Available online at
ScienceDirect
www.sciencedirect.com
Elsevier Masson France
EM|consulte
www.em-consulte.com/en
Diabetes & Metabolism xxx (2016) xxx–xxx

Research letter

Branched-chain amino acids are associated with odd-chain fatty acids in normoglycaemic individuals

1. Introduction

Odd-chain fatty acids (OCFAs), such as margaric acid (C17:0) and pentadecylic acid (C15:0), and the lipids derived from them have traditionally been used as internal standards in lipidomic studies, thereby precluding their detection. It has been suggested that OCFAs might be produced by microbial fermentation in the rumen of dairy cattle and, in humans, might originate from the intake of dairy products. However, the C15:0-to-C17:0 ratio of circulating OCFAs does not match the 2:1 ratio measured in dairy products, suggesting either an endogenous synthesis of C17:0 or a preferred metabolism of C15:0 over C17:0 [1].

Recently, the OCFA C17:0 was suggested to be produced by the branched-chain amino acids (BCAAs) valine and isoleucine in adipocytes [2]. In general, free fatty acid (FFA) synthesis is initiated by a two-carbon precursor in the form of an acetyl coenzyme A (acetyl-CoA). However, in the catabolism of isoleucine and valine, three-carbon propionyl-CoA can be produced and used as a substrate for synthesis of OCFAs [2].

Elevated levels of BCAAs have been associated with insulin resistance and a future risk of type 2 diabetes (T2D) and cardiovascular disease (CVD) [3]. On the other hand, elevated levels of phospholipids containing odd-chain acyls have been associated with a reduced risk of future CVD [4] and T2D [5]. Thus, the incorporation of BCAAs into OCFAs links disease-associated metabolites to their disease-preventative counterparts.

The present study examined the association of circulating BCAA levels with FFA levels, including the OCFA C17:0. Included were 364 subjects, comprising people with normal glucose tolerance (NGT) as well as those with different types of diabetes and levels of obesity.

Abbreviations: BCAA, Branched-chain amino acid; BCAT, Branched-chain amino transferase; BMI, Body mass index; CVD, Cardiovascular disease; FFA, Free fatty acid; HOMA-IR, Homoeostasis model assessment for insulin resistance; LADA, Latent autoimmune diabetes in adults; NGT, Normal glucose tolerance; OCFA, Odd-chain fatty acid; OPLS, Orthogonal projections to latent structures; T1D, Type 1 diabetes; T2D, Type 2 diabetes.

2. Material and methods

2.1. Study populations

Plasma and serum samples were collected from the MEDIM study ($n = 171$) [6], the ANDIS (All New Diabetics in Scania) project ($n = 149$) and a weight-loss intervention study performed at Skåne University Hospital in Malmö, Sweden ($n = 44$; Table 1) [7]. All participants gave their written informed consent, and each of the cohort studies was approved by the ethics committee at Lund University, Sweden.

2.2. Analysis of branched-chain amino acids and free fatty acids

The BCAAs leucine, isoleucine and valine, the FFAs C14:0, C16:0, C17:0, C18:0, C18:1, C18:2 and C20:4, and α-hydroxybutyrate were analyzed by gas chromatography/mass spectrometry, as previously described in detail [8].

2.3. Statistical analysis

Orthogonal projections to latent structures (OPLS) were performed with SIMCA 13.0.2 (MKS Data Analytics Solutions, Malmö, Sweden), multilinear regression was performed with the R limma package and all other analyses used GraphPad Prism software (La Jolla, CA, CA). All data were log2-transformed and scaled to unit variance to obtain the standardized partial regression coefficient (β), with analyses focusing on effects rather than levels. *P* values were corrected for multiple comparisons using the false discovery rate (FDR) method.

3. Results

3.1. BCAAs covary with C17:0 in normoglycaemic individuals

First, the association between plasma metabolites and the BCAAs isoleucine and valine were examined by OPLS in the 98 subjects with NGT in the MEDIM study. Two models were calculated, one each for isoleucine and valine, and the resulting loadings, scaled as correlations, were combined in a shared and unique structure (SUS)-like plot (Fig. 1A). This plot demonstrates that both isoleucine and valine are strongly associated with the third BCAA, leucine and the aromatic amino-acids

http://dx.doi.org/10.1016/j.diabet.2016.12.008

Table 1
Characteristics of the studied cohorts.

	MEDIM study[d]		ANDIS study			Weight-loss intervention[e]	
	NGT	IGT/T2D	T1D	LADA	T2D	Before	After
	($n=98$)	($n=73$)	($n=50$)	($n=49$)	($n=50$)	($n=44$)	($n=44$)
Male/female (n/n)	46/52	47/26	40/10	23/26	27/23	14/30	14/30
Age (years)	54.1 ± 5.8	57.0 ± 5.3***[a]	24.0 ± 4.4	56.0 ± 13	63.7 ± 13.4***[b]	43.0 ± 11.8	43.9 ± 11.9***[c]
BMI (kg/m^2)	27.3 ± 4.2	30.1 ± 5.1***[a]	21.0 ± 3.5	28.4 ± 6.0	31.5 ± 5.0***[b]	42.2 ± 6.3	35.3 ± 7.1***[c]
HbA_{1c} (mmol/mol)	39.3 ± 6.0	48.1 ± 14.3***[a]	100 ± 4.8	64 ± 4.4	53 ± 3.5***[b]		
C-peptide (nmol/L)	0.64 ± 0.3	0.84 ± 0.4***[a]	0.14 ± 0.06	0.78 ± 0.4	1.3 ± 0.5***[b]		
Insulin (pmol/L)						15.5 ± 9.8	9.5 ± 5.6***[c,d]
Fasting glucose (mmol/L)	5.3 ± 0.5	7.3 ± 2.2***[a]	10.4 ± 4.5	9.6 ± 4.5	8.8 ± 3.4***[b]	6.1 ± 2.9	5.4 ± 0.9
HOMA-IR	1.7 ± 0.9	4.1 ± 3.9***[a]				4.2 ± 3.4	2.3 ± 1.5***[c]

Data are presented as means ± standard deviation (SD) unless otherwise specified. NGT: normal glucose tolerance; IGT: impaired glucose tolerance; T2D/T1D: type 2/1 diabetes; LADA: latent autoimmune diabetes in adults; BMI: body mass index; HOMA-IR: homoeostasis model assessment for insulin resistance.

[a] Compared with NGT, Student's t test: *$P<0.05$, **$P<0.01$, ***$P<0.001$.

[b] By ANOVA of T1D, LADA, T2D: *$P<0.05$, **$P<0.01$, ***$P<0.001$ vs T1D.

[c] vs before weight loss, paired Student's t test: **$P<0.01$, ***$P<0.001$.

[d] Fifty-four percent of study population born in Iraq, 46% in Sweden.

[e] Includes only participants who lost weight and/or improved HOMA-IR.

phenylalanine, tryptophan and tyrosine. Of the FFAs, C17:0 showed the strongest associations with isoleucine and valine. This was confirmed by linear regression, which revealed that BCAAs have strong associations with C17:0, including after adjusting for ethnicity, age, gender and body mass index (BMI; Table S1, see supplementary material associated with this article online).

3.2. Association between BCAAs and FFAs is lost in T2D patients

Next, the association between BCAAs and FFAs was investigated close to the time of diagnosis in 149 subjects with diabetes in the ANDIS study. These samples show the metabolic perturbations associated with diabetes, as metabolic control has not yet been fully re-established by antihyperglycaemic treatment. In patients with type 1 diabetes (T1D), C17:0 showed the second strongest association with valine and leucine, and remained associated with these BCAAs after adjusting for age, gender and BMI. Also, in patients with latent autoimmune diabetes in adults (LADA), C17:0 showed strong associations with these BCAAs and remained significant after adjustments for age, gender and BMI. In contrast, in patients with T2D, none of the FFAs were significantly associated with the BCAAs. However, after adjusting for age, gender and BMI, all FFAs except for C17:0 showed significant associations with either valine or isoleucine (Table S1, see supplementary material associated with this article online).

3.3. HOMA-IR, fasting glucose, and elevated levels of BCAAs and C17:0

In the MEDIM study, BCAAs were most strongly associated with the homoeostasis model assessment for insulin resistance (HOMA-IR; $\beta \geq 0.25$ but <0.28, $P<0.01$), followed by C17:0 ($\beta=0.24$, $P<0.01$). Associations were stronger for FFAs, with C17:0 showing the third strongest association ($\beta=0.23$, $P<0.01$), than for BCAAs ($\beta \geq 0.16$ but <0.19, $P<0.05$) after adjusting for age, gender and ethnicity. Levels of the insulin-resistance marker α-hydroxybutyrate were positively associated with HOMA-IR ($\beta=0.24$, $P<0.01$) and also after adjusting for age, ethnicity and gender ($\beta=0.24$, $P<0.01$).

Fasting glucose levels were most strongly associated with C17:0 ($\beta=0.26$, $P<0.001$), followed by the BCAAs ($\beta \geq 0.19$ but <0.24, $P<0.01$). After adjusting for age, gender and ethnicity, C17:0 retained the strongest association with fasting glucose ($\beta=0.28$, $P<0.01$), followed by five of the other six measured FFAs ($\beta \geq 0.2$ but <0.25, $P<0.05$) and the BCAAs isoleucine ($\beta=0.18$, $P<0.05$) and leucine ($\beta=0.17$, $P<0.05$).

3.4. Weight loss, levels of BCAAs and FFAs, and normalization of the association between BCAAs and C17:0

Alterations in levels of BCAAs and FFAs, and their association in obese subjects before and after a weight-loss intervention, were then examined. Those participating in the intervention lost, on average, 19.7 ± 12.5 kg ($P<0.001$), corresponding to a 6.9 ± 4.5-unit reduction in BMI ($P<0.001$). Fasting plasma glucose was unchanged whereas insulin levels were reduced by 6.0 ± 6.8 pmol•L^{-1} ($P<0.01$), resulting in a 33 ± 34% ($P<0.01$) improvement in HOMA-IR. A 26% ($P<0.001$) reduction in levels of α-hydroxybutyrate confirmed improved insulin sensitivity.

Levels of all BCAAs and FFAs, except for C20:4, were reduced after the intervention (Fig. 1B). None of the FFAs were associated with BCAA levels at baseline. However, following the weight-loss intervention, C17:0 was weakly associated with leucine ($\beta=0.39$, $P=0.051$) after adjusting for age, gender and BMI, while C18:0 showed the second strongest association to leucine ($\beta=0.38$, $P=0.051$), whereas no other FFAs were significantly associated.

Please cite this article in press as: Al-Majdoub M, et al. Branched-chain amino acids are associated with odd-chain fatty acids in normoglycaemic individuals. Diabetes Metab (2016), http://dx.doi.org/10.1016/j.diabet.2016.12.008