RESEARCH ARTICLE

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Diabetes Remission Is Modulated by Branched Chain Amino Acids According to the Diet Consumed: From the CORDIOPREV Study

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Scope: Branched Chain Amino Acids (BCAA) plasma levels may be differentially associated with type 2 diabetes mellitus (T2DM) remission through the consumption of the Mediterranean diet (Med) and a low-fat (LF) diet.

Methods: One hundred eighty-three newly diagnosed T2DM patients within the CORDIOPREV study are randomized to consume the Med or a LF diet. BCAA plasma levels (isoleucine, leucine, and valine) are measured at fasting and after 120 min of an oral glucose tolerance test (OGTT) at the baseline of the study and after 5 years of the dietary intervention.

Results: Isoleucine, leucine, and valine plasma levels after 120 min of an OGTT in the Med diet ($N = 80$) are associated by COX analysis with T2DM remission: HR per SD (95% CI): 0.53 (0.37–0.77), 0.75 (0.52–1.08), and 0.61 (0.45–0.82), respectively; no association is found in patients who consumed a LF diet ($N = 103$). BCAA plasma levels combined in a score show a HR of 3.33 (1.55–7.19) of T2DM remission for patients with a high score values in the Med diet, while in those with a LF diet, no association is found.

Conclusion: The study suggests that BCAA measurements potentially be used as a tool to select the most suitable diet to induce T2DM remission by nutritional strategies.

1. Introduction

Type 2 diabetes mellitus (T2DM) represents a serious health problem worldwide, with grave social and economic repercussions. Its mechanisms are not fully understood, but insulin resistance and beta-cell dysfunction are the two major pathophysiologic abnormalities, which underlie most cases of T2DM.[1]

Despite the clinical relevance, the current clinical goals for T2DM only include prevention or delay of complications,[2] rather than diabetes remission. Early T2DM has now been proved to be reversible,[3–5] using different strategies such as an intense weight loss by bariatric surgery,[6,7] very low-calorie[6]/low-carbohydrate diets,[8,9] or the Mediterranean (Med) dietary model[10] even without a calorie restriction.[11,12] These patients improved their hepatic insulin sensitivity and presented a recovery of beta-cell functionality[13] and significant HbA1c reductions.[8,14]

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Several studies have shown the relationship between the plasma levels of branched chain amino acids (BCAAs) and the incidence of diabetes.[15–17] In fact, high BCAA plasma levels have been associated with metabolic abnormalities such insulin resistance[18] and are currently considered as potential biomarkers for T2DM risk.[19,20] Moreover, high levels of plasma BCAAs have been associated with obesity, insulin resistance, impaired glucose tolerance and type 2 diabetes.[17,18,21,22]

Previous studies have shown that consumption of the Med diet reduced fasting plasma BCAA levels (valine, leucine, and isoleucine), which was negatively associated to cardiovascular disease (CVD)[23] and T2DM risk.[24] In fact, these studies found an association between high BCAA plasma levels and the development of these diseases when an olive oil-enriched Med diet was consumed, whereas no association was found when the participants either consumed the Med diet supplemented with nuts, or a control diet in which the participants were advised to reduce their intake of all types of fat.

Based on this, we hypothesized that BCAA plasma levels may be associated with T2DM remission in patients with coronary heart disease, and set out to discover whether this potential relationship was influenced by a specific dietary pattern. We therefore aimed to evaluate the relationship between BCAA plasma levels and the remission of T2DM in newly diagnosed T2DM patients after five years of the consumption of two healthy dietary patterns, a low-fat (LF) diet and the Med diet. Moreover, we tested the relationship between BCAA plasma levels at fasting state and after the performance of a dynamic test, an oral glucose tolerance test (OGTT), with the T2DM remission. To conclude, the potential translational value of this study lies in the fact that patients with acute myocardial infarction and T2DM have a higher risk of developing a new cardiovascular event than those without T2DM.[25]

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2. Experimental Section

2.1. Study Patients

The Coronary Diet Intervention with Olive Oil and Cardiovascular Prevention study (CORDIOPREV) is an ongoing prospective, randomized, open, controlled trial of 1002 patients receiving conventional treatment for coronary heart disease (CHD) who had their last coronary event over 6 months before enrolment in one of two different dietary models [a Med diet and a LF diet] over a period of 7 years. This clinical trial has been registered as legislation requires (Clinical Trials.gov.Identifier: NCT00924937). The eligibility criteria, design, and methods of the CORDIOPREV clinical trial have been reported elsewhere.[26] All patients gave their written informed consent to participate in the study and to the inclusion of material pertaining to themselves, that they acknowledge that they cannot be identified via the paper. The trial protocol and all amendments were approved by the Reina Sofia University Hospital Ethics Committee of Human Experimentation, following the Helsinki's Declaration and Rules of Good Clinical Practices. The experimental and clinical work conducted in this study has complied all mandatory health and safety procedures.

Newly diagnosed type 2 diabetes patients who had not been receiving glucose-lowering treatment at the beginning of the study were included in the CORDIOPREV-DIRECT study (190 out of 1002 patients). Of these, seven patients were lost due to the inability to perform the diagnostic test used in this work; diabetes remission was evaluated in the remaining 183 patients during the 5-year follow-up for each participant. Moreover, three participants died during the follow-up period without achieving diabetes remission. Thus, the remaining 183 newly diagnosed patients were classified as Responders, patients who reverted from type 2 diabetes during the 5 years of dietary intervention without the use of diabetes medication (n = 73); or Non-Responders, who did not achieve diabetes remission during the follow-up period (n = 110). Remission was defined as glycemia below the diabetic range for at least two consecutive years (HbA1c <6.5%, fasting plasma glucose <126 mg dL^{-1}, and 2 h plasma glucose in the OGTT <200 mg dL^{-1}) in absence of active pharmacological or surgical therapy according to the American Diabetes Association.[27] The baseline characteristics of the CORDIOPREV-DIRECT subjects are shown in **Table 1**.

2.2. Study Design

The study design has been previously described.[26] Briefly, participants were randomized to receive two diets: a Med diet or a LF diet. The LF diet consisted of <30% total fat [12–14% monounsaturated fatty-acid (MUFA), 6–8% polyunsaturated fatty-acid (PUFA), and <10% saturated fatty-acid (SFA)], 15% protein, and a minimum of 55% carbohydrates. The Med diet comprised a minimum 35% of calories as fat (22% MUFA, 6% PUFA, and <10% SFA), 15% proteins, and a maximum of 50% carbohydrates. In both diets, the cholesterol content was adjusted to <300 mg day^{-1}.

Table 1. Baseline anthropometric, clinical and metabolic characteristics according to T2DM remission and the diet consumed.

	LOW-FAT DIET			MEDITERRANEAN DIET		
	Non-Responders ($n = 63$)	Responders ($n = 40$)	p value	Non-Responders ($n = 47$)	Responders ($n = 33$)	p value
Male, n (%)	54 (85.7)	30 (75)	0.172	38 (80.9)	30 (90.9)	0.215
Age [years]	59.6 ± 1.2	60.0 ± 1.5	0.850	58.8 ± 1.4	61.9 ± 1.4	0.123
Weight [kg]	89.9 ± 1.7	78.7 ± 1.7	**<0.001**	87.6 ± 2.4	82.1 ± 2.0	0.103
Body mass index [kg m^{-2}]	31.9 ± 0.6	29.5 ± 0.6	**0.005**	32.3 ± 0.7	30.3 ± 0.7	0.057
Waist circumference [cm]	109 ± 1	100 ± 1	**<0.001**	107 ± 2	103 ± 2	0.086
Systolic blood pressure [mmHg]	141 ± 2	133 ± 3	**0.040**	134 ± 3	142 ± 4	0.080
Diastolic blood pressure [mmHg]	77.1 ± 1.4	75.1 ± 2.1	0.413	77.2 ± 1.4	78.4 ± 2.3	0.766
Glucose [mg dL^{-1}]	121 ± 4	99 ± 2	**<0.001**	115 ± 3	99 ± 3	**<0.001**
Insulin [mU L^{-1}]	12.4 ± 0.9	10.2 ± 1.1	0.131	14.8 ± 2.3	8.2 ± 1.2	**0.026**
HbA1c [%]	6.86 ± 0.13	6.43 ± 0.11	**0.023**	6.70 ± 0.08	6.65 ± 0.13	0.704
HDL-cholesterol [mg dL^{-1}]	40.5 ± 1.1	43.7 ± 2.1	0.147	41.1 ± 1.3	42 ± 1.5	0.627
LDL-cholesterol [mg dL^{-1}]	93.1 ± 3.3	87.9 ± 4.2	0.334	93.5 ± 4.4	90.6 ± 3.5	0.634
Triglycerides [mg dL^{-1}]	150 ± 9	147 ± 14	0.862	149 ± 9	112 ± 8	**0.005**
C-reactive protein [mg L^{-1}]	3.32 ± 0.48	4.64 ± 0.88	0.158	3.77 ± 0.60	2.99 ± 0.60	0.383

One-way ANOVA p-values ($p < 0.05$). Abbreviations: HDL-c, high-density lipoprotein; LDL-c, low-density lipoprotein. Differences in gender were tested by chi-square method. Statistically significant differences are in bold.

2.3. Dietary Assessment

Participants in both intervention groups received the same intensive and sustained dietary counseling during the whole period of the trial. At the beginning of the study and every 6 months, each patient had a face-to-face interview with a nutritionist to fill in a 137-item semi-quantitative food frequency questionnaire, validated in Spain.[28] The dietary evaluation was calculated by the validated 14-item Med Diet Adherence Screener (MEDAS), which was used for measuring adherence to the Med diet.[29] Moreover, a 9-item dietary adherence screener was used to measure adherence to the LF diet guidelines. A more detailed report on the dietary adherence has been published recently by the research group.[30] The Med and LF diet were designed to provide a wide variety of foods, including vegetables, fruit, cereals, potatoes, legumes, dairy products, meat, and fish. The participants in both intervention groups received the same intensive dietary counseling. The nutritionists administered personalized individual interviews at inclusion and every 6 months, and quarterly group education sessions were held with up to 20 participants per session and separate sessions for each group.

2.4. Clinical Plasma Parameters

Venous blood from the participants was collected in tubes containing EDTA after a 12-h overnight fast. Lipid variables, serum insulin, and plasma glucose were determined as previously reported.[31]

2.5. Methodology of the Two Metabolic Challenges

An OGTT was performed at the beginning of the study and every year. OGTT (75 g dextrose monohydrate in 250 mL water) was performed with 0, 30, 60, 90, and 120 min sampling to establish plasma glucose and insulin levels. OGTT-derived insulin sensitivity indexes (HOMA-IR, ISI, IGI, HIRI, MISI, and DI) were all calculated as previously described.[31] An oral fat tolerance test was performed at the beginning using a weight-adjusted meal (0.7 g fat and 5 mg cholesterol per kg body weight) with 12% SFA, 10% PUFA, 43% MUFA, 10% protein, and 25% carbohydrates. The meal composition was designed by a group of nutritionists with olive oil, skimmed milk, white bread, cooked egg yolks, and tomatoes. After the meal, the volunteers rested and did not consume food for 5 h but were allowed to drink water. Blood samples for biochemical testing were collected before the meal and every hour during the next 4 h, following recommendations for an oral fat tolerance test proposed by Mihas et al.[32]

2.6. Methodology of the BCAA Determination

2.6.1. Sample Preparation

Plasma samples (60 µL) were randomly deproteinized with 200 µL of 3:1 MeOH:ACN (*v/v*). The mixture was vortexed for 1 min and subsequently cooled at −20°C for 3 min. The resulting precipitate was separated by centrifugation at 14 000 × *g* for 15 min at 4°C and the supernatant phase was isolated. This phase was dried by evaporation. The residue was reconstituted with 20 µL of methoxyamine in pyridine (20 mg mL^{-1}) and maintained at 30°C for 90 min. Then, 180 µL of a 98:2 (*v/v*) BSTFA–TMCS mixture were added to the reconstituted analytical sample, which was shaken for 30 s and maintained at 37°C for 60 min. All samples were analyzed in triplicate.

2.6.2. GC-MS Analysis

GC–TOF/MS analyses were performed by EI ionization mode at 70 eV. Chromatographic separation was carried out with a

fused silica DB-5MS-UI (30 m × 0.25 mm i.d., × 0.25 μm film thickness) capillary column. The gas chromatography (GC) oven temperature program started at 60°C (1 min held), followed by a temperature ramp of 10°C min^{-1} to final 300°C (2 min held). Post-run time was programmed for 4 min up to 310°C to assure complete elution of the injected sample. Pulsed splitless injections of 1 μL of sample were carried out at 250°C and ultrapure grade helium was used as carrier gas at 1.0 mL min^{-1} flow rate. The injector needle was washed five times among injections with n-hexane and acetone to avoid contamination. The interface, ion source and quadrupole temperatures were set at 300°C, 300°C, and 200°C, respectively. A solvent delay of 5 min was used to prevent damage in the ion source filament. The TOF detector was operated at 5 spectra s^{-1} in the mass range m/z 50–550 and data were acquired in centroid mode. According to the manufacturer, daily mass calibration was performed with PFTBA to ensure mass accuracy and the resolution was 8500 full width half maximum (FWHM) at m/z 501.9706.

2.6.3. Data Treatment and Identification of Metabolites

Unknown Analysis software (version 7.0, Agilent Technologies, Santa Clara, CA, USA) was used to unzip all data files obtained by GC–TOF/MS in full scan mode. Then, MassHunter software was used to process GC–TOF/MS data files. Treatment of raw data files started by deconvolution of chromatograms to obtain a list of MFs considered as potential compounds defined by all m/z values with a common peak profile and its RT. For this purpose, the deconvolution algorithm was applied to each sample by considering all ions exceeding 1500 counts for the absolute height parameter, the accuracy error at 50 ppm and the window size factor at 150 units. The list of MFs obtained for each analysis was exported as data files in compound exchange format (.cef files). Tentative identification of compounds was performed by searching each mass spectrum in the NIST database (version 11) and also using the RI value. The identification of branched amino acids was firstly carried out by searching MS spectra on the NIST database and confirmed with standards. A table with the peak area values of branched amino acids in the samples was obtained as a result. Once the data set was extracted from the raw data files, data normalization was performed. This normalization was based on mass spectrometry total useful signal (MS-TUS) method and attempts to limit the contributions of xenobiotics and endogenous substances to the normalization factor by including only peaks present in all samples.

2.7. Statistics

SPSS statistical software (IBM SPSS Statistics version 21.0) and R software (version 3·5·0.; The R Foundation, Vienna, Austria) were used for statistical analysis of data. The normal distribution of variables was assessed using the Kolmogorov–Smirnov test. The statistical differences in the metabolic variables between groups were evaluated by one-way ANOVA. Qualitative variables were compared using the Chi-square test. A repeated-measures ANOVA test was used to determine the statistical differences between variables at baseline and during the follow-up period. A Cox proportional hazards regression analysis was performed to measure the probability of diabetes remission according to BCAA individually or combined as a score and adjusted by age, gender, diet, insulin, body mass index, triglycerides, HDL, and treatment (according to dose) with statins. The data are represented as the mean ± SEM for continuous variables and as frequencies for categorical variables. p values ≤ 0.05 were considered statistically significant.

3. Results

3.1. Baseline Characteristics of the Participants

We found no significant differences in the anthropometrics or biochemical baseline characteristics between the patients assigned to each diet (Table 1, Supporting Information). When we compared the Responders and Non-Responders groups (Table 2, Supporting Information), we observed that the Responders group had lower BMI, waist circumference, weight and HbA1c, glucose, and insulin levels than the Non-Responders group. Similar differences were found between the Responders and Non-Responders assigned to each diet (Table 1).

3.2. BCAA Plasma Levels According to T2DM Remission is Different Between Diets

We found lower isoleucine, leucine, and valine plasma levels in the OGTT (fasting and 120 min plasma levels) performed at the beginning of the study in Responders than in Non-Responders assigned to the Med diet, while we observed no such differences in Responders versus Non-Responders assigned to the LF diet (**Figure 1**). The same profile was found after 3 years of dietary intervention (Figure 1, Supporting Information). In fact, no significant changes were observed for any of the BCAA (isoleucine, leucine, and valine) after the consumption for 3 years of the LF or Med diets in the Responders and Non-Responders groups (Figure 2A and 2B, Supporting Information).

3.3. The Probability of T2DM Remission According to BCAA Depends on Diet

We tested the potential association of each BCAA plasma level at 120 min in the OGTT (**Table 2**) with the probability of T2DM remission, using Cox proportional hazards regression analysis. We found an HR per SD (95% CI) of 0.70 (0.56–0.89), 0.81 (0.64–1.03), and 0.74 (0.59–0.91) for isoleucine, leucine, and valine, respectively, with an HR (95% CI) of 0.71 (0.56–0.91), 0.80 (0.62–1.02), and 0.77 (0.62–0.96) after adjustment by covariates (age, gender, diet intensity of statins consumption, insulin, triglycerides, HDL-c, and BMI) when we included the whole population.

In contrast, we found no associations when we included the group of patients who consumed the LF diet in the Cox analysis. However, in patients who consumed the Med diet, we found an HR per SD (95% CI) of 0.53 (0.37–0.77), 0.75 (0.52–1.08), and 0.61 (0.45–0.82) for isoleucine, leucine, and valine, respectively, with an HR of 0.49 (95% CI 0.32–0.75), 0.65 (0.44–0.97), and 0.61 (0.44–0.85) after adjustment by clinical variables (Table 2).

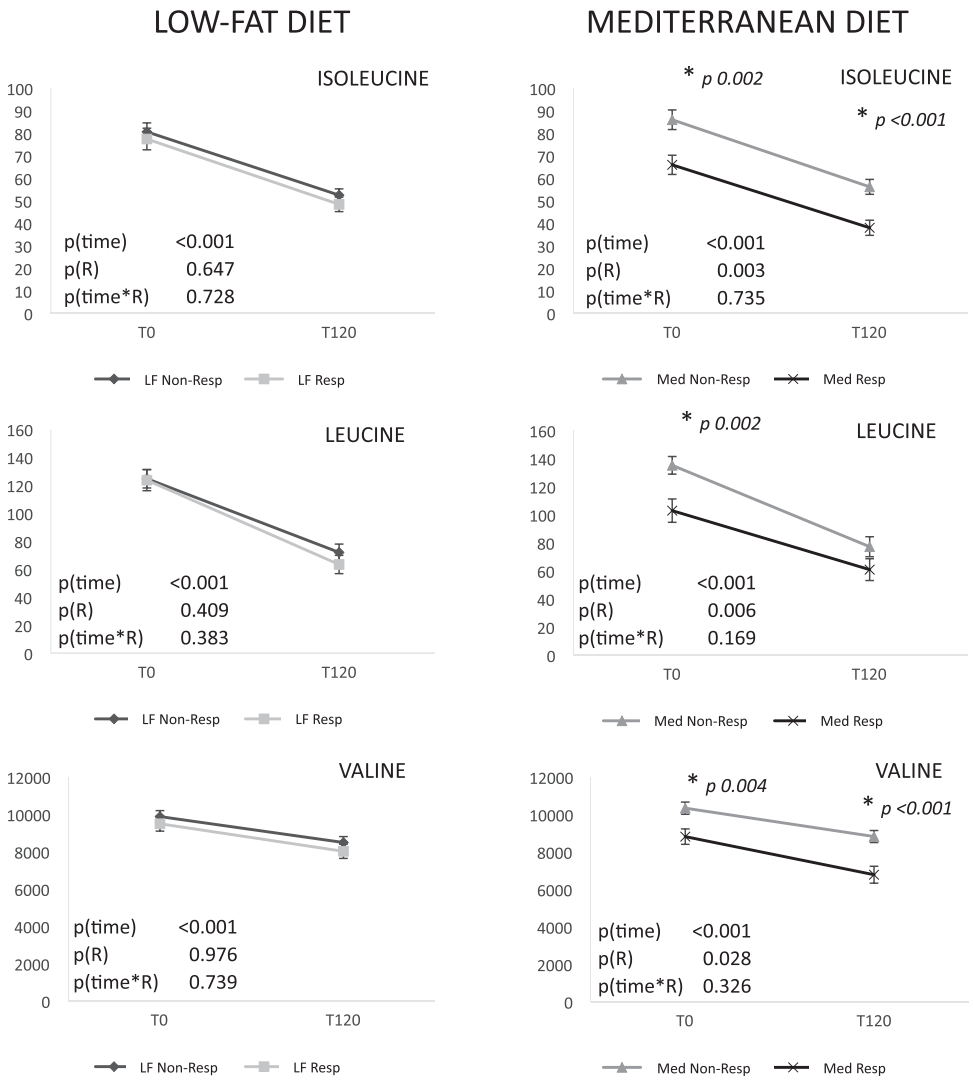

Figure 1. Baseline BCAA plasma levels in arbitrary units according to T2DM remission in the OGTT for each diet consumed. T0: fasting levels, T120: 120 min after the glucose intake. Non-Resp: Non-Responders patients. Resp: Responders patients. ANOVA for repeated measures *p*-values: p(time): OGTT time effect; p(R): remission effect; p(time*R): OGTT time by remission interaction. * $p < 0.05$ between groups in the Post Hoc Bonferroni's multiple comparison tests.

Table 2. Association of individual baseline BCAA plasma levels at 120 min in the OGTT with T2DM remission by COX regression analysis per standard deviation (SD).

		Isoleucine HR (95% CI)	Leucine HR (95% CI)	Valine HR (95% CI)
WHOLE POPULATION	*Unadjusted*	0.704 (0.555-0.894)	0.812 (0.639-1.03)	0.736 (0.594-0.913)
	*Adjusted**	0.708 (0.556-0.902)	0.796 (0.619-1.023)	0.768 (0.618-0.954)
LOW-FAT DIET	*Unadjusted*	0.880 (0.640-1.210)	0.865 (0.628-1.192)	0.887 (0.654-1.202)
	*Adjusted**	0.954 (0.686-1.327)	0.952 (0.682-1.328)	0.932 (0.678-1.281)
MEDITERRANEAN DIET	*Unadjusted*	0.531 (0.365-0.774)	0.751 (0.524-1.076)	0.611 (0.454-0.822)
	*Adjusted**	0.494 (0.326-0.748)	0.650 (0.435-0.972)	0.609 (0.436-0.851)

The analysis was adjusted by age, gender, intensity of statins treatment, insulin, HDL-c (high density lipoproteins cholesterol), and triglycerides plasma levels. HR: hazard ratio. CI: confidence interval.

Table 3. Association between diabetes remission and BCAA-based score.

			HR	(95% CI) for HR	
				Lower	Higher
WHOLE POPULATION	*Low score (ref.)*		1	1	1
	High score	*Unadjusted*	1.907	1.185	3.069
		*Adjusted**	1.822	1.121	2.963
LOW-FAT DIET	*Low score (ref.)*		1	1	1
	High score	*Unadjusted*	1.277	0.685	2.380
		*Adjusted**	1.060	0.559	2.009
MEDITERRANEAN DIET	*Low score (ref.)*		1	1	1
	High score	*Unadjusted*	3.333	1.545	7.190
		*Adjusted**	3.133	1.385	7.087

The score was built with the BCAA plasma levels in the OGTT (120 min) score in the whole population and by diet separately. A Cox regression analysis were performed with patients classified by medians of a score for each population. HR: hazard ratio. CI: confidence interval. High score values represent low BCAA levels taking into account the negative value of the COX coefficients of BCAA in both analyses, unadjusted and adjusted by age, gender, diet, intensity of statins consumption, insulin, triglycerides, HDL-c, and BMI.

3.4. A BCAA-Profile Associated to T2DM Remission by Med Diet Consumption

In order to assess the relationship between plasma BCAA levels at 120 min OGTT and T2DM remission, we built a BCAA score for each patient by multiplying the coefficients of a BCAA, obtained in the previous COX analysis and then adding the products obtained for each patient (Supplementary Materials and Methods, Supporting Information). Thus, high score values represent low BCAA levels, taking into account the negative value of the BCAA coefficients obtained in the previous COX analysis.

To assess the potential association between BCAA-based scores and the probability of T2DM remission, we performed a Cox regression analysis with patients classified by median scores (**Table 3**). Considering the whole population, patients with a high score (lower BCAA levels) presented 1.91 (1.19–3.07) a higher probability of T2DM remission than those with a low score (higher BCAA levels), 1.82 (1.12–2.96) when adjusted by covariates. Regarding the diets administered, the Med diet group with a high score (lower BCAA levels) presented 3.33 (1.55–7.19) a higher probability of T2DM remission than those with a low score, and a HR of 3.13 (95% CI 1.39–7.09) when adjusted by covariates, whereas no association was found in the LF diet group.

3.5. BCAA-Profile is Related with Insulin Resistance and Beta-Cell Functionality According to the Diet Consumed

In addition, we evaluated changes in the insulin resistance and beta-cell functionality indexes derived from OGTT according to BCAA levels and the diet consumed (**Figure 2**). We found an increase in the adipose and hepatic insulin resistance indexes in patients with high BCAA plasma levels ($p = 0.031$ and $p = 0.019$, respectively), whereas no changes were observed in patients with low BCAA levels consuming the Med diet. In contrast, we found an increase in hepatic and muscle insulin resistance indexes in patients with low BCAA levels ($p = 0.033$ and $p = 0.022$, respectively), whereas no changes were observed in patients with high BCAA plasma levels when the LF diet was consumed. In terms of beta-cell functionality, we found that both diets increased the DI in patients with low BCAA plasma levels ($p = 0.012$), while in patients with high BCAA plasma levels, the consumption of the Med diet decreased the DI compared with the consumption of the LF diet ($p = 0.032$), while no statistically significant changes were observed in the consumption of either of the diets in patients with low BCAA plasma levels.

4. Discussion

Our study showed that baseline BCAA plasma levels were negatively associated with T2DM remission in the group of patients who consumed the Med diet, whereas no association was found in the group of patients who consumed the LF diet. Moreover, the association between BCAA levels and T2DM in the Med diet group, individually or combined as a score, was stronger after the dynamic test from the BCAA plasma levels at 120 min after an OGTT than using fasting BCAA plasma levels. In addition, baseline plasma levels of BCAA were able to discern which dietary model, LF or Med diet, was more suitable for inducing T2DM remission.

Several studies have showed the relationship between BCAA plasma levels and the incidence of diabetes.[15–17] In fact, high BCAA plasma levels have been associated to metabolic abnormalities such as insulin resistance[18] by promoting an activation of the mTOR/S6K1 kinase pathway and phosphorylation of IRS1 on multiple serines, leading to incomplete fatty acid oxidation at the mitochondria[33] and the expression of several genes related to BCAA catabolism.[34] Moreover, increased BCAA catabolic flux may contribute to increased gluconeogenesis and glucose intolerance via glutamate transamination to alanine. In addition, T2DM incidence has been associated with BCAA by an overstimulation of beta cell secretion, not only by those amino acids but also by serum lipids, which contribute as secretagogues. This leads to endoplasmic reticulum stress,[35] which may ultimately contribute to beta cell dysfunction and subsequent impairment of glucose-stimulated insulin secretion (GSIS).[36]

Previous studies have shown that the consumption of Med diet reduced fasting plasma levels of BCAAs (valine, leucine, and isoleucine), which was negatively associated to CVD[23] and T2DM risk.[24] Our study showed a reduction in BCAA plasma levels after the glucose intake in the OGTT performed at baseline and after 3 years of dietary intervention, but no changes either in fasting levels or in the levels after glucose intake were observed during these years after the consumption of the Med and the LF diets, presumably due to the fact that our study included T2DM patients with CHD, whose BCAA plasma levels had been found to be abnormally high.[23]

Our study was performed in a population of newly diagnosed diabetic patients with coronary heart disease, which consumed a LF or the Med diet, without differences in the remission rate between diets. However, our results showed that baseline BCAA plasma levels, individually and combined, were associated with T2DM remission in those newly diagnosed diabetic patients who consumed the Med diet, whereas we observed no such association in those who consumed the LF diet. In fact, our results

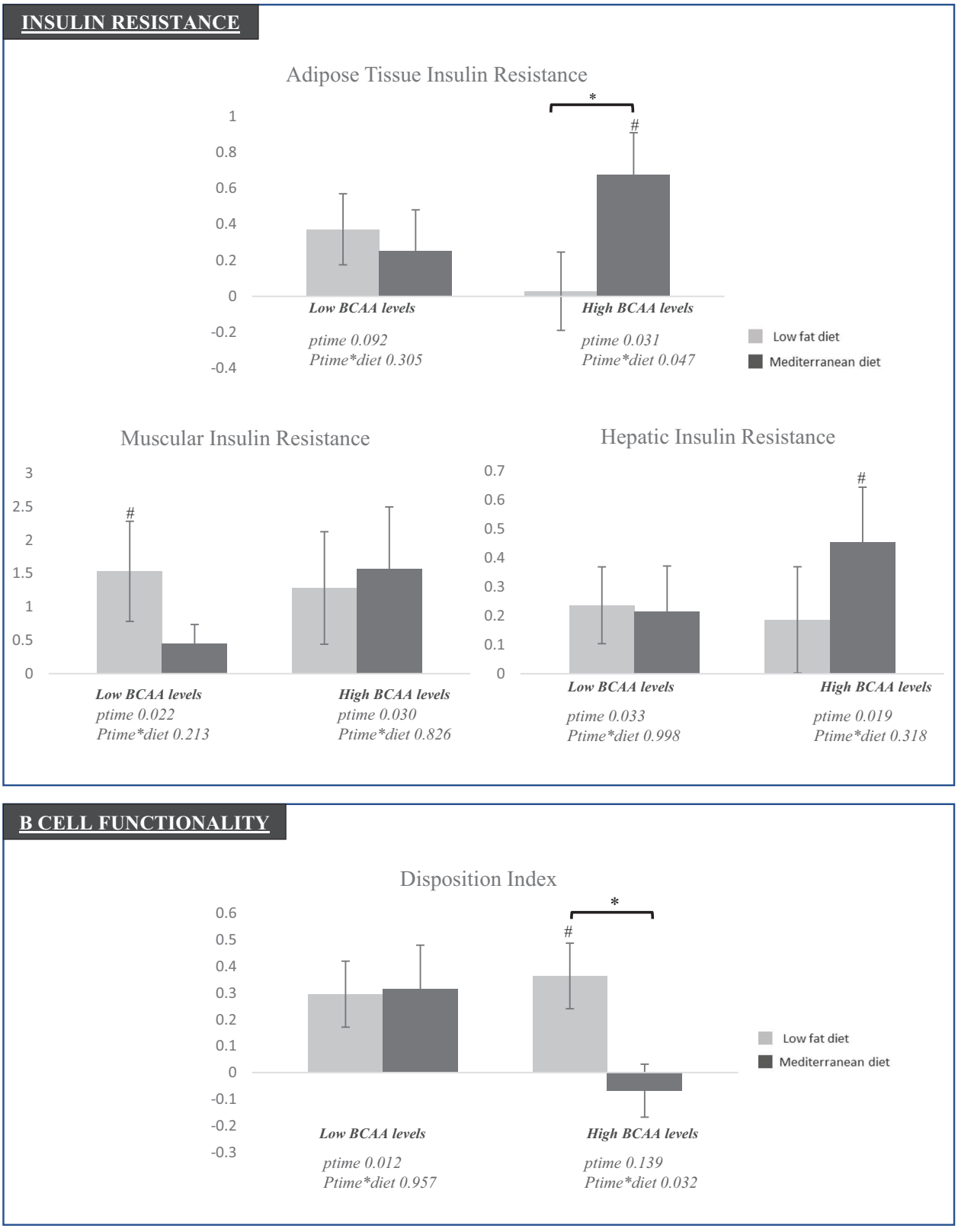

Figure 2. Percentage of change in beta-cell function and insulin resistance assessing indexes after 5 years of dietary intervention according to BCAA-based score median. Mean and standard error of adipose tissue insulin resistance index, muscular insulin resistance index, hepatic insulin resistance index and disposition index. ANOVA for repeated measures *p*-values. $^{\#}$ $p < 0.05$ compared with baseline condition in the post hoc Bonferroni's multiple comparison test. * $p < 0.05$ between diet groups in the post hoc Bonferroni's multiple comparison test.

showed that the probability of remission score, built combining the three BCAA plasma levels in addition to each BCAA individually, was negatively associated by COX regression analysis with T2DM remission in Med diet, whereas no such association was found for the LF diet. In fact, high scores, characterized by low BCAA levels, taking into account the negative value of the BCAA coefficients obtained in the previous COX analysis, were associated to a high probability of T2DM remission. Our results are consistent with previous studies in which an association was found between BCAAs, T2DM incidence[24] and cardiovascular disease[23] when an olive oil-enriched Med diet was consumed, while no association was found when participants consumed the Med diet supplemented with nuts or a control diet in which participants were advised to reduce the intake of all types of fat.

Although observational studies have shown the relationship between high BCAA levels and an impairment of glucose homeostasis,[15–17] little has been written on the potential mechanisms linking BCAA and dietary patterns. However, it seems that high circulating concentrations of BCAAs may be explained by an obesity-related catabolism in adipose tissue and a disruption in liver and skeletal muscle signaling.[37]

Some studies suggest that the deleterious effect of BCAAs on insulin sensitivity are enhanced when the fat content in the diet is high,[37] a condition in which their action as secretagogues that potentiates GSIS,[38] together with the pancreatic accumulation of fatty acid, promotes the dysfunction of pancreatic islets and hence beta-cell dysfunction.[36,39–42] Taking into account this effect, we hypothesized that the higher fat content in the Med diet compared with the LF diet may be responsible for the lower probability of remission in patients with high BCAA levels despite the beneficial effects of a Mediterranean-style diet on obesity,[43] diabetes,[44] and cardiovascular risk factors,[45,46] and, more specifically, the potential protective effect of extra-virgin olive oil.[47–49] However, low BCAA levels were associated to a higher probability of T2DM remission when a Med diet was consumed, and the consumption of the LF diet did not discriminate between high and low BCAA levels.

Taken together, our results suggest that BCAA levels are a pathophysiological key for T2DM remission and could be a crucial element in dietary pattern recommendations. BCAA levels may therefore be determinant in the design of nutritional strategies to induce T2DM remission. In fact, our study showed that patients with low BCAAs have a higher probability of T2DM remission than patients with high BCAA plasma levels who consumed the Med diet and patients with low BCAAs who consumed the LF diet. In this context, the consumption of the Med diet should be recommended to those patients with low BCAA levels, while those with higher BCAA levels would benefit more from adherence to the LF diet, which could reduce the associated insulin resistance and improve beta-cell functionality.[37] This idea is supported by the fact that consumption of the Med diet increased ATI and HIRI in patients with high BCAA levels, whereas consumption of the LF diet increased adipose tissue insulin resistance index (ATI) and muscular insulin resistance index (MISI) in patients with low BCAA levels but increased disposition index (DI) in patients with high BCAA levels.

Certain limitations of the current study must be mentioned. One limitation lies in the fact that this research is based on a long-term, well-controlled dietary intervention, which ensures the quality of the study but may not reflect the level of compliance in a free-living population. Another limitation lies in the fact that the remission of T2DM was not the primary endpoint of the CORDIOPREV trial, but was rather a secondary analysis conducted in the subgroup of newly diagnosed diabetic patients with CVD, with a sample size that did not allow us to splits data in training and test sets to validate the results. Moreover, our findings are limited to patients with CVD and precludes its generalization to healthy individuals.

In conclusion, our results imply that the dietary pattern is crucial in determining the role of BCAAs in T2DM remission. Our study also suggests that the differential relationship found between BCAA levels and T2DM remission according to the diet consumed, may potentially be used as a tool to select the most suitable dietary recommendations to induce T2DM remission by nutritional strategies. As a result, diabetes remission should definitely be the first therapeutic goal for recent-diagnosed short-duration T2DM patients, and it may be enhanced by a BCAA study at baseline, to help to provide better dietary counseling.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

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Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

M.P.C. and J.F.A.-D. contributed equally to this work. A.C. and J.L.-M. equally contributing senior authors. M.P.C. and J.F.A-D. wrote the draft manuscript. M.P.C., J.F.A-D., F.M.G-M., and J.L.-M. collected data and performed the classification of participants M.P.C., J.F.A-D., F.M.G-M., A.VG., A.P.A-L. performed the experiments. M.P.C., J.F.A-D., S.C-A., J.D-L., F.R-C., and R.M.L. performed the medical revisions of participants and clinical databases, and performed the statistical analysis. M.P.C. and J.F.A-D.,

J.M.O., A.C., and J.L.-M. interpreted the data and contributed to the discussion. A.C., J.M.O., and J.L.-M. contributed to the writing of the manuscript and revised it critically for important intellectual content. J.L.-M. and A.C. are the guarantors of this work and, as such, had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Keywords

branched chain amino acids, CORDIOPREV study, Mediterranean diet, type 2 diabetes remission

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