit performance via a wide range of mechanisms [9]. As such, the presence of intracellular  
77 pH buffers such as carnosine are essential to maintain high intensity muscle contraction, and hence to sustain performance.

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79 Given the importance of carnosine to athletic performance, considerable research efforts have been made to investigate both means to increase it, and  
80 in what situations such increases are ergogenic. In 2006, Harris and colleagues [10] published a series of studies indicating that  $\beta$ -alanine (BA) availability  
81 was the limiting factor in intramuscular carnosine synthesis, and that supplementation with this amino acid could substantially increase muscle carnosine  
82 content (MCarn). Shortly after, the same group reported that BA supplementation (mean of 5.2 g·day<sup>-1</sup> for 4 weeks) was ergogenic to high-intensity exercise  
83 performance [11]. Since then, the ergogenic benefits of this supplementation strategy have been tested and proven in a wide range of models and recently,  
84 our group published a meta-analysis showing that BA supplementation is most ergogenic in capacity-based exercise tests that last between 30 seconds  
85 and 10 minutes [12]. This strong evidence supporting the ergogenic potential of BA supplementation has earned it its place as one of the world's most  
86 popular sports supplements, and it is one of just five ergogenic supplements endorsed by the International Olympic Committee [13].

87

88 It seems that substantial amounts of BA are required to increase MCarn content, with most studies using doses of approximately 3.2 – 6.4 g·day<sup>-1</sup>, for  
89 periods ranging from 4 – 24 weeks. But many questions about the nature of the muscle carnosine response to BA supplementation remain open, and  
90 substantial research efforts are being made to refine BA dosing strategies in order to optimize its efficacy and applicability [14]. For example, inter-  
91 individual variation in response to supplementation is high [12], yet little is known about what factors underpin this [15]. What is the capacity of the muscle  
92 to uptake BA and increase MCarn? What is the individual proportion of response to BA supplementation, and do baseline levels dictate the extent of this?  
93 Do sex or age influence response to supplementation? To address these questions, we employed a comprehensive analysis that included individual  
94 participant data from multiple studies conducted within our laboratory; and combined these findings with summary published data using a frequently  
95 used dose-response model (Emax).

96

97 **METHODS**

98 The protocol for this study was designed according to the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) guidelines. The  
99 *Population, Intervention, Comparator, Outcomes and Study Design (PICOS)* approach was used to guide the inclusion and exclusion of studies for this  
100 review and are described in Table 1.

101

102 **Table 1:** Study Inclusion and Exclusion Criteria

<b>Population:</b>	Healthy individuals of any age or physical activity level.
<b>Intervention:</b>	Original studies investigating the effects of oral BA supplementation on skeletal MCarn content.
<b>Comparator:</b>	No human comparators were required in the studies included in this review, although the data from placebo groups were used to quantify biological variability across the times periods investigated, when available.
<b>Outcomes:</b>	The primary outcome was the effect of BA supplementation on skeletal MCarn concentration. Potential moderators to this response included dose, sex, age, baseline MCarn and the method used to measure MCarn.
<b>Study Design:</b>	Controlled or uncontrolled intervention studies.

103

104 *Search Strategy*

105 The search strategy was based on a three-step screening (title/abstract screening, full-text screen and full text appraisal), independently undertaken by  
106 two reviewers. This search was originally conducted to inform a systematic risk assessment on the use of BA supplementation [16]. This risk assessment  
107 included all BA supplementation studies (including both human and animal models). One hundred and one human studies were included in that  
108 investigation, and were subsequently screened to identify those that included a MCarn measurement. The search strategy, including databases and  
109 keywords used has been described in detail elsewhere [16] and the protocol for that review was prospectively registered ((PROSPERO registration no.  
110 CRD42017071843).

111

112 *Data Analysis*

113 The present study comprised both individual and aggregate data meta-analyses from a Bayesian perspective. Individual data were pooled using Bayesian  
114 mixed effects multilevel models. Analyses were performed on the outcome variable MCarn (absolute value) to quantify the effects of beta-alanine  
115 supplementation and random noise due to biological variation and measurement error. Additionally, proportion of response was estimated across  
116 controlled studies by calculating interindividual difference in response to supplementation and comparing this to a non-zero increase in MCarn [17].  
117 Bayesian estimates of the standard deviation in observed change from active and placebo groups were used to obtain the intervention response standard  
118 deviation ( $\hat{\sigma}_{IR}$ ) describing interindividual difference in response. Aggregate data meta-analyses were performed using published pre- and post-intervention  
119 mean and standard deviation values. Values were transformed into standardized mean differences (SMD) and sampling variance calculated using methods  
120 described previously [18]. Three-level mixed effects models were used to quantify the effects of supplementation dose. Insufficient data were available to  
121 allow investigation of the interaction between daily dose and intervention duration and so the total cumulative dose ingested was selected as the primary  
122 outcome, which previous research has identified as being more influential than either daily dose or intervention duration [16,19,20]. Subset analyses using  
123 study covariates were used to assess the effects of sex, age or measurement method on the main effect of BA supplementation. Finally, a model-based  
124 approach was employed to investigate the dose-response relationship between cumulative BA supplementation and the SMD. A standard four parameter  
125 sigmoid predicted maximum effect (Emax) model was estimated with:

126

$$E = E_0 + \frac{E_{max} \times C^\gamma}{EC_{50}^\gamma + C^\gamma}$$

127 Where  $E$  is the effect size (SMD),  $E_0$  is the baseline effect,  $E_{max}$  is the maximum effect,  $EC_{50}$  is the cumulative dose that provides 50% of the maximum  
128 effect,  $C$  is the input (cumulative dose) and  $\gamma$  is the Hill coefficient controlling the slope of the sigmoid response. Inferences from all models were  
129 performed on posterior samples generated by Markov Chain Monte Carlo with Bayesian 95% credible intervals (CrIs) constructed to enable probabilistic  
130 interpretations of parameter values. Models were run in OpenBUGS (version 3.2.3, MRC Biostatistics Unit) and in R (version 3.3.1 R Development Core  
131 Team) using the R2OpenBugs package.

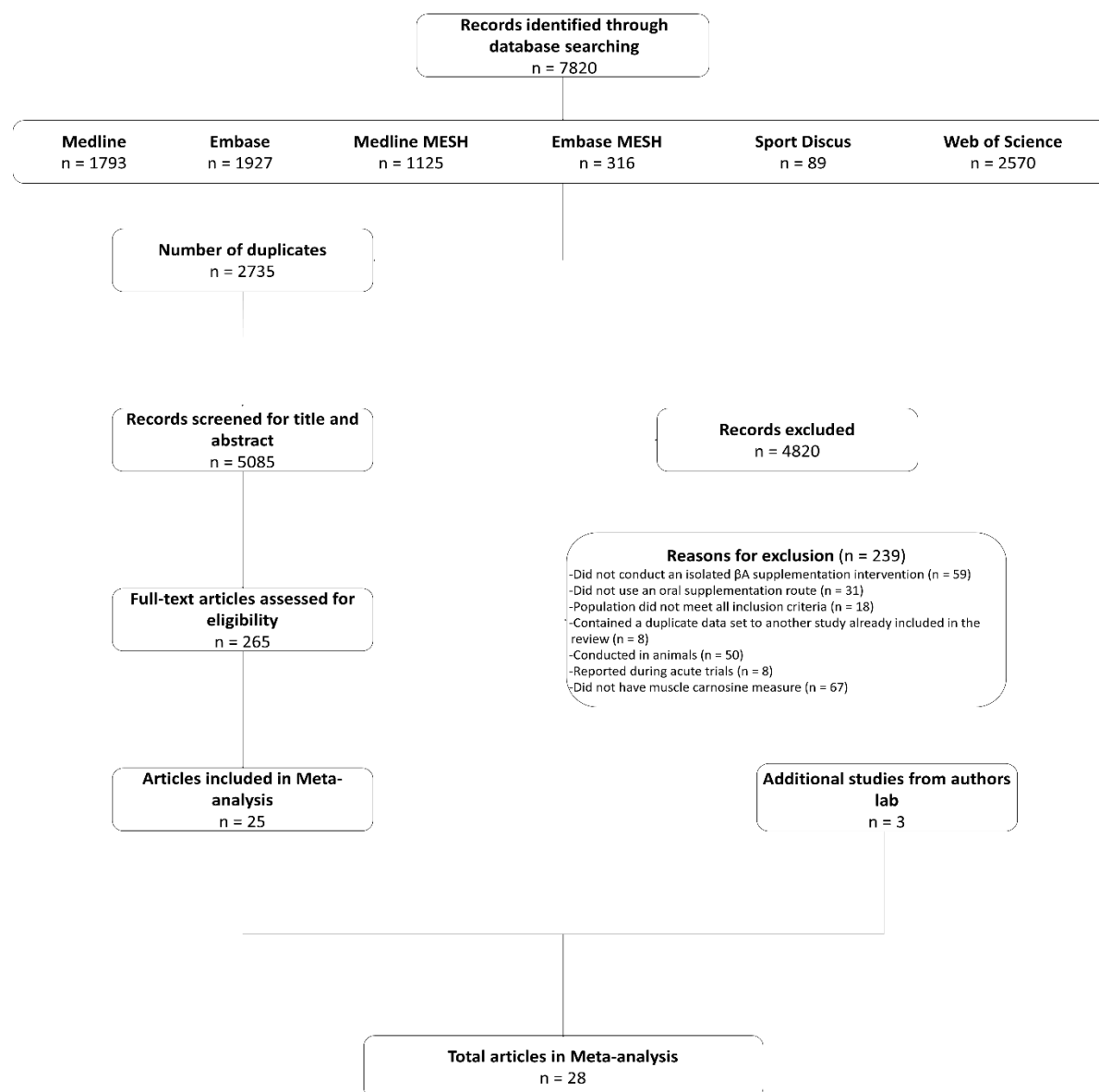
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### 133 **RESULTS:**

#### 134 *Study characteristics*

135 Twenty-five studies were identified in the systematic search and included in the meta-analysis [10–12,19–40], along with three, currently unpublished,  
136 data sets from the authors' lab (see Figure 1 for search flow diagram). In total, 575 participants (comprising 486 men and 89 women) were included, of  
137 which 382 consumed BA, with the remaining 193 allocated to a placebo intervention. The majority of studies were conducted on healthy young adults  
138 (mean age (yrs) = 23.89, SD = 5.46), with only one study conducted on elderly (mean age (yrs) = 64.34, SD = 4.99, [26]). An overview of all included studies  
139 is presented in Supplementary File 1. Analyses were completed on subsets of the data depending on the specific analysis and suitability of each study set,  
140 as described below.

141



142

143 **Figure 1:** Search Flow Diagram

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145 **Individual Data:**

146 Complete individual data sets were obtained from five studies conducted within the authors laboratory. Two of these studies were identified in the  
 147 systematic search [12,22], while the remaining three are currently unpublished. Ninety-nine participants were available ( $BA_n = 67$ ,  $PLA_n = 32$ ) comprising a  
 148 total of 232 observations. All studies provided a BA dose of  $6.4 \text{ g}\cdot\text{day}^{-1}$  with observations ranging from 4 to 24 weeks post baseline. BA supplementation  
 149 increased MCarn on average by  $16.0 \text{ mmol}\cdot\text{kgDM}^{-1}$  ([95%CrI: 12.4 – 19.6] compared to placebo. Regression analyses with duration centred at 4 weeks were  
 150 completed to determine if the effects of supplementation increased beyond this point ( $BA_n = 50$ , 134 total observations). The mean change in MCarn at 4  
 151 weeks was  $14.0 \text{ mmol}\cdot\text{kgDM}^{-1}$  [95%CrI: 10.1 – 18.1], with a positive regression slope indicating a further  $0.5$  [95%CrI: 0.2 – 0.7]  $\text{mmol}\cdot\text{kgDM}^{-1}$  increase per  
 152 week. Analyses of the same data also demonstrated that baseline levels of MCarn were not associated with changes due to supplementation ( $-0.1$  [95%CrI:  
 153  $-0.3 – 0.1$ ]). The amount of random noise in MCarn values due to biological variation and measurement error (*i.e.*, typical variation) was estimated using

154 observations from placebo groups. The standard deviation of residuals from the multilevel model representing typical variation was 4.1 mmol·kgDM<sup>-1</sup>  
155 ([95%CrI: 3.4-5.1], PLAn = 18, 61 total observations). Calculation of proportion of response first required an estimate of the intervention response standard  
156 deviation ( $\hat{\sigma}_{IR}$ ), which determines the variability of individual change centered on the group pre to post change. The intervention response standard  
157 deviation ( $\hat{\sigma}_{IR}$ ) was estimated as 6.6 mmol·kgDM<sup>-1</sup> [95%CrI: 3.4 – 9.4] and the proportion of response was 99.3% [95%CrI: 96.2 - 100].

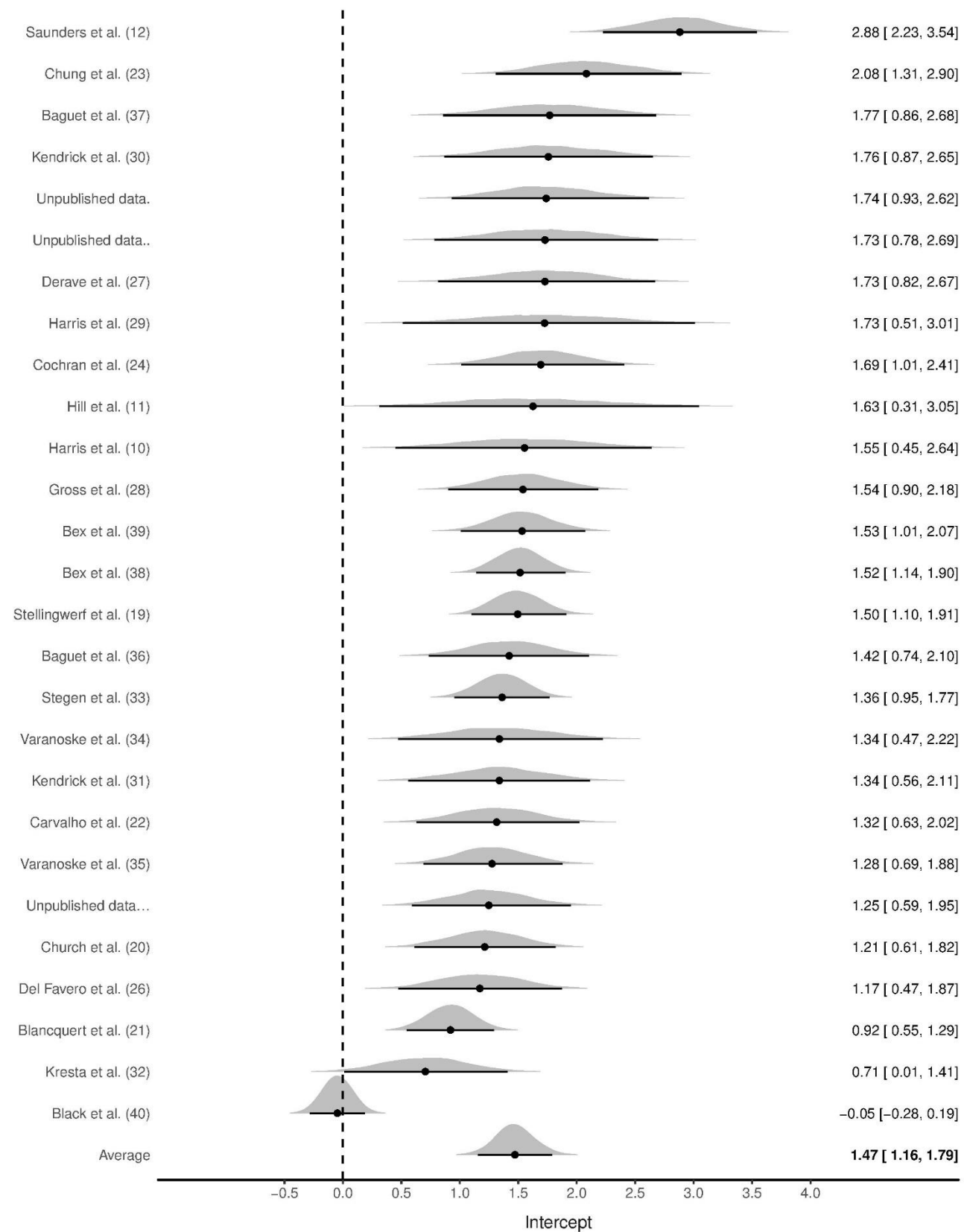
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#### 159 **Aggregate Data:**

160 Aggregate analyses were based on effect sizes calculated from all available studies using the SMD pre to post change in MCarn levels. One hundred and  
161 eight effect sizes were available from BA groups only, 6 of which were removed as they were outliers (ES > 5). The multilevel meta-analysis with no study  
162 covariates estimated a large pooled effect size of 1.5 [95%CrI 1.2 – 1.8], with substantial between ( $\tau^2_{0.5} = 0.6$ ) and within ( $\epsilon^2_{0.5} = 0.7$ ) study variance (Figure  
163 2). The same model applied to effect sizes calculated with supplementation and control group data (22 studies and 56 effect sizes) also produced a large  
164 pooled effect size of 1.7 [95%CrI: 1.3 – 2.1], with substantial between ( $\tau^2_{0.5} = 0.8$ ) and within ( $\epsilon^2_{0.5} = 0.5$ ) study variance (Figure 3). Using a simple linear  
165 model, the effects of cumulative BA dose was assessed by centering on the mean value (208g). Results demonstrated a large effect at the mean cumulative  
166 dose (1.5 [95%CrI: 1.2 – 1.8]) and an estimated 0.23 [95%CrI: 0.06 – 0.49] increase in effect size per additional 100g. Similar results were obtained for  
167 effect sizes calculated with supplementation and control group data (effect at mean: 1.7 [95%CrI: 1.3 – 2.1]; effect per additional 100g: 0.16 [95%CrI: 0.01  
168 – 0.31]). Insufficient data were available to ascertain if age altered the effects of BA supplementation, but subset analyses were conducted to investigate  
169 the impact of sex and the method used to measure MCarn, using effect sizes generated from supplementation groups only. Sixteen studies were selected  
170 that used the most common dosing protocol (cumulative dose between 130 and 180g) comprising a total of 56 effect sizes. For the sex comparison there  
171 were 8 effect sizes from a female only group, 38 effect sizes from a male only group and 10 effect sizes from a mixed group. No substantive evidence of a  
172 gender effect was obtained (male vs female: -0.32 [95%CrI: -1.1 – 0.43]; male vs mixed: -0.00 [95%CrI: -0.95 – 0.88]). Across the 16 studies, 40 effect sizes  
173 were obtained from MCarn values measured with non-invasive scanning devices (*i.e.*, HR-MRS) and 16 effect sizes obtained with muscle biopsy based  
174 analyses (mainly assessed by HPLC, with one study using UPLC and one using mass spectrometry), with some evidence of increased effects with HPLC (0.16  
175 [95%CrI: 0.01 – 0.43]).

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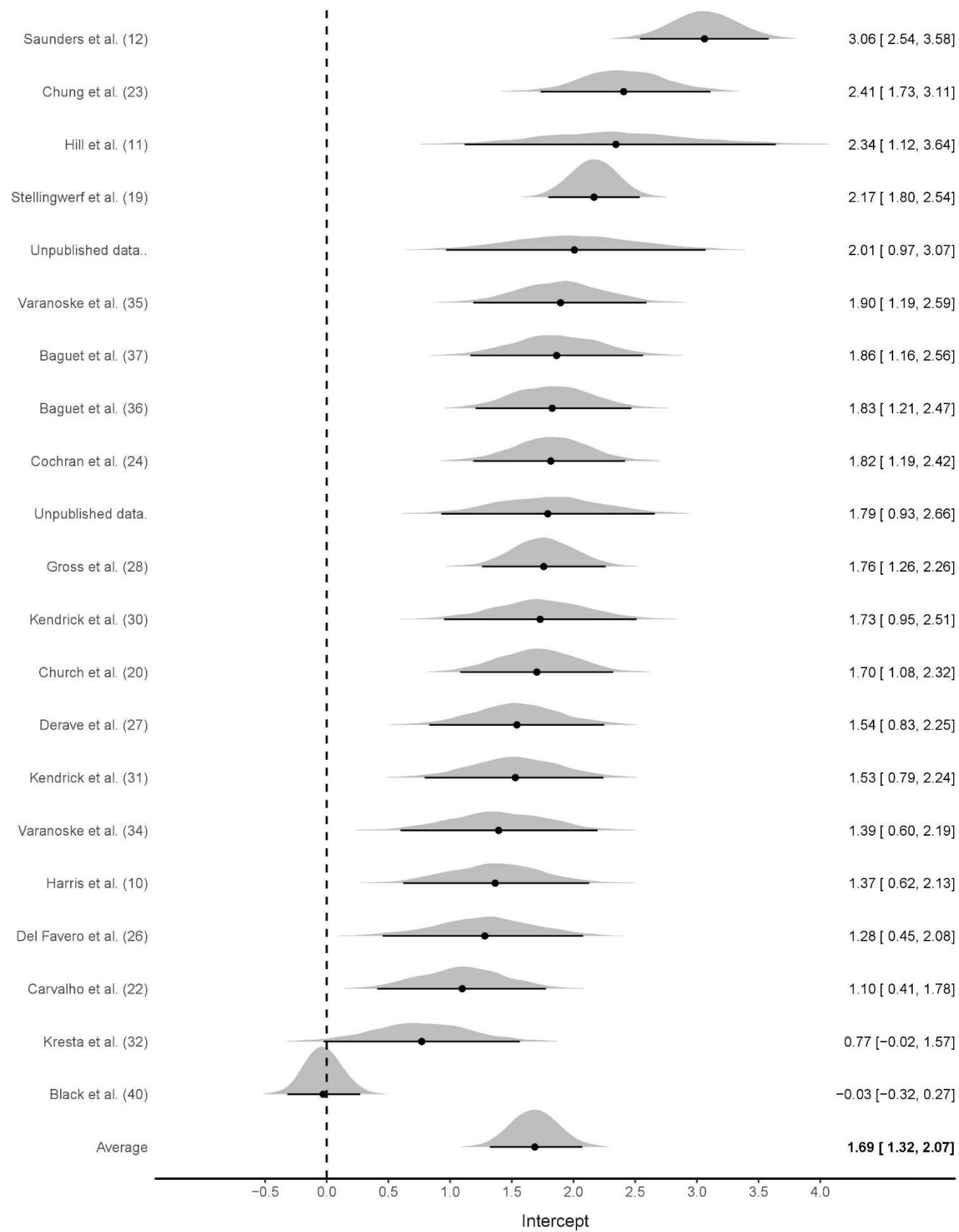


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178 **Figure 2: Bayesian Forest Plot of multilevel meta-analysis with non-controlled effect sizes**

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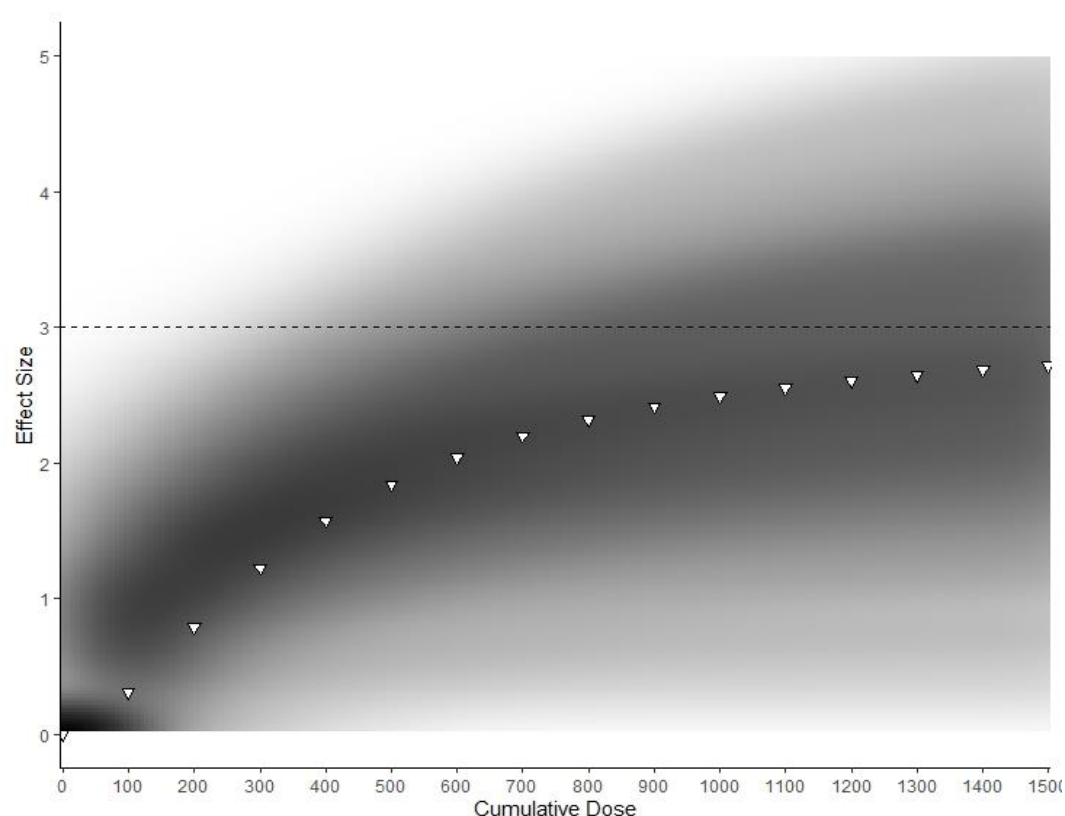
182 **Figure 3:** Bayesian Forest Plot of multilevel meta-analysis with controlled effect sizes

183

184 **E<sub>max</sub> Model**

185 The predicted maximum effect of BA supplementation (E<sub>max</sub>) was 3.0 (50%CrI 2.2 – 3.7) and the estimated total cumulative dose (g) required to achieve  
 186 50% of this maximum effect (ED<sub>50</sub>) was 377g (50%CrI 210 – 494). A density plot with the E<sub>max</sub> curve generated from median parameter values is provided  
 187 in Figure 4. An extrapolation of posterior samples from the E<sub>max</sub> model was performed to estimate probabilities that percentage of maximum effect could  
 188 be achieved with cumulative doses ranging from 1000 to 1500g (see Table 2). These results estimated, for example, that the probability of obtaining at  
 189 least 70% of maximum effect with a cumulative dose of 1000g was 0.68.

190



191

192 **Figure 4:** Density plot of Bayesian E<sub>max</sub> model predicting effect of cumulative BA supplementation on muscle carnosine content. **Note:** Darker areas  
 193 represent more common E<sub>max</sub> trajectories. White triangles represent E<sub>max</sub> generated with median parameter values.

194

195 **Table 2:** Probability table representing the chance that various cumulative doses (columns) create a response greater than the specified percentage of  
 196 E<sub>Max</sub> (rows) based on Bayesian model generated.

Percentage of E <sub>Max</sub>	1000g	1100g	1200g	1300g	1400g	1500g
70%	0.68	0.73	0.77	0.80	0.83	0.85
80%	0.45	0.48	0.51	0.54	0.56	0.59
90%	0.31	0.33	0.35	0.37	0.38	0.40

197 Values in table represent probabilities (p)  $0 \leq p \leq 1$ .

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201 **Discussion:**

202 The purpose of this study was to conduct a comprehensive analysis with various modelling techniques to synthesize existing knowledge about the MCarn  
203 response to BA supplementation. Collectively, our findings and models employed indicated that human skeletal muscle has large capacity for MCarn  
204 accumulation, and that commonly used protocols (*e.g.*, 4 weeks at 6.4 g·day<sup>-1</sup>) may not come close to saturating muscle carnosine content. Baseline values  
205 do not appear to influence subsequent response to supplementation and the non-linear response to supplementation was not influenced by sex. Analysis  
206 of individual data indicated that muscle carnosine content is relatively stable in the absence of the intervention, and that effectually all (99.3% [95%CrI:  
207 96.2 – 100]) participants respond to BA supplementation.

208

209 Our observation that humans have large capacity for non-linear MCarn accumulation align with a recent theoretical model proposed by Spelnikov & Harris  
210 [41]. Their model describes changes in MCarn over time with BA supplementation, and was based on three studies that used a slow release BA formulation.  
211 The authors described absolute increases in MCarn as a product of the rates of synthesis and decay, with carnosine synthesis considered to be constant in  
212 relation to time and first order to daily BA dose. Similarly, carnosine decay was also considered to be first order, but in relation to total MCarn content. As  
213 such, carnosine decay increases when absolute content is higher and so the rate of MCarn accumulation due to BA induced elevations in synthesis will  
214 slow. Tissue saturation represents the point at which the rates of synthesis match decay, and so content will remain constant despite continued  
215 supplementation. The lack of data at higher BA doses limits precision regarding the point at which human skeletal muscle saturation may occur; however,  
216 our analyses suggest that humans have very large capacity to accumulate MCarn and that naturally occurring baseline levels are far below that which the  
217 muscle is capable of maintaining.

218

219 Our analyses indicate that the nature of individual MCarn response to BA supplementation differs from other commonly used supplements, such as  
220 creatine. Human skeletal muscle appears to reach creatine saturation at approximately 140 – 160 mmol·kgDM<sup>-1</sup> [42] and this can be achieved within 5 days  
221 of high-dose supplementation. Response to creatine supplementation is largest in those with lowest baseline levels, whereas individuals whose creatine  
222 content is habitually closer to this saturation point gain smaller benefit from supplementation [42]. In contrast, we observed no evidence of an influence  
223 of baseline carnosine content on the subsequent response to supplementation. This makes sense when considered in relation to our predictive model, as  
224 it appears that we have very large capacity to accumulate MCarn – far greater than is achieved with commonly used protocols (*e.g.*, 179.2 grams provided  
225 as 6.4g·day<sup>-1</sup> for 4 weeks). This may be because baseline MCarn contents (approximately 25 mmol·kgDM<sup>-1</sup>) are substantially lower than those observed for  
226 creatine, with many individuals having baseline contents close to the proposed creatine saturation limit of 140 – 160 mmol·kgDM<sup>-1</sup>. These data, in turn,  
227 raise another interesting question. Does human skeletal muscle have a largely uniform saturation point, after which no further increases can be attained  
228 (as seems to be the case with creatine)? Or does capacity to accumulate MCarn vary widely between individuals, with each having their own upper limit?  
229 Currently, insufficient data using very high BA protocols on MCarn precludes the answering of this question, but one thing that is clear is that human  
230 skeletal muscle has large capacity to uptake BA and to increase MCarn above naturally occurring, non-supplemented levels.

231

232 Our data indicated that humans have large capacity for MCarn accumulation, and this, in turn, raises other important questions, *e.g.*, is maximal MCarn  
233 accumulation necessary, or even desirable? Theoretically, the greater the increase in MCarn content, the greater its ability to buffer, and to contribute to  
234 other processes, and so intuitively, attaining the largest increases possible seems desirable. Two studies did report that larger MCarn increases were

235 associated with greater performance effects [11,12], but meta-analytic data does not support this, and the total dose ingested was previously reported  
236 not to influence the effect on exercise performance [18]. It would be counterintuitive to believe that performance benefits would continue to linearly  
237 increase with ever-increasing MCarn, given that numerous factors, apart from acidosis, contribute to fatigue [43], and so it makes sense that at some  
238 point, performance benefits would plateau. Numerous studies have reported that approximately 179g of BA can be ergogenic, and so it seems MCarn  
239 saturation is not essential for BA supplementation to be ergogenic, although it does remain to be seen whether greater increases can elicit greater effects  
240 on exercise performance. A very important avenue for future research is the identification of the lowest MCarn increase necessary to elicit an ergogenic  
241 effect, along with the point after which no further benefits can be obtained. This information could have large potential to enhance the applicability and  
242 efficacy of BA supplementation strategies. For example, it seems that the largest gains in MCarn are attained in the earlier phases of supplementation (see  
243 Figure 4). It would be of interest to identify if strategies such as meal co-ingestion [33], intake in proximity to training [39] or intake in slow-release capsules  
244 [35] can influence the early response to supplementation [14] and whether this, in turn, meaningfully impacts exercise performance.

245

246 The current analysis also brought to light some interesting points about the nature of carnosine response to supplementation, which has implications for  
247 future study design. It seems that in the absence of intervention carnosine is relatively stable in the muscle, likely due to low intramuscular carnosinase  
248 and roughly equivalent synthesis and degradation rates [3]. Our analysis of individual data indicated typical variation of approximately 4 mmol·kgDM<sup>-1</sup>  
249 across a 4-week period. Previously, two muscle samples taken from the same biopsy cut showed a variation of approximately 1mmol·kgDM<sup>-1</sup> [44], and so  
250 measurement error likely accounts for at least a quarter of this variation (and potentially more). It was interesting to observe that both within and between  
251 study variance were large and similar. A large proportion of this sampling error is likely due to small sample sizes. Typically the use of a control group  
252 would be recommended to normalize the effects of the intervention against those of usual biological variability [17]. But in this situation we observed little  
253 variation in placebo group MCarn, while the effects of intervention studies when analysed both with and without controlling for the effects of the placebo  
254 group were similar (ES (95%CrI): 1.7 (1.3 – 2.1) versus 1.5 (1.2 – 1.8). This implies that the control group adds little value to the analysis, likely because of  
255 MCarn stability and the large effect of supplementation. In future investigations of the MCarn response to BA in young healthy males (and particularly  
256 those for which resources are limited) it may be prudent to direct resources toward the intervention group, in order to reduce within study variance. It is  
257 important to note that this recommendation applies only to studies on the MCarn response to BA supplementation. The influence of BA supplementation  
258 on exercise performance is far less well-characterized and subject to substantially more sources of internal and external variability and so control groups  
259 are essential in studies for which exercise is the primary outcome of interest.

260

261 In addition to characterizing the nature of MCarn response to BA supplementation, we also considered the influence of various potential moderators on  
262 this response. In relation to the method of assessment, it seems that lower effect estimates are generally observed when MCarn is measured using the  
263 HR-MRS technique when compared to those obtained using HPLC analysis of muscle biopsies. When considering the influence of non-modifiable factors  
264 on the MCarn response to supplementation (namely age and sex), we were unable to conduct analyses on the influence of age, as most studies were  
265 conducted in young men, and insufficient data in older and younger groups was available. Women have previously been reported to have lower MCarn  
266 than men [45,46], but our data indicate that both men and women have a similar response to BA supplementation. This implies that the lower values  
267 previously reported in women are unlikely to relate to an inherent gender dysmorphism in the biological factors that underpin carnosine metabolism.

268

269 In conclusion, our findings indicate that human skeletal muscle has large capacity to accumulate carnosine. MCarn remains stable in the absence of  
270 intervention and neither low baseline MCarn levels, nor sex, influence the subsequent response to BA supplementation. In turn, these findings lead to  
271 other questions, the response to which may have large implications for future practice. From the point of view of athletic performance, key questions  
272 include: what is the absolute MCarn increase required to elicit an ergogenic effect, along with the point after which no further benefits are attained? It is  
273 clear that 4 weeks of BA supplementation can be ergogenic, but can this be achieved earlier? Can strategies to enhance the early response to BA  
274 supplementation meaningfully impact the subsequent ergogenic benefits? The response to these questions may progress practical application of this  
275 supplementation strategy, with potential benefit to many athletic and clinical populations.

276

277 **Conflict of Interest Statement:** Bryan Saunders has previously received a scholarship from Natural Alternatives International (NAI), San Marcos, California  
278 for a study unrelated to this one. NAI has also partially supported an original study conducted within our laboratory. This company has not had any input  
279 (financial, intellectual or otherwise) into this review. The authors have no other potential conflicts of interest to disclose.

280

281 **Financial Support:** Eimear Dolan (2017/09635-0 and 2019/05616-6), Bruno Gualano (2013/14746-4) Bryan Saunders (2017/04973-4) and Guilherme  
282 Giannini Artioli (2014/11948-8) were supported by research grants from the Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP).

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