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Dietary β-alanine intake assessed by food records does not associate with muscle carnosine content in healthy, active, omnivorous men and women

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ABSTRACT

β-alanine (BA) is one of the most widely used sport supplements, due to its capacity to improve high intensity exercise performance by increasing muscle carnosine (MCarn) content, and consequently, the buffering capacity of the muscle. BA is also available in a variety of animal foods, but little is currently known about the influence of dietary BA intake on MCarn. The aim of the current study was to compile a detailed summary of available data on the BA content of commonly consumed foods, and to explore whether associations could be detected between self-reported dietary BA intake and skeletal MCarn in a group of 60 healthy, active, omnivorous men and women. Dietary BA intake was assessed via 3-day food records and MCarn content assessed by high performance liquid chromatography. A series of univariate and multivariate linear regression models were used to explore associations between estimated dietary BA and MCarn. No evidence of associations between dietary BA intake and MCarn were identified, with effect sizes close to zero calculated from models accounting for key demographic variables ($f^2 \leq 0.02$ for all analyses). These findings suggest that capacity to increase MCarn via dietary strategies may be limited, and that supplementation may be required to induce increases of the magnitude required to improve performance.

Keywords: β-alanine, diet, carnosine, supplement, nutrition, food.

INTRODUCTION

Carnosine is a dipeptide molecule comprising the amino acids L-histidine and β-alanine (BA), and is abundant in human skeletal muscle (Boldyrev et al., 2013). Although investigation into the biological functions of this diverse dipeptide is ongoing, evidence indicates that it contributes to many essential processes, including anti-oxidation (Boldyrev et al., 2004; Boldyrev et al., 2010), Ca^{2+} regulation (Dutka & Lamb, 2004), anti-glycation (Hipkiss & Brownson, 2000) and intracellular buffering (Bate Smith, 1938; Dolan et al., 2019). The latter is one of carnosine's most studied actions, and is the most likely mechanism underpinning its well-documented contribution to high-intensity exercise performance (Blancquaert et al., 2015; Sale et al., 2013). L-histidine is abundant in the skeletal muscle, and BA availability has been reported to be the rate-limiting factor in muscle carnosine (MCarn) synthesis (Harris et al., 2006), with meta-analytic data concluding that BA supplementation increases MCarn content (Rezende et al., 2020), and represents a safe (Dolan et al., 2019) and effective (Saunders et al., 2017) strategy to enhance sustained, high-intensity, exercise capacity.

Given that BA is the main limiting factor in MCarn synthesis, and that supplementation has large capacity to increase MCarn content, it seems logical to assume that individuals with high consumption of foods rich in BA would also have higher MCarn content. Supporting this hypothesis, is evidence that the absolute amount of supplemented BA is the primary moderator of MCarn increases, with manipulations to daily dose or intervention duration not influencing the overall response (Church et al., 2017; Stellingwerff et al., 2012). Supplementation of 168 g of BA as either 6 $g \cdot day^{-1}$ for 4 weeks, or 12 $g \cdot day^{-1}$ for 2 weeks results in comparable MCarn increases (Church et al., 2017). If the absolute amount of BA availability, rather than the daily dose or duration of supplementation, moderates the MCarn response, it seems plausible that differences in dietary BA availability could also influence MCarn content, if sustained for sufficient periods of time. For example, an average chicken breast (~200 g) contains approximately 0.8 g of BA and one additional chicken breast per day for a period of 3 – 7 months (approx. 72 to 168 g), would lead to an equivalent increase in BA availability as occurs

with commonly used dosing protocols (*e.g.,* 3.2 to 6.4 g·day^{-1} for 4 weeks, approx. 89 – 179 g) (Rezende et al., 2020). Indeed, previous research has indicated that supplemental BA of as little as 0.5 g·day^{-1} for 3 months, can measurably increase MCarn (Blancquaert et al., 2018). It is plausible, therefore, that higher consumption of BA rich foods may lead to MCarn increases, potentially allowing for a "food-first" approach to achieving MCarn goals.

It is important to highlight, however, that the biokinetics of supplemental BA, such as absorption and uptake kinetics, may differ from dietary intakes, while little is currently known about how BA variability within and between different food sources, nor how cooking and preparatory practices, may impact BA availability. Relatively little data on this topic exists, and available data on whether dietary BA influences MCarn are somewhat conflicting. Supporting a potential role for dietary BA intake on MCarn is evidence that vegetarians, who rely entirely on exogenous BA production due to a lack of dietary BA, have lower MCarn than their omnivorous counterparts (Everaert et al., 2011). Despite this, 6 months exposure to a vegetarian diet (thus eliminating dietary BA) did not influence MCarn in a group of habitual omnivores (Blancquaert et al., 2018), indicating that dietary intake may not be so important. This finding may also relate to the rate of MCarn wash-out, which is estimated to be approximately 10-fold slower than other important metabolites such as creatine (Baguet et al., 2009). Similarly, no correlation was observed between estimated daily BA intake and MCarn assessed by proton magnetic resonance spectroscopy (H-MRS) in a group of 29 omnivorous males (Baguet et al., 2009; Everaert et al., 2011), although it is worth noting that estimated daily intakes in this group were very low (approximately 0.32 g·day^{-1}). As such, there is currently no consensus related to the influence of dietary BA on MCarn. This area of investigation is impeded both by a lack of empirical data, along with limited knowledge of the BA content of various foods, thus rendering accurate estimation difficult. As such, the aim of this study was to compile a detailed summary of available data on the BA content of commonly consumed foods, and to explore whether associations could be detected between self-reported dietary BA intake, and skeletal MCarn in a group of healthy, active, omnivorous men and women.

METHODS

Experimental design

This cross-sectional study is a secondary analysis of data obtained as part of a larger, on-going, investigation of determinants of MCarn content. Healthy, active, men and women completed 3-day food records for assessment of dietary BA intake and provided a muscle biopsy for assessment of MCarn. Participants were excluded if they currently were, or had been, supplementing BA in the 6 months prior to the study. Complete data sets were available for 60 participants (44 men and 16 women). All participants provided written informed consent prior to participating, and the study was approved by the institutional ethical review committee (CAAE: 55265416.8.0000.5391).

HPLC Analysis of MCarn Content

Muscle biopsies from the *m. vastus lateralis* were taken using a 5 mm Allendale aspiration biopsy needle (Northern Hospital Supplies, Edinburgh, United Kingdom) according to a method adapted from Bergstrom (1975). Whole MCarn concentration was determined by high performance liquid chromatography (HPLC; Hitachi Ltd., Tokyo, Japan) using the method of Mora et al. (2007). The samples were analyzed in duplicate and injected through an automatic sampler, using a cut injection method, which is described in detail elsewhere (Saunders et al., 2017). Quantification was performed using peak areas, which were calculated by the HPLC chromatography data software (CDS) and inspected individually for error and consistency by a trained researcher. Standard curves for carnosine were performed before each analysis session using concentrations of 0.1, 0.5, 1, 2.5 and 5.0 mM and a simple linear regression equation was obtained, from which the interpolations were used to calculate total MCarn content. The lower limit of detection for this method has previously been estimated to be 0.5125 $mmol \cdot kg^{-1}$DM and the intra-assay CV for carnosine measurements to be 4 ± 4.5% (Saunders et al., 2017).

Dietary Analysis

All volunteers were asked to complete a 3-day food diary on non-consecutive days including 2 weekdays and 1 day at the weekend to estimate usual food intake. Instructions were provided about the maintenance of this diary, including an instruction booklet with indicative portion sizes and examples to demonstrate the level of detail required. To improve the representativeness of these records, participants were instructed to maintain these records on days that reflected their usual, habitual, intakes. Diets were checked for completeness, and only dietary records which contained a sufficient level of detail in relation to portion sizes and meal composition were included in the analysis. The quantity of consumed macronutrients was estimated using Webdiet software (WebDiet Health Manager, Rio de Janeiro, Brazil).

BA content of different foods

The BA content of commonly consumed foods was estimated from available literature (Abe, 1983, 2000; Abe et al., 1985; Abe & Okuma, 1995; Aristoy & Toldrá, 2004; Dolan et al., 2018; Harris et al., 1990; Jones et al., 2011). When HCD content was reported as $mmol \cdot kg^{-1}$ this was converted to $g \cdot 100g^{-1}$ assuming a molecular mass of 226.23 and 240.26 $g \cdot mol^{-1}$ for carnosine and anserine. Considering the molecular weight of BA relative to carnosine, BA was assumed to comprise 37 and 39% of total anserine and carnosine content. A muscle water content of approximately 75% was assumed and used to estimate content from lyophilized versus wet tissue. Where multiple estimates were reported for any one food group the mean and standard deviation is reported. Additional articles were identified that reported the HCD content of different meat samples (chicken/turkey or beef hamburgers, minced beef, minced pork and turkey breast (Gil-Agusti et al., 2008) and horse fillet, pork loin, beef fillet, rabbit hindleg, chicken and turkey breast (Peiretti et al., 2011)). These investigations assessed HCD content by pure micellar liquid chromatography ($\mu g \cdot g^{-1}$) or HPLC/MS (%w/w) and attempts to convert these to $gBA \cdot 100g^{-1}$ of meat resulted in estimates that were substantially lower than all other findings

and so these estimates are not included in our summary table.

Statistical Analysis

A series of univariate and multivariate linear regression models were used to explore associations between estimated dietary BA and MCarn. A staged approach was adopted for multivariate models with inclusion of control variables: 1) demographics-adjusted models including age, sex, height, and weight; 2) fully adjusted models including demographics and total energy intake expressed relative to estimated resting metabolic rate calculated using the Harris-Benedict equation (J. A. Harris & Benedict, 1918). The effects of the explanatory factors were quantified comparing parameter estimates in relation to their standard errors and calculation of Cohen's local effect size f^2 (Cohen, 1988). The presence of multicollinearity in multivariate models was evaluated using the variance inflation factor, with a value >5 suggesting its presence (Kutner et al., 2005). Inputs were standardized by scaling relative to sample standard deviations to simplify interpretation of model results. Visual diagnostics of residuals revealed no signs of heteroscedasticity or deviations from a normal distribution. Given that BA is primarily contained within meat and animal products, a sensitivity analysis based on total protein consumption was also conducted. All analyses were conducted with R 3.6.2. (R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

The BA content of commonly consumed foods is summarised in Table 1. In descending order, prawns, beef jerky, turkey and chicken breast, mackerel and tuna (white meat), and beef, horse or pork leg, had the highest BA contents. Content varied in different cuts of the same meat, *e.g.,* turkey breast > wing > leg, while in fish and poultry white meat generally had higher content than red. Inter and intra--cut variability was also observed, although this did not usually affect placement when putting different meats in order according to content. Participant characteristics and descriptions of

nutritional intake are presented in Table 2. Data are reported for the full group (n = 60), and for each sex (men = 44 and women = 16), as previous research indicates that men may have higher MCarn than women (Everaert et al., 2011; Mannion et al., 1992). After adjusting for selected model inputs, neither BA, nor protein intake associated with MCarn ($f^2 \leq 0.02$). Full results are reported in Table 3 with scatter plots presented in the supplementary files.

DISCUSSION

The aim of the current study was to summarise available data on the BA content of food, and to investigate whether dietary BA intake assessed by food records associated with MCarn measured from the *m. vastus lateralis* in a group of healthy, active men and women. We observed no associations between dietary BA intake and MCarn, with effect sizes close to zero obtained from all models including those corrected for key demographic variables and total energy intake ($f^2 \leq 0.02$). There are two potential explanations for the null effect observed in this study. The first is that a relationship between dietary BA intake and MCarn exists, but that a lack of sensitivity precluded its detection. The second is that there is no true effect, or that the effect is so small as to be of no practical relevance. Both these explanations are plausible, although for several reasons we believe that the latter is better supported by the available data.

First, we consider the possibility that there is a true effect of dietary BA intake on MCarn, but that a lack of sensitivity within the method to assess BA precluded detection. Whilst food records are used widely within sport and exercise science, and nutritional sciences, they are an imprecise way to measure habitual dietary intake (Capling et al., 2017; Kubena, 2000). This is due to factors such as reporting errors (*e.g.,* missing foods, errors in portion size estimation), whether discrete measurement of 3 days truly represents “habitual” intake, and the potential for a Hawthorne effect (whereby individuals modify their behaviour in response to awareness of being observed). These issues are exacerbated for non-essential amino acids such as BA, which have been reported to have large inter

and intra-measurement variability, both in foods and within cells (Bergstrom et al., 1974; De Marchi et al., 2021). We compiled detailed summary tables of available data on the BA content of different foods, however it is important to consider that only data on a relatively small sample of foods were available and that estimates for each cut of meat varied both within and between studies. As such, it is important to acknowledge the potential for Type 2 error in this study, due to difficulties in separating signal from amidst various sources of potential noise. Future investigations should seek to overcome these limitations, through developing and validating more accurate means to assess habitual dietary BA intake. This could include evaluation of the BA content of a greater range of foods, to provide better estimates of both inter and intra-food variability, along with how various cooking and processing procedures may impact this. Additionally, MCarn takes time to accumulate in the muscle, and has a long wash-out period (Baguet et al., 2009; Yamaguchi et al., 2020). Food frequency questionnaires with specific questions about frequency of intake of BA rich foods may provide better estimates of habitual BA intake, than do one-off food records, as may longitudinal studies that conduct multiple evaluations over time.

Despite this potential for Type 2 error, we observed effect sizes of effectively zero between dietary BA intake and MCarn ($f^2 \leq 0.02$). Considering that large standardized effects of approximately 0.8 to 1.0, up to a potential of 3.0 are commonly observed in response to BA supplementation (Rezende et al., 2020), it seems unlikely that a lack of sensitivity within the measurement could entirely account for the null findings observed herein. As such, even if small effects were masked by noise within our data, the practical relevance of such small effects is questionable, considering the large capacity of the muscle to accumulate MCarn. This, in turn, leads to another interesting question that could be investigated in future research, which is what is the smallest change in MCarn required to meaningfully influence exercise performance?

A number of mechanistic explanations exist that may explain the lack of relationship observed between dietary BA intake and MCarn. The large capacity of human skeletal muscle to accumulate

MCarn suggests habitual maintenance at a level far below that which the muscle is capable of sustaining. Additionally, the rate of MCarn synthesis is limited by the activity rate of carnosine synthase (de Souza Goncalves et al., 2020) and only 3 to 6% of supplemented BA is estimated to be used to synthesise MCarn (Blancquaert et al., 2015; Perim et al., 2022; Stegen et al., 2013), with the rest assumed to go toward other processes such as transamination or oxidation (Blancquaert et al., 2016). This implies that maximising MCarn content is not a high biological priority for the body, nor is incorporation into MCarn the preferential fate for exogenous BA. Instead, it seems that the body requires the stimulus of supplemental BA far in excess of that to which it is habitually accustomed to, to measurably increase MCarn. Previous research indicated that supplemental BA of as little as 0.5 $g \cdot day^{-1}$ could be sufficient to increase MCarn (Blancquaert et al., 2018). BA intake in the current investigation varied from 0 to 2 $g \cdot day^{-1}$ (mean ± SD = 0.60 ± 0.37), which should, theoretically, be sufficient to lead to larger MCarn in those ingesting larger greater amounts. It is interesting to observe, however, that the Belgian population that took part in that study appeared to have a very low habitual BA intake of just 0.32 ± 0.14 $g \cdot day^{-1}$. As such, an additional 0.5 $g \cdot day^{-1}$ represents a substantial increase on their habitual intake. In contrast, our Brazilian population had a substantially higher estimated daily BA intake of 0.60 ± 0.37 $g \cdot day^{-1}$, which may reflect cultural differences in habitual meat consumption. It would be very interesting for future investigations to directly measure whether this apparent difference in the habitual daily intake would influence the minimum amount of supplemental BA required to increase MCarn. Additionally, and in order to better understand the influence of dietary versus supplemental BA sources, ongoing research could compare the MCarn response to increased BA availability provided through either controlled dietary manipulation, or supplementation.

In summary, we observed no associations between dietary BA intake and MCarn content and it seems that typical BA variation observed within an omnivorous diet is insufficient to measurably impact MCarn. Instead, more extreme interventions, such as the complete absence of dietary BA as occurs when following a vegetarian diet, or a marked increase in BA availability, as occurs by supplementing in quantities far in excess of habitual intake, may be required. These observations support recent calls

240 for the sports nutrition community to consider "*food first, but not always food only*" (Close et al.,
241 2022), given that the benefits observed with some widely used sports supplements, such as BA, may
242 be difficult to achieve through dietary means alone.

243

Authorship: ED and BS conceived the initial idea for this investigation. NSR, GCB, LFO, BCM, FIS and AD collected and analysed the data. PS undertook the statistical analysis. All authors read and approved the final version of the paper.

Conflict of interest: Our research group has previously received financial support, supplements free of charge, and support for open access publication charges from Natural Alternatives International (NAI, a company that produces BA) for studies unrelated to this one. NAI has not had any input (financial, intellectual, or otherwise) to the present investigation. The authors have no other potential conflicts of interest to declare.

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Protocol: The current manuscript used exploratory analyses and the protocol was not pre-registered.

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401 **Table 1**: β-Alanine (BA) content of various foods

Article	Food	BA (g/100g)	SD
	Beef		
Abe et al. (1995)	*Beef Jerky*	0.521	0.010
Aristoy et al. (2003)	*Blend*	0.088	0.012
Abe et al. (1995)	*Corned beef*	0.021	-
Abe et al. (1995)	*Hamburger patty*	0.079	0.012
Abe et al. (1995)	*Leg*	0.283	0.036
Aristoy et al. (2003)	*Loin*	0.167	0.012
Abe et al. (1995)	*Luncheon meat*	0.153	-
Aristoy et al. (2003)	*Neck*	0.108	0.011
Jones et al. (2011)	*Rump*	0.198	0.016
	Pork		
Aristoy et al. (2003)	*Blend*	0.096	0.002
Abe et al. (1995)	*Frankfurter*	0.056	-
Abe et al. (1995)	*Leg*	0.270	0.080
Abe et al. (1995) Aristoy et al. (2003)	*Loin*	0.159	0.050
Abe et al. (1995) Aristoy et al. (2003)	*Luncheon meat*	0.154	0.047
Aristoy et al. (2003)	*Neck*	0.077	0.006
Jones et al. (2011)	*Rump*	0.125	0.007
Abe et al. (1995)	*Sausage*	0.046	-
	Chicken		
Aristoy et al. (2003)	*Blend*	0.224	0.016
Abe et al. (1995) Dolan et al. (2018) Jones et al. (2011) Aristoy et al. (2003)	*Breast*	0.411	0.055
Abe et al. (1995)	*Chicken nugget*	0.165	-
Abe et al. (1995) Abe et al. (2000) Dolan et al. (2018) Aristoy et al. (2003)	*Leg*	0.168	0.049
Abe et al. (1995)	*Luncheon meat*	0.181	0.067
	Turkey		
Abe et al. (1995) Jones et al. (2011)	*Breast*	0.498	0.065

Abe et al. (1995)	*Leg*	0.220	0.200
Aristoy et al. (2003)	*Wing*	0.313	0.020
	Lamb		
Aristoy et al. (2003)	*Blend*	0.080	0.008
Aristoy et al. (2003)	*Neck*	0.081	0.006
Jones et al. (2011)	*Rump*	0.179	0.006
Aristoy et al. (2003)	*Shoulder*	0.027	0.002
	Trout		
Abe et al. (1985)	*White muscle*	0.040	0.001
Abe et al. (1985)	*Red muscle*	0.010	0.002
Abe et al. (1983) Jones et al. (2011) Aristoy et al. (2003)	*Unspecified*	0.030	0.024
	Prawns		
Jones et al. (2011)	*Unspecified*	0.780	0.086
	Tuna		
Abe et al. (2000)	*White muscle*	0.880	0.181
Abe et al. (2000)	*Red muscle*	0.165	0.089
Jones et al. (2011)	*Unspecified*	0.316	0.052
	Salmon		
Aristoy et al. (2003)	*Unspecified*	0.218	0.020
	Mackerel		
Abe et al. (2000)	*White muscle*	0.980	-
Abe et al. (2000)	*Red muscle*	Trace	-
Jones et al. (2011)	*Unspecified*	0.081	0.030
	Boneless ham		
Abe et al. (1995)	*Unspecified*	0.135	-
	Deer		
Abe et al. (1995)	*Leg*	0.150	0.020
	Horse		
Abe et al. (1995) Harris et al. (1990) Bump et al. (1990)	*Leg*	0.272	0.064
	Wiener		
Abe et al. (1995)	*Unspecified*	0.106	0.038
	Fish		
Aristoy et al. (2003)	*Blue whiting*	0.042	-

Abe et al. (1983)	*Japanese char*	0.030	0.010
Abe et al. (1983)	*Japanese smelt*	0.020	-
Aristoy et al. (2003)	*Sardine*	Trace	-
Abe et al. (1983)	*Smeltfish*	0.018	0.001
Abe et al. (1983)	*Tilapia*	Trace	-
	Pacific blue marlin		
Abe et al. (1985)	*White muscle*	0.239	0.027
Abe et al. (1985)	*Red muscle*	0.047	0.016

Data are reported as mean ± standard deviation. When more than one study reported data on a particular cut of meat the means and standard deviations for all estimates were combined.

- Variation data not reported.

406 **Table 2**: Participant characteristics and nutritional intake

Characteristics	**Total** (N=60)	**Men** (N=44)	**Women** (N=16)
Age (yrs)	36±10	34±7‡	43±9
Height (cm)	173.2±9.3	177.1±7.3‡	163.4±6.1
Weight (kg)	74.3±15.3	79.7±13.6‡	59.4±8.0
Energy intake ($Kcal \cdot kg^{-1} \cdot day^{-1}$)	33.1±9.2	33.1±8.0	33.0±12.3
Carbohydrate ($g \cdot kg^{-1} \cdot day^{-1}$)	3.8±1.2	3.9±1.0	3.8±1.7
Protein ($g \cdot kg^{-1} \cdot day^{-1}$)	1.7±0.6	1.7±0.7	1.6±0.5
Fat ($g \cdot kg^{-1} \cdot day^{-1}$)	1.2±0.4	1.2±0.4	1.3±0.6
β-alanine ($g \cdot day^{-1}$)	0.60±0.37	0.67±0.37†	0.41±0.29
β-alanine ($g \cdot kg^{-1} \cdot day^{-1}$)	0.018±0.015	0.022±0.016†	0.010±0.008
MCarn ($mmol \cdot kgDM^{-1}$)	23.2±6.2	24.4±6.6†	19.9±3.6

407 Men vs Women statistical comparisons with Mann-Whitney U test *: $p<0.05$; †: $p<0.01$; ‡: $p<0.001$.

408

409 **Table 3**: Predicting total muscle carnosine content with β-alanine and protein consumption
410 (standardized betas) after controlling for selected model inputs

	Univariate [(A)]	**Model 1** [(B)]		**Model 2** [(C)]	
Primary Variable		-Adj R^2 [(D)]	+Adj R^2 [(E)]	-Adj R^2	+Adj R^2
β-alanine consumption		.061	.068	.090	.083
	Estimate (SE)	Estimate (SE)		Estimate (SE)	
	1.1 (0.80)	1.1 (0.91)		0.70 (0.94)	
Primary Variable		-Adj R^2	+Adj R^2	-Adj R^2	+Adj R^2
Protein consumption		.061	.082	.090	0.079
	Estimate (SE)	Estimate (SE)	Estimate (SE)		
	1.7 (0.78)*	1.3 (0.87)		0.66 (1.1)	

411 *(A) Univariate: Simple linear regression between total muscle carnosine content and primary variable. (B) Model 1: Control for age, sex, height, and weight. (C)*
412 *Model 2: Control for age, sex, height, weight, and total energy intake expressed relative to estimated basal metabolic rate. (D) -Adj R2: Adjusted R2 value*
413 *without primary variable. (E) +Adj R2: Adjusted R2 value including primary variable. * p<0.05. All model resulted in effect sizes close to zero (f2 ≤0.02) with*
414 *variance inflation factor ≤3.1.*
415

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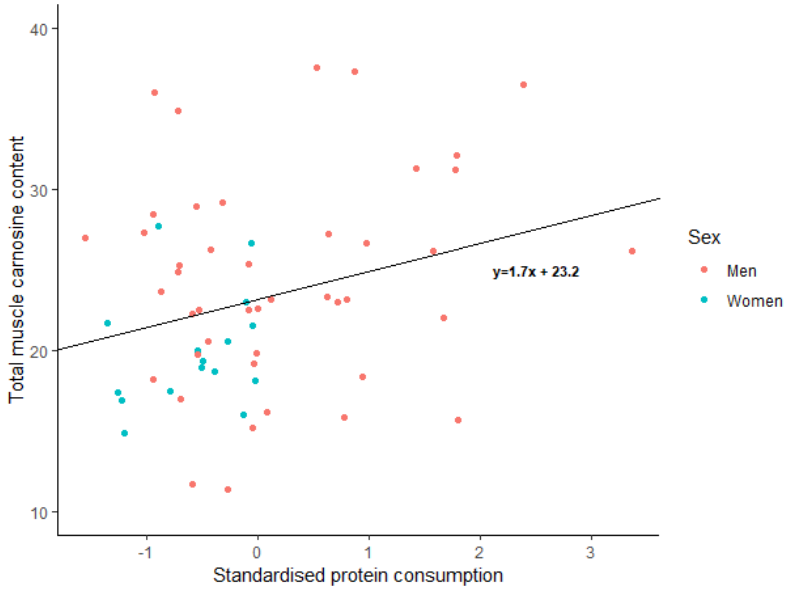
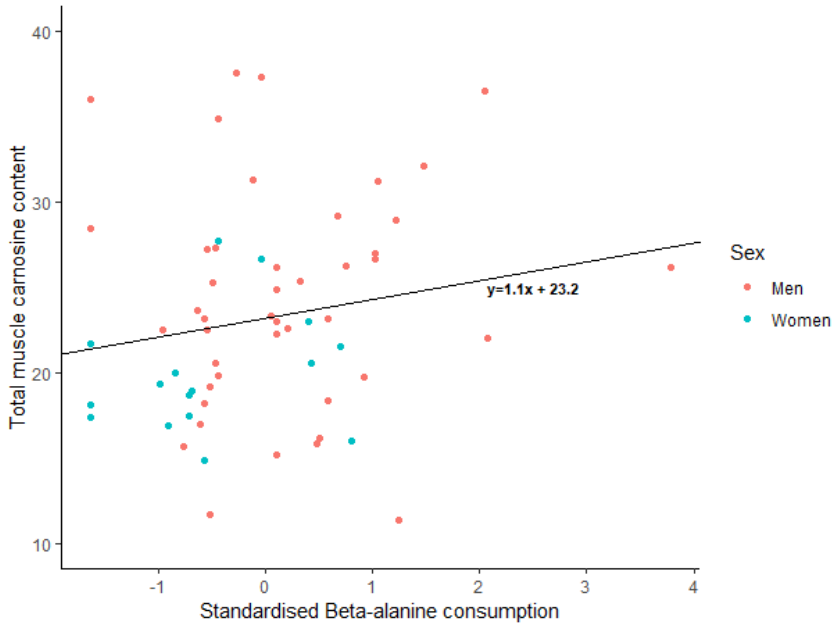
Supplementary Material with scatterplots illustrating regression relationships presented in table 3.

Univariate plots: Protein and β alanine consumption are standardised by centring and dividing by sample standard deviation.





Plots controlling for age, sex, height and weight: Protein and β alanine consumption are standardised by centring and dividing by sample standard deviation. Male and female regression lines with sex specific mean values entered for other predictors.

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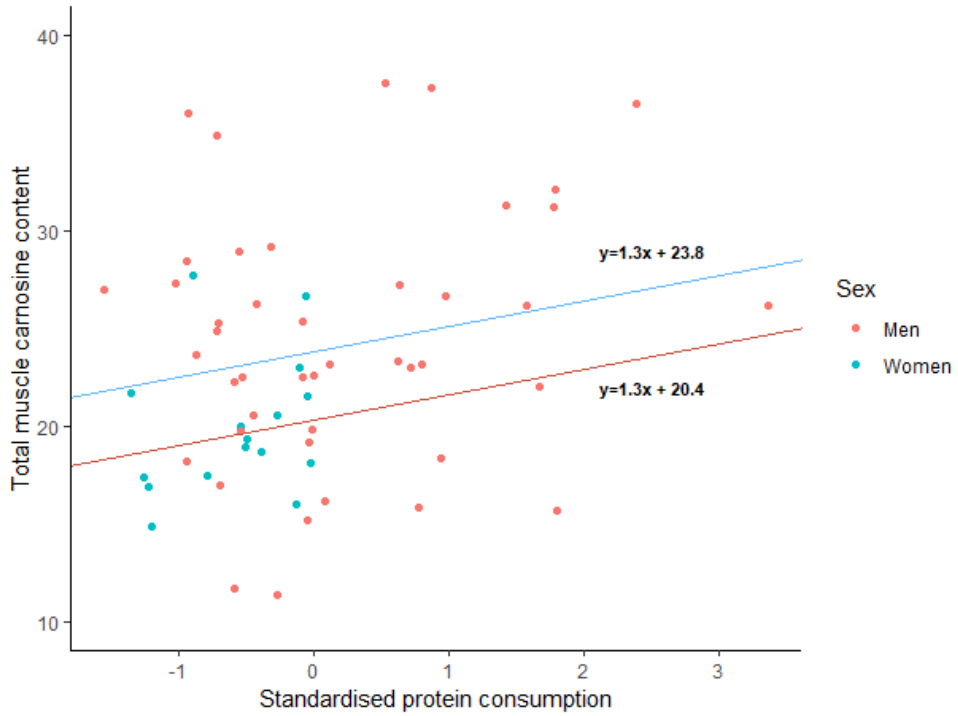

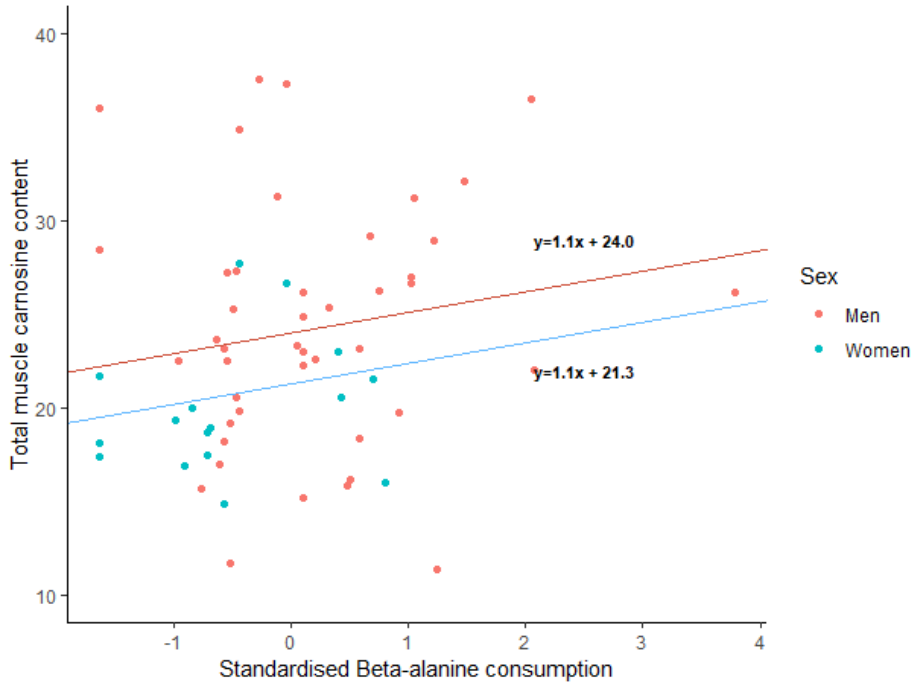

Plots controlling for age, sex, height, weight and total energy intake expressed relative to estimated basal metabolic rate: Protein and β alanine consumption are standardised by centring and dividing by sample standard deviation. Male and female regression lines with sex specific mean values entered for other predictors.

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