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Original article

Low serum glycine strengthens the association between branched-chain amino acids and impaired insulin sensitivity assessed before and after weight loss in a population with pre-diabetes: The PREVIEW_NZ cohort

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SUMMARY

Aim: Accumulation of circulating branched-chain amino acids (BCAA) is a hallmark feature of impaired insulin sensitivity. As intracellular BCAA catabolism is dependent on glycine availability, we hypothesised that the concurrent measurement of circulating glycine and BCAA may yield a stronger association with markers of insulin sensitivity than either BCAA or glycine alone. This study therefore examined the correlative relationships of BCAA, BCAA and glycine together, plus glycine alone on insulin sensitivity-related markers before and after an 8-week low energy diet (LED) intervention.

Methods: This is a secondary analysis of the PREVIEW (PREVention of diabetes through lifestyle Intervention and population studies in Europe and around the World) Study New Zealand sub-cohort. Eligible participants with pre-diabetes at baseline who achieved ≥8 % body weight loss following an LED intervention were included, of which 167 paired (Week 0 and Week 8) blood samples were available for amino acid analysis. Glycemic and other data were retrieved from the PREVIEW consortium database. Repeated measures linear mixed models were used to test the association between amino acids and insulin sensitivity-related markers (HOMA2-IR, glucose, insulin, and C-peptide).

Results: Elevated BCAA was associated with impaired insulin sensitivity ($p < 0.05$), with strength of association ($\eta p2$) almost doubled when glycine was added to the model. However, glycine in isolation was not associated with insulin sensitivity-related markers. The magnitude (β-estimates) of positive association between BCAA and HOMA2-IR, and inverse association between glycine and HOMA2-IR, increased when body weight was higher (Body weight*BCAA, Body weight*glycine, $p < 0.05$, both).

Conclusion: Low serum glycine strengthened the association between BCAA and impaired insulin sensitivity. Given that glycine is necessary to facilitate intracellular BCAA catabolism, measurement of glycine is necessary to complement BCAA analysis to comprehensively understand the contribution of amino acid metabolism in insulin sensitivity.

Clinical trial registration: This study was registered with ClinicalTrials.gov (NCT01777893).

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Abbreviations

BCAA	Branched-chain amino acids
BCAT	Branched-chain aminotransferase
BCKA	Branched-chain ketoacids
BMI	Body mass index
CoA	Coenzyme A ester
DBP	Diastolic blood pressure
DXA	Dual-energy x-ray absorptiometry
En%	Percentage of energy
FFM	Fat-free mass
FINDRISC	Finnish Diabetes Risk Score
FM	Fat mass
FPG	Fasting plasma glucose
HbA_{1c}	Glycated haemoglobin
HOMA2-IR	Homeostatic model assessment 2-insulin resistance
LED	Low energy diet
PREVIEW	PREVention of diabetes through lifestyle Intervention and population studies in Europe and around the World
SBP	Systolic blood pressure
SD	Standard deviation
SEM	Standard error of mean
UoA	University of Auckland

1. Introduction

Body weight loss is a widely adopted strategy demonstrated to improve insulin sensitivity among individuals with obesity and pre-diabetes. This approach not only slows the progression towards type-2 diabetes [1], but also serves as a pivotal intervention in the remission of type-2 diabetes [2]. Whilst weight loss is strongly associated with improved insulin sensitivity, there is substantial inter-individual variability in response [3]. Impaired insulin sensitivity is more than the manifestation of dysglycemia, it also encompasses alterations in amino acid metabolism [4]. Within this context, there is a potential to enhance weight loss-induced improvement in insulin sensitivity through promoting the improvement in amino acid metabolism.

Elevated concentrations of branched-chain amino acids (BCAA) have long been proposed as a “metabolic marker” that differentiates individuals with obesity from their lean counterparts, with BCAA also in turn associated with impaired insulin sensitivity [5]. A recent meta-analysis of prospective studies has confirmed that elevated BCAA can predict type-2 diabetes risk up to 20 years prior to the manifestation of dysglycaemia, whereby 1 standard deviation (SD) higher BCAA was found to be associated with a 40–57 % higher type-2 diabetes incidence [4]. Notably, whilst various amino acids are reported to be positively associated with glycemia, glycine is one of very few shown to be inversely associated such that 1 SD higher glycine was found to lower type-2 diabetes incidence by 21 % [4]. Furthermore, recent animal models have demonstrated an intricate biochemical relationship between BCAA and glycine, proposing that these amino acids together may modulate the pathogenesis of obesity and type-2 diabetes [6].

Diving into the biological mechanism that links glycine, BCAA and insulin sensitivity, it is proposed that glycine facilitates BCAA catabolism [6,7], potentially decreasing BCAA-induced insulin resistance. In individuals with obesity, the chronic upregulation of BCAA catabolism into branched-chain ketoacids (BCKA) and their Coenzyme A esters (CoA) depletes the pyruvate store. Given that glucose is a significant source of pyruvate and that glycolysis is limited in the presence of insulin resistance [8], glycine is depleted to replenish pyruvate for the deamination of BCAA [6]. Consequently, glycine becomes limited to export BCAA-derived CoA from mitochondria into the circulation by forming acyl-glycine, and their eventual excretion via urine [9] (Fig. 1). Hence, excess accumulation of BCAA and their derivatives induce mitochondrial stress, and in turn insulin resistance.

Following weight loss, BCAA concentration is commonly reported to decrease and glycine to increase, although these observations are not uniform across studies [11–20]. Considering the variability of BCAA and glycine improvement following weight loss and the critical role of these amino acids in the context of insulin sensitivity, we were interested in investigating the combined effects of BCAA and glycine on markers of insulin sensitivity, especially since glycine is required to promote BCAA catabolism. We hypothesised that the concurrent measurement of glycine and BCAA may yield a stronger association with insulin sensitivity than either BCAA or glycine alone. The study aimed to examine the association of BCAA, BCAA and glycine, plus glycine alone on markers of insulin sensitivity in a cohort of individuals with pre-diabetes undergoing an 8-week low energy diet (LED) weight loss intervention within the framework of the diabetes prevention program PREVIEW [1,21,22].

2. Materials & methods

2.1. Trial design

This is a secondary analysis of the PREVIEW study, with its detailed methodology previously outlined [22]. The primary aim of PREVIEW was to decrease the incidence of type-2 diabetes in individuals with pre-diabetes through a diet and lifestyle intervention. Briefly, the intervention commenced with an 8-week rapid weight loss phase using a commercial low energy diet (LED, Cambridge Weight Plan® Ltd., Northants, UK), aiming to achieve ≥8 % body weight loss. The present analysis reports data from the PREVIEW_New Zealand (Auckland) cohort which was one of the eight participating countries.

All participants provided written informed consent before participating. The New Zealand site received ethical approval from Northern B Health and Disability Ethics Committee (13/NTB/41) and the PREVIEW Study was prospectively registered at ClinicalTrials.gov (NCT01777893). The trial conduct adhered to the Declaration of Helsinki and the International Conference on Harmonisation for Good Clinical Practice.

2.2. Participant recruitment

Participants were recruited via advertisements on social media platforms and public notice boards in Auckland, New Zealand. Participants enrolled in the PREVIEW Study were 25–70 years of age, with body mass index (BMI) ≥ 25 kg/m^2 and pre-diabetes. According to the American Diabetes Association criteria, pre-diabetes was characterised by either (i) impaired fasting glucose (5.6–6.9 mmol/L), and/or (ii) impaired glucose tolerance (7.8–11.0 mmol/L following a 2 h oral glucose tolerance test after administration of 75 g glucose, and fasting plasma glucose, FPG <7.0 mmol/L). Detailed inclusion and exclusion criteria were previously described [22].

2.3. Interventions

All participants underwent an 8-week LED weight loss intervention (Cambridge Weight Plan® Ltd., Northants, UK). Participants

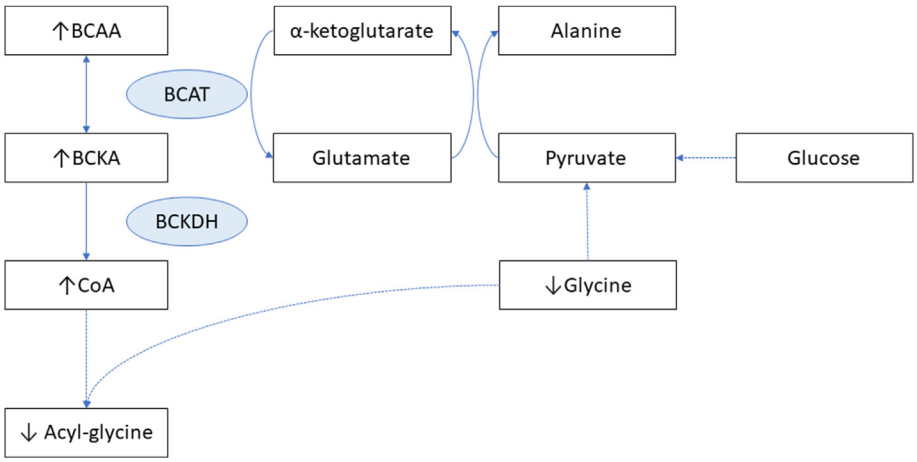

Fig. 1. Glycine is depleted to transaminate BCAAs into BCKAs and becomes limited to form acyl-glycine from BCAA-derived CoAs. Simplified biochemical pathway adapted from Supruniuk, Żebrowska and Chabowski [10]. Solid line represents direct pathway, dotted line represents simplified pathway. BCAA, branched-chain amino acids; BCKA, branched-chain ketoacids; CoA, coenzyme A; BCAT, branched-chain aminotransferase; BCKDH, branched-chain ketoacids dehydrogenase.

were required to consume 4 sachets of LED meal replacement products every day, of which 3 sachets were reconstituted in low-fat milk (250 mL for each sachet), and 1 in water. Participants were also allowed 375 g of non-starchy vegetables and recommended to drink 2 L/d of water. Psyllium husk was provided to prevent bowel discomfort. The energy intake of the LED was 3.4 MJ/d (43.7 En% protein, 41.2 En% carbohydrate, 15.1 En% fat), whereby the total daily energy intake including additional non-starchy vegetables was <4.0 MJ/d. To promote adherence, participants attended fortnightly group counselling sessions led by Registered Dietitians. No advice on physical activity was provided during this phase and participants were advised to adopt a sedentary lifestyle. The intervention occurred between September 2013 and May 2015.

2.4. Clinical and biochemical outcomes

Baseline (Week 0) and post-LED (Week 8) assessments included measurement of body weight, height, and body composition fat mass (FM) and fat-free mass (FFM) using dual-energy X-ray absorptiometry (DXA, iDXA software version 15, GE-Lunar, Madison, WI, USA). Fasting venous samples were collected, with the extracted serum and plasma stored at −80 °C until batch analysis, which occurred in 2017. Data retrieved from the PREVIEW Consortium were glycated haemoglobin (HbA_{1c}) and indicators of insulin sensitivity including plasma glucose, serum insulin, and serum C-peptide, which were all analysed at the PREVIEW central laboratory (National Institute for Health and Welfare, Helsinki, Finland). Homeostatic model assessment-2 method was used to estimate insulin resistance (HOMA2-IR) and pancreatic beta-cell function (HOMA2-β), calculated from FPG and fasting serum insulin [23]. Serum amino acids were analysed at the Liggins Institute, University of Auckland, New Zealand using an ultra-high performance liquid chromatography (UPLC) assay. Briefly, serum samples hydrolysed with sulphuric acid were extracted after sodium tungstate precipitation [24]. Free amino acids were derivatised by adding borate buffer and tagging with 6-aminoquinolyl N hydroxysuccinimidyl carbamate mixture, subsequently injected through a separation column (Kinetex EVO C18 1.7 μm 150 × 2.1 mm, Thermo Scientific Bionex Ultimate 3000 pump; Thermo Fisher Scientific, Dornierstrasse, Germany), attached to a fluorescence detector. L-norvaline was used as an internal standard. Chromeleon 7.1 software (Thermo Fisher Scientific Inc, Waltham, Massachusetts, USA) was used to quantify the amino acids based on standard curves.

2.5. Statistical analysis

Descriptive data are expressed as mean ± SD, and efficacy outcomes as mean ± standard error of mean (SEM). Paired t-test was used to compare clinical and biochemical outcomes at Week 0 and Week 8. A repeated measures linear mixed model was used to evaluate the association between amino acids (BCAA and glycine) and glycaemia-related endpoints used as indirect markers of insulin sensitivity (HOMA2-IR, glucose, insulin, and C-peptide). These indirect markers of insulin sensitivity were included in the model as outcome variables, amino acids as fixed variables, week as a repeated variable, and participant as a random intercept. Additional covariates included in the repeated linear mixed model were body weight, fat-mass, fat-free mass, age, and gender. Outcomes were reported as β-estimates and partial eta-squared (η_p^2). Partial eta-squared was interpreted as the proportion of variance in outcome variables accounted by a fixed variable or covariate after controlling for other variables in the model. Statistical analyses were performed using Statistical Package for Social Sciences (SPSS) software (version 28, IBM Corp., Armonk, NY, USA). Statistical significance was set at $p < 0.05$.

Sample size calculation for the original PREVIEW Study was powered based on type-2 diabetes risk reduction [22]. Sample size calculation showed that a total of 2403 participants were required in this multi-centre study, whereby the University of Auckland site enrolled 321 eligible participants. In this secondary analysis, data from all available paired (Week 0 and Week 8) blood samples from the University of Auckland site were used. Post-hoc power calculation revealed that the power to detect an increment in 0.05 unit of HOMA2-IR for each 100 μmol/L increment in BCAA was 91.2 % [95 % Confidence Interval (89.3 %, 92.2 %)] for 167 participants with 2 repeated observations. The power calculation was performed using simr R package [25], which assumed Z-test was used for the effect of BCAA, an alpha level of 0.05, and following 1000 simulations.

3. Results

3.1. Participant characteristics

At the New Zealand site, 305 eligible participants were enrolled into the 8-week LED phase, of which 267 completed the intervention. Among the 267 completers, 247 participants achieved ≥8 % body weight loss, of which 167 paired (Week 0 and Week 8)

serum samples available for amino acid analysis were included in the current analysis, as shown in the flow chart Fig. 2). The participants were predominantly female (n = 130, 77.8 %), 48.9 ± 11.1 years of age, BMI 37.4 ± 6.4 kg/m^2, and with elevated FPG 5.77 ± 0.55 mmol/L.

3.2. Clinical and biochemical outcomes before and after LED intervention

Participants included in this analysis achieved a statistically and clinically significant body weight loss (−11.6 ± 0.2 kg, $p < 0.001$), equivalent to 11 % of baseline body weight (Table 1). The weight loss was predominantly contributed by fat mass (−8.1 ± 0.2 kg, $p < 0.001$), but accompanied by a decrease in fat free mass (−3.0 ± 0.1 kg, $p < 0.001$). The LED intervention also resulted in a significant decrease in waist circumference, systolic blood pressure (SBP), and diastolic blood pressure (DBP) ($p < 0.05$, all). HbA_{1c} was significantly decreased, and indicators of insulin sensitivity were significantly improved following LED intervention ($p < 0.001$, all). Specifically of interest was the marked improvement in HOMA2-IR which was decreased by 0.63 ± 0.06 unit following the LED, equivalent to 39 % decrease from baseline. Contrary to the hypothesis that body weight loss promotes a decrease in circulating BCAA, no significant effect on serum leucine, isoleucine, or valine concentration was observed in our analysis ($p > 0.05$, all), nor total BCAA ($p = 0.096$). Conversely, the LED intervention significantly increased serum glycine concentration by 32.3 ± 8.7 μmol/L ($p < 0.001$), equivalent to 10 % increase from baseline. Consequently, a decrease in BCAA-glycine ratio was detected (−0.42 ± 0.05, $p < 0.001$), equivalent to 16 % decrease from baseline, a magnitude higher than either BCAA or glycine alone.

3.3. Repeated measures associations between amino acids and insulin sensitivity-related markers

Repeated measures association between amino acids and insulin sensitivity-related markers, including HOMA2-IR, FPG, fasting serum insulin, and fasting serum C-peptide, are summarised in Table 2.

BCAA and glycine were included as sole predictors of markers related to insulin sensitivity in Model 1 and Model 2, respectively. BCAA were significantly associated with impaired insulin sensitivity, exemplified by the positive association with HOMA2-IR, FPG, fasting serum insulin, and fasting serum C-peptide ($p < 0.05$, all), which accounted for 2–7 % of variance in these measures. Unexpectedly, despite glycine being significantly higher following the LED intervention, it had no significant association with any of HOMA2-IR, FPG, fasting serum insulin, or fasting serum C-peptide ($p > 0.05$, all). When BCAA and glycine were both included in a single model (Model 3), BCAA maintained the significant positive association with HOMA2-IR, FPG, fasting serum insulin, and fasting serum C-peptide ($p < 0.001$, all). Moreover, BCAA accounted for 7–12 % of variance in these insulin sensitivity-related markers when controlling for the concurrent glycine concentration. Hence, the variance accounted for by BCAA almost doubled compared to Model 1 with BCAA as the sole predictor. Also in Model 3, glycine had a significant negative association with HOMA2-IR, FPG, fasting serum insulin, and fasting serum C-peptide, but accounted for only 4–5 % of variance in insulin sensitivity-related markers when controlling for the concurrent BCAA concentration, an effect size much smaller than for BCAA. There was no significant interaction of BCAA and glycine on most markers of insulin sensitivity (HOMA2-IR, $p = 0.729$; insulin, $p = 0.659$; C-peptide, $p = 0.263$) and did not improve the model ($\eta p2$) (Model 4), hence the BCAA-glycine

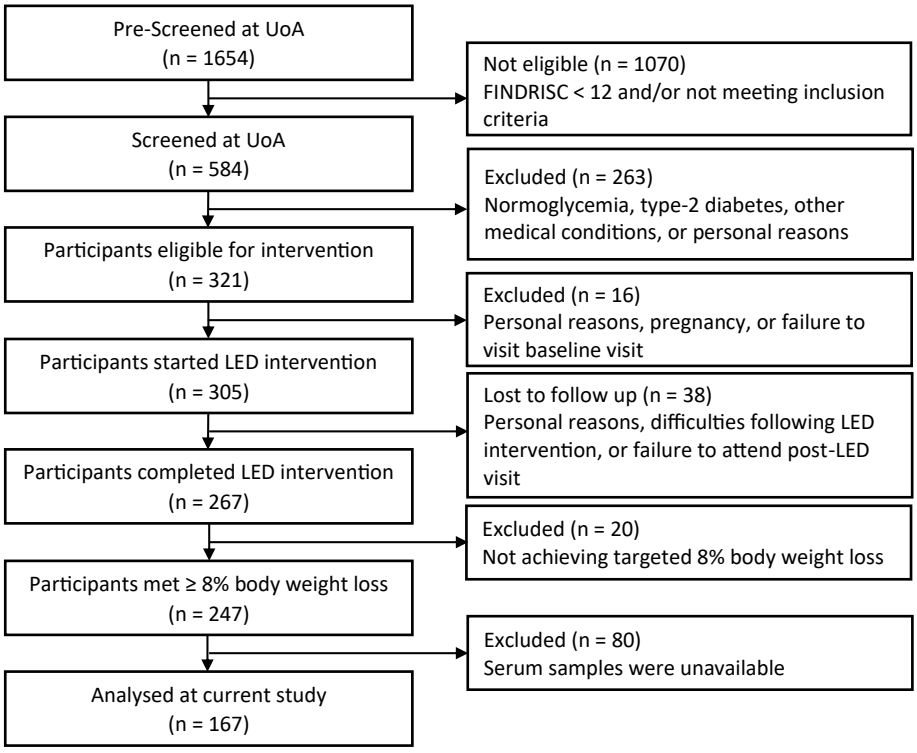

Fig. 2. Flow diagram of participants. UoA, University of Auckland; FINDRISC, Finnish Diabetes Score Risk [26]; LED, low energy diet.

Table 1
Anthropometry and fasting blood parameters at baseline and post-LED.

	Week 0	Week 8	ΔWeek 8	*p*-value
Anthropometry				
Body weight (kg)	104.2 ± 21.4	92.6 ± 19.2	−11.6 ± 0.2	<0.001
Waist Circumference (cm)	109.9 ± 14.7	98.6 ± 13.7	−11.4 ± 0.5	<0.001
BMI (kg/m^2)	37.4 ± 6.4	33.3 ± 5.9	−4.2 ± 0.1	<0.001
FFM (kg)	55.9 ± 11.5	52.9 ± 10.7	−3.0 ± 0.1	<0.001
FM (kg)	47.7 ± 13.3	39.6 ± 12.3	−8.1 ± 0.2	<0.001
SBP (mmHg)	122.4 ± 17.2	113.7 ± 14.6	−8.7 ± 1.0	<0.001
DBP (mmHg)	67.3 ± 8.9	65.3 ± 8.9	−2.0 ± 0.6	0.001
Biochemical outcomes				
HbA_{1c} (mmol/mol)	36.0 ± 3.4	34.3 ± 3.1	−1.7 ± 0.2	<0.001
Glucose (mmol/L)	5.77 ± 0.55	5.43 ± 0.49	−0.34 ± 0.04	<0.001
Insulin (mU/L)	12.1 ± 7.5	7.4 ± 4.1	−4.7 ± 0.5	<0.001
C-Peptide (pmol/L)	800.0 ± 280.3	618.0 ± 245.1	−182.0 ± 18.0	<0.001
HOMA2-IR (unit)	1.61 ± 0.96	0.98 ± 0.54	−0.63 ± 0.06	<0.001
HOMA2- β (unit)	96.2 ± 41.0	77.0 ± 26.3	−19.1 ± 2.4	<0.001
Leucine (μmol/L)	367.3 ± 194.6	348.3 ± 183.4	−18.9 ± 13.1	0.152
Isoleucine (μmol/L)	99.7 ± 31.3	96.2 ± 28.8	−3.5 ± 33.5	0.183
Valine (μmol/L)	323.5 ± 97.7	309.5 ± 79.7	−14.0 ± 7.5	0.064
BCAA (μmol/L)	791.8 ± 309.6	753.9 ± 283.1	−37.8 ± 22.6	0.096
Glycine (μmol/L)	321.0 ± 109.5	353.3 ± 96.0	32.3 ± 8.7	<0.001
BCAA-Glycine Ratio	2.60 ± 0.94	2.18 ± 0.70	−0.42 ± 0.05	<0.001

Data are presented as mean ± SD, and the change from Week 0 to Week 8 (ΔWeek 8) presented as mean ± SEM for n = 167. Paired T-test was used to test the significance of change. BMI, body mass index; FFM, fat-free mass; FM, fat mass; SBP, systolic blood pressure; DBP, diastolic blood pressure; HbA_{1c}, glycated haemoglobin.

Table 2
Repeated measures associations between amino acids and of insulin sensitivity-related markers.

	HOMA2-IR (unit)			FPG (mmol/L)			Fasting serum insulin (mU/L)			Fasting serum C-peptide (pmol/L)		
	Estimates ± SEM	ηp2	p-value	Estimates ± SEM	ηp2	p-value	Estimates ± SEM	ηp2	p-value	Estimates ± SEM	ηp2	p-value
Model 1												
BCAA (*x*100 μmol/L)	0.054 ± 0.013	0.072	< **0.001**	0.036 ± 0.010	0.040	< **0.001**	0.404 ± 0.098	0.070	< **0.001**	14.601 ± 5.158	0.024	**0.005**
Model 2												
Glycine (*x*100 μmol/L)	−0.023 ± 0.038	0.002	0.547	−0.037 ± 0.029	0.005	0.192	−0.170 ± 0.289	0.001	0.556	−24.223 ± 14.403	0.001	0.094
Model 3												
BCAA (*x*100 μmol/L)	0.083 ± 0.015	0.118	< **0.001**	0.057 ± 0.012	0.076	<**0.001**	0.621 ± 0.117	0.115	<**0.001**	28.832 ± 6.103	0.068	< **0.001**
Glycine (*x*100 μmol/L)	−0.151 ± 0.043	0.048	**0.001**	−0.117 ± 0.033	0.040	< **0.001**	−1.128 ± 0.330	0.047	**0.001**	−68.430 ± 16.673	0.049	< **0.001**
Model 4												
BCAA (*x*100 μmol/L)	0.098 ± 0.047	0.018	**0.038**	−0.016 ± 0.033	0.001	0.628	0.770 ± 0.358	0.017	**0.033**	45.745 ± 16.254	0.028	**0.005**
Glycine (*x*100 μmol/L)	−0.120 ± 0.097	0.006	0.218	−0.267 ± 0.071	0.042	<**0.001**	−0.832 ± 0.738	0.005	0.261	−33.494 ± 35.299	0.003	0.343
BCAA*Glycine	−0.004 ± 0.011	0.000	0.729	0.019 ± 0.008	0.014	**0.019**	−0.038 ± 0.086	0.001	0.659	−4.476 ± 3.987	0.005	0.263
Model 5												
Body Weight (kg)	0.016 ± 0.002	0.300	<**0.001**	0.004 ± 0.002	0.025	**0.024**	0.125 ± 0.014	0.298	< **0.001**	7.087 ± 0.762	0.287	<**0.001**
BCAA (*x*100 μmol/L)	0.051 ± 0.014	0.060	< **0.001**	0.051 ± 0.012	0.061	<**0.001**	0.380 ± 0.108	0.049	**0.001**	13.273 ± 5.689	0.018	**0.020**
Glycine (*x*100 μmol/L)	−0.060 ± 0.040	0.010	0.139	−0.098 ± 0.034	0.027	**0.004**	−0.431 ± 0.305	0.009	0.160	−26.889 ± 15.504	0.009	0.084
Model 6												
Fat Mass (kg)	0.020 ± 0.004	0.155	< **0.001**	0.012 ± 0.003	0.077	< **0.001**	0.152 ± 0.027	0.151	< **0.001**	9.823 ± 1.468	0.171	< **0.001**
Fat-Free Mass (kg)	0.012 ± 0.006	0.026	**0.044**	−0.003 ± 0.005	0.002	0.585	0.091 ± 0.044	0.026	**0.043**	4.378 ± 2.509	0.018	0.083
Age (years)	0.000 ± 0.004	0.000	0.986	0.013 ± 0.003	0.097	< **0.001**	−0.003 ± 0.028	0.000	0.925	2.559 ± 1.628	0.015	0.118
Female	−0.012 ± 0.136	0.000	0.931	−0.082 ± 0.117	0.003	0.483	−0.112 ± 1.036	0.000	0.914	−15.778 ± 59.267	0.000	0.790
BCAA (*x*100 μmol/L)	0.050 ± 0.014	0.058	**0.001**	0.049 ± 0.012	0.060	< **0.001**	0.373 ± 0.109	0.055	**0.001**	12.641 ± 5.710	0.016	**0.028**
Glycine (*x*100 μmol/L)	−0.061 ± 0.040	0.010	0.134	−0.094 ± 0.033	0.027	**0.005**	−0.440 ± 0.308	0.009	0.155	−25.502 ± 15.538	0.008	0.102

BCAA, branched-chain amino acids; FPG, fasting plasma glucose.

interaction effect was excluded from all subsequent models. Since body weight loss is known to improve insulin sensitivity, body weight was included as a covariate in Model 5 in addition to BCAA and glycine as predictors. Body weight loss was significantly associated with lower HOMA2-IR, fasting serum insulin, and fasting serum C-peptide ($p < 0.001$, all), accounting for nearly 30 % of variance in these parameters. Whilst body weight loss was also significantly associated with lower FPG ($p = 0.024$), it only accounted for 2.5 % of variance in glucose. After controlling for body weight, BCAA remained its significant positive association with HOMA2-IR, FPG, fasting serum insulin, and fasting serum C-peptide ($p < 0.05$, all); whereas glycine remained its significant negative association with FPG only ($p = 0.004$). Model 6 included fat mass, fat-free mass, age, and gender as covariates. Despite the increasing number of variables included in the model, it is noteworthy that BCAA remained its significant positive association with HOMA2-IR, FPG, fasting serum insulin, and fasting serum C-peptide ($p < 0.05$, all); whereas glycine retained its significant negative association with glucose ($p = 0.005$).

3.4. Associations between amino acids and insulin sensitivity-related markers were stronger at higher body weight

Since body weight has been shown to be a single strong predictor of most insulin sensitivity-related markers, we used Model 5 as the "base model" to investigate if the association between amino acids and insulin sensitivity-related markers varied based on body weight. Hence, interaction effects between body weight and amino

acids were included in Model 7 (Table 3). There was significant body weight*BCAA and body weight*glycine effects on HOMA2-IR and insulin ($p < 0.05$, all). A similar trend was observed for fasting serum C-peptide, but not for FPG. Using HOMA2-IR as a marker of insulin resistance, our model indicated that under conditions of a fixed decrease in BCAA and fixed increase in glycine, higher body weight resulted in greater decrease in HOMA2-IR (Fig. 3a, b). Our predictive models in Fig. 3a and b shows the progression of HOMA2-IR along the continuum of BCAA and glycine when body weight is fixed at the mean baseline body weight for this PREVIEW_NZ cohort, and also ±1 SD. Notably, in practice, body weight also changes as BCAA and glycine change from Week 0 to Week 8.

4. Discussion

In this analysis of the PREVIEW data, body weight decreased by~11 % following the LED intervention concurrent with a significant improvement in insulin sensitivity-related markers, including ≈40 % decrease in HOMA2-IR. The decrease in both FPG and fasting serum insulin were markers for the improvement in insulin sensitivity following weight loss. Despite the modest inverse association between BCAA and markers of insulin sensitivity, and the seemingly limited association between glycine and markers of insulin sensitivity when assessed in isolation, notably our analysis demonstrated that modelling BCAA and glycine together almost doubled the strength of association between BCAA and insulin sensitivity. This observation underscores intricate BCAA-glycine dynamics in governing insulin sensitivity. Our analysis also identified that a fixed decrease in BCAA concentration was associated with a more pronounced improvement in insulin sensitivity in individuals with higher body weight. Generally, under the conditions of a fixed energy LED (<4 MJ/day), it is expected that individuals with higher body weight would experience greater energy deficit, in turn a greater decrease in body weight and a greater improvement in insulin sensitivity. Hence, interpretation of the data must also take into account the simultaneous dynamic change in all of these variables.

4.1. The effect of a dietary weight loss intervention on BCAA and glycine concentrations

In our cohort of individuals with pre-diabetes, perhaps unexpectedly, the 8-week LED intervention had no significant effect on BCAA concentrations, but significantly increased glycine concentration. Prior studies examining the impact of dietary weight loss interventions on these amino acids however have reported mixed results. Previous dietary weight loss studies which achieved an average of 7–8 % body weight loss reported no change in BCAA and glycine concentrations [12,18]. Whilst these studies included individuals with normo- and dysglycaemia, these studies highlighted that macronutrient content of the diet had no significant effect on BCAA or glycine concentration.

In contrast, a cohort of 91 individuals with obesity and mean elevated fasting glycaemia (mean ± SD FPG = 6.10 ± 2.70 mmol/L) who achieved ≥10 % body weight loss following a 1-year behavioural weight loss program reported a concurrent decrease in BCAA and increase in glycine concentration [11]. Similar results were also replicated in a cohort of individuals with type-2 diabetes [19]. Importantly, the decrease in BCAA concentration could be maintained up to 2 years with successful weight maintenance [15]. Yet, a small cohort of 22 older adults with mean normal fasting glycaemia who achieved 7 % body weight loss undergoing an 8-week personalised dietary intervention observed decreased isoleucine concentration but without changes in valine, leucine, or glycine concentrations [14]. Whilst acknowledging that the dietary intervention for weight loss is highly variable, such as variable macronutrient composition, rate of weight loss, and the use of meal replacements, this collective evidence suggests that solely inducing an energy deficit through diet does not consistently lead to alterations in circulating BCAA or glycine.

In a review which compared bariatric surgery versus behavioural interventions for weight loss, bariatric surgery resulted in a greater decrease in BCAA and increase in glycine compared to behavioural interventions, concurrent with better improvement in markers of insulin sensitivity [17]. This observation was also true when weight loss was matched at −10 kg, although it is acknowledged that individuals who received bariatric surgery achieved more rapid weight loss than dietary intervention [13]. Therefore, other contributing factors might have influenced the levels of these amino acids, underscoring the complexity of their regulation during dietary weight loss interventions.

4.2. Targeting circulating BCAA and glycine to improve insulin sensitivity

In individuals with obesity, the cellular mitochondria cannot cope with increased substrate availability, including the elevated BCAA, hence resulting in mitochondrial stress and impaired insulin sensitivity [9]. Hence, in individuals with obesity who already exhibit elevated mitochondrial stress, further accumulation of BCAA may aggravate insulin sensitivity, in agreement with our observation that the association between BCAA-glycine and insulin sensitivity is more pronounced in individuals of greater body weight.

Decreasing BCAA availability by decreasing BCAA intake has been shown to improve insulin sensitivity [27,28]. Within the context of energy balance, restricting the dietary supply of BCAA by 45 % in Zucker-fatty rats successfully increased the excretion of acyl-glycine adducts via urine, which is concurrent with an improvement in muscle insulin sensitivity [27]. BCAA-restricted diet in individuals with type-2 diabetes has also successfully decreased fasting and postprandial BCAA concentration, concurrent with improvement in markers of insulin sensitivity without weight loss [28,29]. However, BCAA-restricted diet is extremely difficult to

Table 3
Repeated measures association between body weight*amino acids interaction and insulin sensitivity-related markers.

	HOMA2-IR (unit)			FPG (mmol/L)			Fasting serum insulin (mU/L)			Fasting serum C-peptide (pmol/L)		
	Estimates ± SEM	ηp2	*p*-value	Estimates ± SEM	ηp2	*p*-value	Estimates ± SEM	ηp2	*p*-value	Estimates ± SEM	ηp2	*p*-value
Model 7												
Body Weight (kg)	0.007 ± 0.006	0.006	0.234	0.013 ± 0.005	0.021	**0.010**	0.047 ± 0.044	0.005	0.281	6.030 ± 2.190	0.024	0.006
BCAA (*x*100 μmol/L)	−0.243 ± 0.064	0.054	< **0.001**	0.040 ± 0.055	0.002	0.467	−1.924 ± 0.489	0.059	< **0.001**	−53.849 ± 25.022	0.015	**0.032**
Glycine (*x*100 μmol/L)	0.355 ± 0.162	0.023	**0.030**	0.189 ± 0.148	0.006	0.204	2.732 ± 1.228	0.024	**0.027**	101.206 ± 67.004	0.009	0.132
Body Weight*BCAA	0.003 ± 0.001	0.072	< **0.001**	0.000 ± 0.001	0.000	0.800	0.025 ± 0.005	0.081	< **0.001**	0.686 ± 0.248	0.023	**0.006**
Body Weight*Glycine	−0.005 ± 0.002	0.030	**0.008**	−0.003 ± 0.002	0.013	**0.048**	−0.036 ± 0.013	0.031	**0.008**	−1.343 ± 0.692	0.013	0.053

BCAA, branched-chain amino acids; FPG, fasting plasma glucose; HOMA2-IR, homeostatic model assessment 2-insulin resistance.

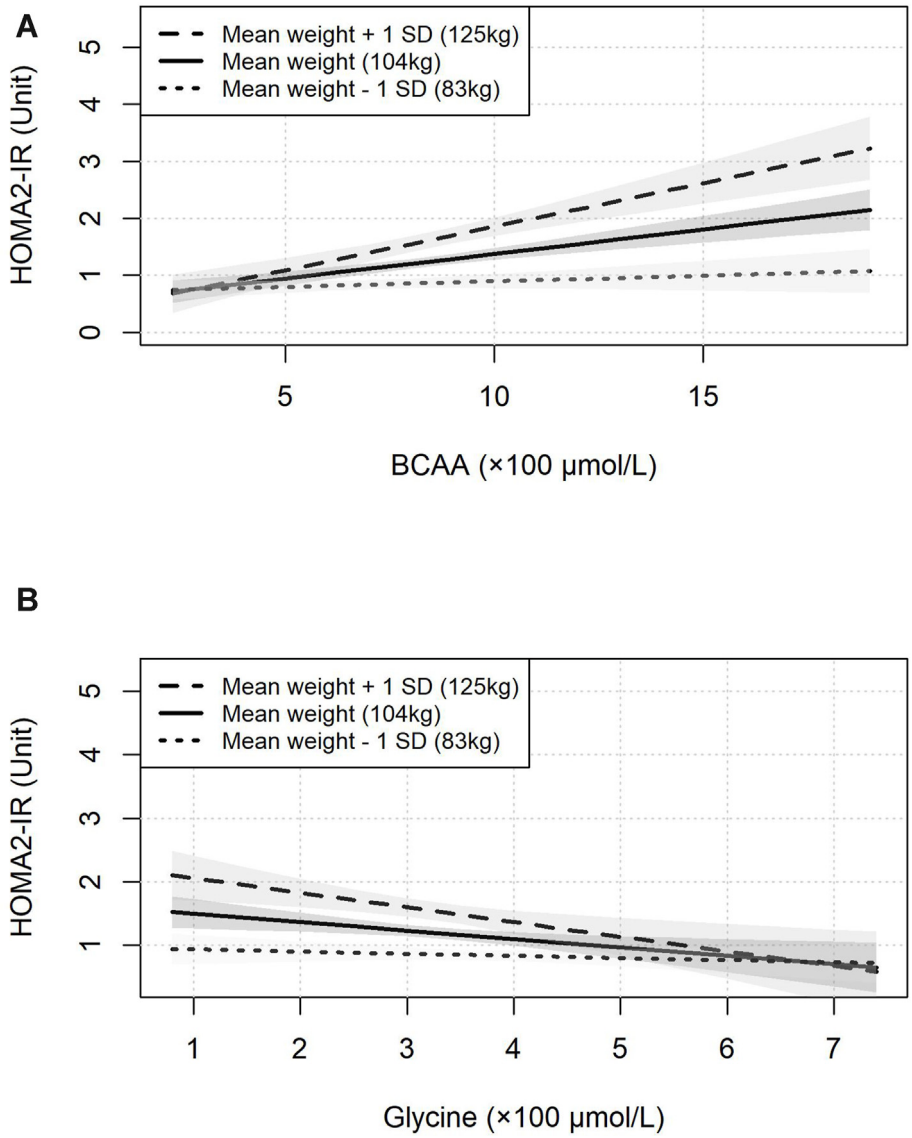

Fig. 3. Predictive models demonstrate that the magnitude (slope, β-estimates) of associations between (a) BCAA and HOMA2-IR, and (b) glycine and HOMA2-IR increase when body weight is higher. For the purpose of illustration, body weight is fixed at the mean baseline body weight (104.2 kg) for the PREVIEW_NZ sub-cohort ± 1SD.

achieve in real life using whole foods. Nevertheless, these studies provide a framework for investigating whether a commercial LED with modified dietary amino acid profile may provide additional benefits for improvement in insulin sensitivity compared to the existing LED.

Paradoxically, there is an abundance of human studies investigating the benefits of BCAA supplementation or high protein diets on body weight loss and the subsequent improvement in markers of insulin sensitivity and metabolic health [30]. This is exemplified by whey protein's role in lowering postprandial glucose excursions in healthy individuals and those with dysglycemia [31]. However, whey protein is high in BCAA but limited in glycine, a characteristic deemed as undesirable in the context of type-2 diabetes. Our group previously reported that increasing doses of oral whey protein significantly increased postprandial BCAA, whereby glycine decreased below fasted baseline [32]. The discrepancy between the potential positive and negative effects of dietary BCAA on glycemic variables may be reconciled by the hypothesis that negative outcomes associated with dietary BCAA may be evident when circulating BCAA availability exceeds its catabolic capacity [10]. Based on this rationale, we speculate that the positive effect of BCAA supplementation on glycemic variables in sedentary individuals with obesity and dysglycemia is limited in the absence of weight loss or exercise, a hypothesis that warrants further investigation in the future.

Impaired BCAA catabolism also plays a pivotal role in circulating BCAA accumulation in individuals with obesity and dysglycemia. Many studies have investigated novel dietary, exercise, and pharmaceutical interventions to target BCAA catabolism and, where BCAA catabolism is successfully improved, there is evidence of decreased insulin resistance or improved insulin sensitivity [33,34]. Although there has been emerging evidence that glycine plays a central role in the catabolism of excess BCAA, interventions which increased glycine concentration have not been as successful as BCAA restriction in improving insulin sensitivity [6,35,36]. Nevertheless, a small pilot study found a decrease in insulin resistance after administering a therapeutic oral dose of glycine (100 mg/kg/day) in conjunction with N-acetylcysteine (100 mg/kg/day) for 14

days to individuals with type-2 diabetes [37]. These observations align with our current analysis whereby glycine alone may not be sufficient to improve insulin sensitivity.

4.3. Strengths and limitations

The main strength of this study is the within-participant repeat measures outcomes at baseline and post-LED. Therefore, our model considers both within- and between-participant variability in the association between amino acids and glycemic variables. This contrasts with many cross-sectional studies which test the association between amino acids and glycemic variables at a single snapshot. Our current study is limited by its observational design, hence cause–effect relationships could not be established.

5. Conclusion

In conclusion, this study demonstrated a BCAA-glycine interplay in association with insulin sensitivity in our study population of adults with overweight and dysglycaemia. Low serum glycine strengthened the association between higher BCAA and impaired insulin sensitivity. Furthermore, a modelled fixed decrease in BCAA concentration was associated with a more pronounced improvement in insulin sensitivity in individuals with higher body weight. It is noteworthy that the association between glycine and measures of insulin sensitivity appears to be dependent on concurrent BCAA concentrations. In order to gain a more detailed insight into the contribution of disturbed amino acid metabolism during states of impaired insulin sensitivity and increased type-2 diabetes risk, we propose measurement of the conditionally essential amino acid glycine is necessary to complement BCAA analysis. Whilst we acknowledge that weight loss remains the strongest predictor of improvement in insulin sensitivity, future research that focuses on lifestyle or pharmaceutical interventions to promote glycine availability to induce BCAA catabolism may be integral to improving insulin sensitivity in clinical practice.

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Author's contributions

JJL – Conceptualisation, formal analysis, methodology, writing – original draft, writing – review and editing; UKP – Formal analysis, writing – original draft, writing – review and editing; MPS – Data curation, investigation, project administration, validation, writing – review and editing; AYL – Data curation, investigation, project administration, writing – review and editing; JM – Software, validation, visualisation; MF – Conceptualisation, funding acquisition, methodology, resources, writing – review and editing; AR – Conceptualisation, funding acquisition, methodology, resources, writing – review and editing; SDP – Conceptualisation, funding acquisition, methodology, resources, supervision, validation, writing – review and editing; DCS – Conceptualisation, resources, supervision, validation, writing – review and editing. All authors have reviewed the manuscript and approved the manuscript to be published. DCS accepts full responsibility for the work, had access to the data, and controlled the decision to publish.

Data availability

The lead author has full access to the data reported in the manuscript. De-identified data may be shared and made available upon reasonable request to DCS and subject to an approved proposal and data access agreement.

Conflict of interest

SDP was the Fonterra Chair in Human Nutrition during the PREVIEW intervention; AR has received honorariums from Nestle, Unilever and the International Sweeteners Association. Other authors declare no relevant competing interests.

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