Associations between branched chain amino acid intake and biomarkers of adiposity and cardiometabolic health independent of genetic factors: A twin study

Amy Jennings a,1, Alex MacGregor a,1, Tess Pallister b,1, Tim Spector b,1, Aedín Cassidy a,⁎,1

a Department of Nutrition and Preventive Medicine, Norwich Medical School, University of East Anglia, Norwich, UK
b Department of Twin Research and Genetic Epidemiology, Kings College London, London, UK

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Background: Conflicting data exist on the impact of dietary and circulating levels of branched chain amino acids (BCAA) on cardiometabolic health and it is unclear to what extent these relations are mediated by genetics.

Methods: In a cross-sectional study of 1997 female twins we examined associations between BCAA intake, measured using food frequency-questionnaires, and a range of markers of cardiometabolic health, including DXA-measured body fat, blood pressure, HOMA-IR, high-sensitivity C-reactive protein (hs-CRP) and lipids. We also measured plasma concentrations of BCAA and known metabolites of amino acid metabolism using untargeted mass spectrometry. Using a within-twin design, multivariable analyses were used to compare the associations between BCAA intake and endpoints of cardiometabolic health, independently of genetic confounding.

Results: Higher BCAA intake was significantly associated with lower HOMA-IR (∼0.01, P-trend 0.02), insulin (∼0.5 μU/mL, P-trend 0.03), hs-CRP (∼0.3 mg/L, P-trend 0.01), systolic blood pressure (∼2.3 mmHg, P-trend 0.01) and waist-to-height ratio (∼0.01, P-trend 0.04), comparing extreme quintiles of intake. These associations persisted in within-pair analysis for monozygotic twins for insulin resistance (P < 0.01), inflammation (P = 0.03), and blood pressure (P = 0.04) suggesting independence from genetic confounding. There was no association between BCAA intake and plasma concentrations, although two metabolites previously associated with obesity were inversely associated with BCAA intake (alpha-hydroxyisovalerate and trans-4-hydroxyproline).

Conclusions: Higher intakes of BCAA were associated, independently of genetics, with lower insulin resistance, inflammation, blood pressure and adiposity-related metabolites. The BCAA intake associated with our findings is easily achievable in the habitual diet.

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1. Introduction

Conflicting results have been reported on the relationship between branched chain amino acids (BCAA), which includes the essential amino acids leucine, isoleucine and valine, and cardiometabolic health. Higher fasting plasma concentrations of BCAA have been associated with an increased risk of developing type 2 diabetes and insulin resistance in both rodent models and humans [1] with several longitudinal studies observing that higher circulating levels of BCAA are associated with a threefold higher risk of type 2 diabetes and 1.3-fold higher risk of metabolic syndrome [2,3]. Together these data have led to the suggestion that increased blood levels are predictive of future insulin resistance or type 2 diabetes.

In sharp contrast, BCAA supplementation and diets rich in BCAA intake have been shown to be beneficial for metabolic health (regulation of body weight and glucose homeostasis), especially in rodent models. Specifically, increasing leucine intake in mice fed a high-fat diet resulted in a 32% reduction in weight gain, a 25% decrease in adiposity and a 53%...
decrease in LDL-cholesterol concentrations independent of adiposity [4]. Improved body weight, glucose tolerance and insulin sensitivity were also observed in mice fed a BCCA-rich protein source (whey protein isolate) [5]. A previous small-scale study in 296 males reported higher BCAA intake (0.03 g/kg body weight) was associated with reduced weight (13.8 kg), total body fat (4.5%), insulin concentrations (2.1 μU/mL), diastolic blood pressure (3.4 mmHg) and a 47% lower prevalence of metabolic syndrome [6].

One proposed explanation for these conflicting findings is that circulating BCAA are a biomarker of impaired insulin action, rather than a causative factor of insulin resistance, via activation of mammalian target of rapamycin complex 1 signalling or accumulation of mitotoxic metabolites that subsequently cause mitochondrial dysfunction and apoptosis associated with type 2 diabetes [1]. Direct and indirect mechanisms for the positive effects of BCAA intake have been suggested including direct effects on hypothalamus derived processes associated with satiety and body weight and enhanced insulin-stimulated AKT phosphorylation and glucose uptake related to improved insulin sensitivity and glucose metabolism [7,8].

To our knowledge there are no comprehensive population based studies examining the associations between BCAA intake and parameters of adiposity and cardiometabolic health and no studies utilizing a twin model to explore the relative contributions of diet and genetics and reduce confounding. We firstly examined the associations between intake and circulating levels of BCAA and then investigated the relationship between intakes and a range of parameters of metabolic health, including overweight, insulin resistance, inflammation and blood pressure, using this unique approach. On the basis of previous research, we expected that participants with higher BCAA intake would have lower adiposity and healthier parameters of cardiometabolic health; additionally we hypothesized that the associations would remain significant within monozygotic (MZ) and dizygotic (DZ) twin-pairs, suggesting a lack of genetic confounding.

2. Methods

2.1. Study population

Participants included in these analyses were twins enrolled in the TwinUK cohort [9]. The participants were not selected for particular diseases or traits and were unaware of the specific hypotheses being tested. The cohort has previously shown to be representative of the general UK population in terms of disease- and lifestyle-related characteristics, including diet [10,11]. In this study we included female twins, aged 18–76 years, who had completed a food frequency questionnaire (FFQ) and attended a clinical assessment for cardiometabolic risk factors between 1996 and 2000. In total, 4181 unique participants completed a FFQ, of which 222 (n = 919) were either incomplete (<10 food items were left blank) or participants reported implausible energy intake (defined as the ratio of energy intake to estimated basal metabolic rate falling ±2SD from the population mean). Of the 3262 participants who completed a valid FFQ, 61% (n = 1997) attended for a relevant clinical assessment. Although 3299 participants had metabolomics data, clinical data were unavailable for 2360 of these participants, leaving us with 28% (n = 939) to include in the current analyses. Although the sample size was fixed before the start of the study a formal power calculation revealed that a moderate association with blood pressure of 2 mmHg (α = 0.05, 95% power) would require 913 participants in these multivariate analyses. The study was approved by the St. Thomas' Hospital Research Ethics committee and all subjects provided informed written consent.

2.2. Assessment of cardiometabolic biomarkers

Anthropometric measurements were made in light clothing, height was measured using a wall-mounted stadiometer to the nearest 0.5 cm, weight (light clothing only) was measured with digital scales to the nearest 0.1 kg and waist circumference (cm) was measured at the level midway between the lower rib margin and the iliac crest. These data were used to derive BMI (kg/m2) and waist to height ratio (WHtR). Body weight was measured at the level midway between the lower rib margin and the iliac crest, using a wall-mounted stadiometer to the nearest 0.5 cm, weight (light clothing only) was measured at the time of the current analyses. Although the sample size was fixed before the start of the study a formal power calculation revealed that 939 participants would be required to establish significant associations within MZ and DZ twin-pairs, suggesting a lack of genetic confounding.

3. Results

Participants completed a 131-item validated FFQ [16,17]. Nutrient values were assigned to each item in the FFQ or for mixed dishes a value for each ingredient, using data from UK food composition tables [18]. Amino acid content was derived predominantly using UK food composition data with additional data from the US Department of Agriculture if UK data was not available [19,20]. If the sum of the individual amino acids and the values for protein from the latest UK food composition tables differed for a specific food item the amino acid values were rescaled to match the most recent protein content [21]. Participants were ranked into quintiles of BCAA intake expressed as a percentage of total protein and associations with cardiometabolic parameters were determined using ANCOVA. Prevalence ratios for insulin resistance, metabolic syndrome, inflammation and overweight were estimated by quintile of BCAA intake using Poisson regression.
In further analyses we excluded singletons from the analyses (n = 77) and examined associations between BCAA intake, in quintiles, and cardiometabolic variables in models that included the twin-pair mean for BCAA intake as follows:

\[ E(Y_{ij}) = \beta_0 + \beta_1 X_{ij} + \beta_2 \Sigma_i \]

where \( Y_i \) and \( X_i \) represent the cardiometabolic (Y) and BCAA intake variable (X) of twin \( j \) from family \( i \) and \( T \) is the twin-pair mean [26]. \( \beta_1 \) can be interpreted as the result of a one-quintile increase in the pair-average of BCAA intake on cardiometabolic health with the individual intake held fixed (between-pair association). \( \beta_2 \) is the result of a one-quintile increase in the individuals BCAA intake on cardiometabolic parameters with the pair average held fixed (within-pair association). Within-pair associations are inherently controlled for shared environmental factors and, in MZ pairs, genetic confounding.

All models were adjusted for age (years), current smoking (yes or no), physical activity (inactive, moderately active, active), BMI (kg/m^2), use of hormone replacement therapy (yes or no), use of diabetes or cholesterol lowering drugs (yes or no), use of vitamin supplements (yes or no), menopausal status (pre- or post-menopausal), under-reporting (yes or no) and intakes of energy (kcal), carbohydrate (g), saturated fat (g), wholegrains (g), alcohol (g), and protein (g). Natural-log-transformed values were used as the distribution of cardiometabolic outcome measures were skewed, with the exception of lipid levels expressed as logTG/HDL-C. Values in the text are means or geometric means (95%CI) except for values from within-pair analyses which are the geometric means expressed as a percentage of the non-transformed values, calculated as:

\[ (e^{\text{exp}b} - 1) \times 100 \]

Linear regression was used in multivariable analyses to determine associations between BCAA intake and the 75 metabolites associated with amino acid metabolism [15]. A separate random intercept model was performed for each metabolite with age, BMI, batch effects, family relatedness, smoking status and energy intake included as covariates:

\[ Y_{ij} = \beta_0 + \beta_1 X_{ij} + \gamma_1 age_{ij} + \delta_i BMI_{ij} + \epsilon_{ij} \]

where \( Y_{ij} \) is the metabolite and \( X_{ij} \) is the BCAA intake of twin \( j \) from pair \( i \) and \( \epsilon_{ij} \) is the family-specific error component that captures the unobserved heterogeneity or family characteristics.

P-values <0.05 were considered statistically significant for all analyses except for the metabolomic models where we accounted for multiple testing using Bonferroni correction, giving a significance threshold of 6.67 × 10^-4. Statistical analyses were performed with Stata statistical software version 11 (StataCorp, Texas, USA).

### 3. Results

The characteristics, cardiometabolic risk factors and dietary intakes of the study participants are shown in Table 1. BCAs contributed to 18.1% (SD 0.4 range 16.5 to 20.3) of protein intake with leucine (43.7% SD 0.4 range 41.7 to 46.3) contributing more than isoleucine (25.9% SD 0.4 range 24.2 to 27.0) and valine (30.4% SD 0.4 range 29.3 to 32.7) to total BCAA intake (data not shown). Compared to the participants included in the hs-CRP analyses (n = 1432) those excluded for having missing data (n = 565) were significantly younger (45.2 y vs. 47.9 y, t = 4.49, P < 0.01) and therefore less likely to be post-menopausal (\( \chi^2 = 18.2 P < 0.01 \)) or to take HRT (\( \chi^2 = 8.1 P < 0.01 \)). These participants also had higher saturated fat intakes (27.5 g vs. 26.0 g, t = 2.9 P < 0.01), more likely to smoke (\( \chi^2 = 5.5 P < 0.02 \)) and less likely to take vitamin supplements (\( \chi^2 = 6.7 P = 0.01 \)). There were no significant differences between those participants included (n = 1972) or excluded (n = 205) from the body fat analyses (data not shown).

There was a difference in BCAA intake between extreme quintiles of 1.3 g or 1.2% of protein (Table 2). In multivariable analyses a higher BCAA intake was associated with a lower WHtR (Q5-Q1 = 0.01, P-trend 0.04) and there was a trend towards a lower body weight although this finding did not reach levels of statistical significance (Q5-Q1 = 0.7 kg, P-trend 0.05). Higher BCAA intake was also associated with significantly lower insulin resistance as indicated by lower HOMAIR (Q5-Q1 = -0.1, P-trend 0.02) and insulin concentrations (Q5-Q1 = -0.5 μU/mL, P-trend 0.03) in addition to lower hs-CRP levels (Q5-Q1 = -0.3 mg/L, P-trend 0.01) and lower SBP (Q5-Q1 = -2.3 mmHg, P-trend 0.01) comparing those in the highest and lowest quintiles of intake.

### Table 1

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>41.7 ± 12.1 (39, 56)</td>
</tr>
<tr>
<td>Smoking</td>
<td>37.1% (759, 72.5)</td>
</tr>
<tr>
<td>Physical activity</td>
<td>26.7% (513)</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>1.6 ± 0.8 (1.7, 18)</td>
</tr>
<tr>
<td>Systolic BP</td>
<td>121 ± 16.6 (109, 131)</td>
</tr>
<tr>
<td>Insulin</td>
<td>7.8 ± 6.8 (6, 8.6)</td>
</tr>
<tr>
<td>Insulin resistance</td>
<td>4.5 ± 0.5 (4.2, 4.8)</td>
</tr>
<tr>
<td>Metabolic syndrome</td>
<td>9.1 (152)</td>
</tr>
</tbody>
</table>

### 4. Discussion

In our cardiometabolic risk factor analyses participants with higher BCAA intake had a lower prevalence of overweight, insulin resistance, systemic inflammation, and hypertension (Fig. 1 and Supplemental Table 2). There was no association between BCAA intake and prevalence of metabolic syndrome, dyslipidemia or abdominal obesity.

In analyses controlling for genetic and shared environmental confounders in twin-pairs, significant within-pair associations between BCAA intake and markers of insulin resistance, inflammation and blood pressure were observed (Table 3). Specifically, a one-quintile higher intake of BCAA was associated with lower HOMA-IR (2.1% 95% CI = 3.9, 0.2 P = 0.03), insulin (2.0%, 95% CI = 3.8, -0.2 P = 0.03) and hs-CRP (7.1% 95% CI = 11.7, -2.3 P < 0.01). After stratifying by zygosity we observed a greater magnitude of association for MZ than DZ twins, suggesting that the associations were free of genetic confounding (HOMA-IR = 5.0% 95% CI = -8.1, 1.9 P < 0.01; insulin = 4.8% 95% CI = -7.7, -1.8 P < 0.01; hs-CRP = 9.7% 95% CI = -17.7, 1.0 P = 0.03; diastolic blood pressure = 1.2% 95% CI = -2.3, -0.1 P = 0.04). No within-pair associations were observed for weight status variables or cholesterol levels. We observed no significant associations in our
between-twin analyses when all twins were considered together or when stratified by zygosity (data not shown).

BCAA intake was unrelated to circulating levels in the 3299 participants for whom data were available (leucine $\beta = 0.005$ 95% CI $-0.07, 0.05$ $P = 0.91$, valine $\beta = -0.01$ 95% CI $-0.06, 0.09$ $P = 0.76$ and isoleucine $\beta = -0.04$ 95% CI $-0.11, 0.04$ $P = 0.35$, Supplemental Table 1). After Bonferroni correction, two of the 75 metabolites identified to be representative of amino acid metabolism were significantly inversely associated with BCAA intake, alpha-hydroxyisovalerate ($\beta = -0.15$ 95% CI $-0.23, -0.07$ $P = 2.99 \times 10^{-4}$) and trans-4-hydroxyproline ($\beta = -0.20$ 95% CI $-0.28, -0.12$ $P = 1.48 \times 10^{-6}$).

4. Discussion

In the current study, using a co-twin design, significant inverse associations were observed in female twins between dietary intakes of BCAA and measures of insulin resistance, inflammation and blood pressure, associations that were independent of a wide range of known cardiometabolic risk factors including smoking, physical activity, BMI and medication use. To our knowledge, this was the first study to examine these associations within a twin population which provided us with a unique opportunity to control for genetic confounding. With stronger findings observed in MZ compared to DZ twins, these results suggest that shared environmental and genetic factors do not confound the reported associations. Additionally, in metabolomic analyses, we identified two metabolites that were inversely associated with BCAA intake; alpha-hydroxyisovalerate and trans-4-hydroxyproline. Interestingly higher circulating levels of alpha-hydroxyisovalerate have previously been associated with greater adiposity and blood pressure and circulating levels of trans-4-hydroxyproline have shown to be elevated in patients with dietary-induced non-alcoholic fatty liver disease, a cardio-metabolic risk factor for the development of diabetes [27–29].
We found no association between BCAA intake and circulating levels confirming previous findings in children and adding evidence to support the theory that plasma BCAA levels are not a direct reflection of diet-derived intake [30].

Genetic factors are strong determinants of dietary habits and cardiometabolic health, with estimates of heritability reported between 43 and 57% [11,31]. Our within-twin analysis confirmed our individual-level findings for insulin resistance and inflammation but not those for measures of weight status which may indicate that previously reported associations between BCAA intake and weight status are influenced by genetics or other lifestyle factors not considered [6,32]. We found no associations between BCAA intake and lipid levels or prevalence of metabolic syndrome in our individual- or twin-level analyses. It is noteworthy that the prevalence of clinical dyslipidemia and metabolic syndrome were very low in this apparently healthy cohort (9%).

Our results are consistent with a short-term (2 month) intervention trial which observed improvements in insulin resistance and β-cell function in men with chronic liver disease but no significant effect on body composition following supplementation with 3.2 g of BCAA (0.8 g valine, 1.6 g leucine and 0.8 g isoleucine) [33]. Our findings however, only partly support those of a longer-term supplementation trial (7.1 g leucine for 6 months) in which both glycemic control and body composition were not changed in diabetic males [34]. Our novel data also provide mechanistic support for the reported association between higher BCAA intake (0.9% of protein) and a 43% decreased risk of diabetes in Japanese women [35]. Furthermore, our findings are plausible given the findings from mechanistic studies; Macotela et al. recently reported, using rodent models, that doubling dietary leucine for eight weeks reversed many of the metabolic abnormalities associated with high fat diet-induced obesity, improving glucose tolerance, insulin signalling and inflammation in the adipose tissue. Interestingly, they found that serum cholesterol levels were not changed by leucine administration [36]. Our associations between BCAA intake and cardiometabolic health were independent of total protein intake and it is noteworthy that there were no associations between total protein intake and any of the cardiometabolic factors examined in these analyses (data not shown). This suggests an independent effect of BCAA rather than BCAA acting as a marker of total protein intake.

Our study results have potential clinical significance. Previous studies have shown that reduced insulin resistance and inflammation are associated with lower rates of obesity, type 2 diabetes and cardiovascular disease [37]. In our study, the highest quintile of BCAA intake compared with the lowest quintile was associated with 19–37% lower insulin resistance, inflammation, overweight and hypertension. The presence of insulin resistance (HOMA-IR > 2) was previously related to an increased odds ratio of 1.54 (95% CI 0.91–2.62) for coronary artery disease and elevated CRP (hs-CRP > 3 mg/L) was associated with odds ratios of 1.46 (95% CI 1.05–2.04) for cardiovascular disease and 3.12 (95% CI 1.77–5.48) for type 2 diabetes [38–40]. Although the associations we observed in absolute terms may be considered modest it has previously been shown that small changes in risk factors such as cholesterol, blood pressure and obesity are associated with potentially clinically relevant changes to cardiovascular risk [41].

To place our findings into context we examined the standardized beta coefficients for the HOMA-IR model which allowed us to compare the magnitude of associations for the various predictors within our model. The size of the standardized coefficient for BCAA intake (−0.05 95% CI −0.09, −0.01) was more than half that of smoking, a major risk factor for poor cardiometabolic status, and 1.6 and 7 times greater than alcohol intake and age, respectively. The major dietary sources of BCAA in this cohort were milk (16.3% of intake), red meat (11.1% of intake), poultry (8.8% of intake) and high fat dairy products (5.8% of intake). The difference in intakes of BCAA between extreme quintiles of intake in the current study was 1.3 g/day, to incorporate these levels of BCAA into the diet people would need to consume either a glass of milk (185 g), one small piece of cheddar cheese (20 g), one small portion of cashew nuts (35 g), or approximately one quarter of a beef or salmon steak (28 g). Although this establishes that the relationships we have reported are related to dietary achievable intakes of BCAA further research is required before recommendations on BCAA intakes in relation to metabolic health can be made.

Strengths of the current study include the large sample of well characterised participants and the use of the co-twin model which allowed us to examine these associations independently of genetic confounding. It was notable that we excluded participants with high glucose levels (>7 mmol/L) and there were low proportion of participants with metabolic syndrome and dyslipidaemia (<10%), as it is plausible that we would have observed greater associations if participants with impaired metabolic function were included. A further strength was the range of robust measures of cardiometabolic status, which included dual-energy X-ray absorptiometry measured body composition and HOMA-IR which is a reliable measure of in vivo insulin resistance and correlates well with scores obtained from the hyperinsulimemic-euglycemic clamp technique [42]. There were also limitations. There is a lack of evidence to show the FFQ used in the current study is able to accurately quantify BCAA intake although it has the ability to reflect habitual dietary intake and rank participants according to amino acid sources of BCAA in this cohort were milk (16.3% of intake), red meat (11.1% of intake), poultry (8.8% of intake) and high fat dairy products (5.8% of intake). The difference in intakes of BCAA between extreme quintiles of intake in the current study was 1.3 g/day, to incorporate these levels of BCAA into the diet people would need to consume either a glass of milk (185 g), one small piece of cheddar cheese (20 g), one small portion of cashew nuts (35 g), or approximately one quarter of a beef or salmon steak (28 g). Although this establishes that the relationships we have reported are related to dietary achievable intakes of BCAA further research is required before recommendations on BCAA intakes in relation to metabolic health can be made.
was still the possibility of residual or unmeasured confounding from additional unmeasured factors. Although we made a number of hypothesis-driven comparisons in this study we believe they were justified given the novel and exploratory nature of the analyses.

In conclusion, these novel data suggest that habitual intake of BCAA was inversely associated with parameters of insulin resistance, inflammation and blood pressure independent of shared genetic and common environmental factors. In addition, from our metabolomic dataset we identified two biomarkers of BCAA intake that have previously been associated with adiposity, although these findings need to be replicated in other data sets. Our results have clinical and public health relevance as our findings were related to dietary achievable intakes. These findings support the hypothesis that BCAA have cardioprotective effects through improvements in insulin resistance, inflammation and blood pressure and highlight the need for more intervention trials examining dietary attainable levels of BCAA and metabolic health.

Conflict of interest

The authors report no relationships that could be construed as a conflict of interest.

Acknowledgements

TS and AMaCG established and coordinated the collection of all the data. AJ performed the statistical analysis and TP provided the metabolomic dataset. AJ and AC conducted the dietary analyses and drafted the paper. All authors read and approved the final manuscript. AC has primary responsibility for the final content.

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.ijcard.2016.08.307.

References

