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Increased Plasma Branched Short-Chain Fatty Acids and Improved Glucose Homeostasis: The Microbiome and Insulin Longitudinal Evaluation Study (MILES)

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Short-chain fatty acids (SCFAs) have been extensively studied for potential beneficial roles in glucose homeostasis and risk of diabetes; however, most of this research has focused on butyrate, acetate, and propionate. The effect on metabolism of branched SCFAs (BSCFAs; isobutyrate, isovalerate, and methylbutyrate) is largely unknown. In a cohort of 219 non-Hispanic White participants and 126 African American participants, we examined the association of BSCFA with dysglycemia (prediabetes and diabetes) and oral glucose tolerance test–based measures of glucose and insulin homeostasis, as well as with demographic, anthropometric, lifestyle, and lipid traits, and other SCFAs. We observed a bimodal distribution of BSCFAs, with 25 individuals having high levels (H-BSCFA group) and 320 individuals having lower levels (L-BSCFA group). The prevalence of dysglycemia was lower in the H-BSCFA group compared with the L-BSCFA group (16% vs. 49%; *P* = 0.0014). This association remained significant after adjustment for age, sex, race, BMI, and levels of other SCFAs. Consistent with the lower rate of dysglycemia, fasting and postprandial glucose levels were lower and the disposition index was higher in the H-BSCFA group. Additional findings in H-BSCFA versus L-BSCFA included lower fasting and postprandial C-peptide levels and lower insulin clearance without differences in insulin levels, insulin sensitivity, insulin secretion, or other variables examined, including diet and physical activity. As one of the first human studies associating higher BSCFA levels with lower odds of

ARTICLE HIGHLIGHTS

- This study was undertaken to explore the relationship between short-chain fatty acids (SCFAs) and dysglycemia and to explore detailed measures of glucose and insulin homeostasis.
- We sought to determine whether less frequently studied SCFAs have a role in glucose homeostasis.
- Individuals with higher levels of branched SCFAs had a lower prevalence of dysglycemia and had improved glucose tolerance.
- The study findings indicate further investigation is warranted of branched SCFA as novel targets for prevention or treatment of diabetes.

dysglycemia and improved glucose homeostasis, this study sets the stage for further investigation of BSCFA as a novel target for prevention or treatment of diabetes.

Type 2 diabetes (T2D) is characterized by glucose dysregulation due to insulin resistance combined with insufficient insulin secretion. Several studies have shown that glucose regulation is affected by diet and gut microbiota (1). Specifically, the lack of bacterial strains that produce short-chain fatty acids (SCFAs) appears to increase the risk of developing T2D (2,3). SCFAs are produced via bacterial

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fermentation of nondigestible carbohydrates that reach the colon (4). Several studies have implicated SCFAs in improved glucose homeostasis (1).

Branched SCFAs (BSCFA; namely, isobutyric, isovaleric, methylbutyric acids) are metabolites produced by fermentation of the branched-chain amino acids (BCAA; i.e., valine, leucine, and isoleucine) derived from undigested protein in the colon (5). The role of BSCFAs in systemic metabolism and T2D is poorly understood. Fecal levels of BSCFAs were increased in patients who experienced reduction of hemoglobin A_{1c} after Roux-en-Y and gastric sleeve bypass surgeries (6). Circulating levels of isobutyrate and isovalerate were increased 12 months after Roux-en-Y gastric bypass; the increase in isobutyrate was correlated with reduction in BMI and HOMA for insulin resistance (7). In lean individuals, a brown-bean diet was associated with decreased postprandial glucose and insulin concentrations and increased plasma levels of isobutyric acid (8). To our knowledge, no study has examined the association between BSCFAs and detailed measures of glucose homeostasis in humans.

Herein, we evaluated the relationship between plasma BSCFA and prevalence of prediabetes and T2D as well as overall glucose and insulin homeostasis in participants enrolled in the Microbiome and Insulin Longitudinal Evaluation Study (MILES) (9).

RESEARCH DESIGN AND METHODS

Study Participants

This is a report on a cross-sectional study of 129 African American and 224 non-Hispanic White participants from the MILES cohort; enrollment and phenotyping protocols were previously described (9). The oral glucose tolerance test (OGTT) revealed that 136 participants (38.5%) had prediabetes (fasting glucose 100–125 mg/dL and/or 2-h glucose 140–199 mg/dL), 28 (8%) had diabetes (fasting glucose ≥126 mg/dL and/or 2-h glucose ≥200 mg/dL), and 189 (53.5%) had normoglycemia. For logistic regression analyses, those with prediabetes and diabetes were combined under "dysglycemia."

OGTT-Based Measurement of Glucose and Insulin Homeostasis

Venous blood samples were collected for measurement of plasma insulin, glucose, and C-peptide (Cpep) at time 0 (fasting), 30 min, and 120 min after administration of 75 g of oral glucose. The area under the curve (AUC) values of glucose, insulin, and Cpep were calculated using the trapezoidal method. Measurement of insulin secretion was via the AUC for insulin from baseline to 30 min divided by the corresponding AUC for glucose ($AUC\text{-}Ins_{30}/AUC\text{-}Glu_{30}$). The Matsuda insulin sensitivity index (ISI) was used to measure insulin sensitivity (10). The disposition index was calculated as the product of insulin secretion and insulin sensitivity (disposition index = ISI × $AUC\text{-}Ins_{30}/AUC\text{-}Glu_{30}$) (11). Insulin clearance was measured as the AUC of Cpep divided by the AUC of insulin ($AUC\text{-}Cpep/AUC\text{-}Ins$) (12).

Measurement of SCFAs

Plasma samples from 345 participants were analyzed for eight SCFAs (acetic acid, propionic acid, isobutyric acid, butyric acid, 2-methylbutyric acid, isovaleric acid, valeric acid, and hexanoic acid) using liquid chromatography with tandem mass spectrometry, as previously described (13). The BSCFAs are isobutyric, isovaleric, and methylbutyric acids. The remaining five have nonbranched structures.

Dietary Intake

Habitual dietary intake over the past year was assessed using the Diet History Questionnaire, version 2 (14). Participants were asked to report their frequency of consumption and average portion size of 132 foods over the past year. On the basis of their responses, Diet*Calc software (U.S. National Institutes of Health, National Cancer Institute) was used to generate habitual intake of 176 micro- and macronutrients and 124 foods and beverages, based on the U.S. Department of Agriculture's MyPyramid Equivalents Database and Food Patterns Equivalents Database (15). These data were used to generate three diet scores: the Dietary Approaches to Stop Hypertension score (16), the Alternative Mediterranean Diet score (17), and the Healthy Eating Index–2015 version (HEI-15) (18). Total Healthy Eating Index–2015 scores range from 0 to 100, with higher scores indicating higher diet quality. We also estimated total protein intake, animal protein intake, and vegetable protein intake as absolute grams per day.

Physical Activity and Substance Use

The physical activity survey used in MILES is the well-validated instrument used in the Multi-Ethnic Study of Atherosclerosis, which is based on the Cross-Cultural Activity Participation Study (19,20). The survey yields estimates of different degrees of physical activity (light, moderate, and vigorous) in METs per week. Herein, we analyzed total physical activity as the sum of light, moderate, and vigorous activity. The survey was also used to determine whether each participant engaged in any exercise physical activity in the past month. On the basis of participant response to questionnaires, alcohol use and cigarette smoking were categorized as current, former, and never.

Statistical Analysis

Upon finding a bimodal distribution in BSCFA levels, we divided the cohort into two groups: one with higher BSCFA levels (H-BSCFA group) and one with lower BSCFA levels (L-BSCFA group). Primary analyses were based on comparing these groups. To preserve the structure of the data, we did not analyze BSCFAs as continuous variables. Phenotypes between the H-BSCFA and L-BSCFA groups were compared using Student t tests (quantitative traits) and χ^2 tests (qualitative traits). For all analyses, a rank-based inverse normal transformation was applied to normalize the distribution of waist circumference; protein intake; METs; ISI; $AUC\text{-}Ins_{30}/AUC\text{-}Glu_{30}$; disposition index; $AUC\text{-}Cpep/AUC\text{-}Ins$; levels of fasting Cpep, fasting insulin, fasting

glucose, and 120-min glucose; AUC values for glucose, insulin, and Cpep; and triglyceride, AST, ALT, butyrate, propionate, acetate, valerate, and hexanoate levels. However, median values are presented in tables to facilitate interpretation. Association of the BSCFA group with the qualitative trait dysglycemia was further assessed by analyzing BSCFA status as an independent variable along with age, sex, race, BMI, and SCFA levels in logistic regression analyses where dysglycemia was the dependent variable.

Data and Resource Availability

The data are not publicly available because participants did not give consent for the data to be publicly posted. Interested researchers should contact the corresponding author and submit their credentials to the Cedars-Sinai Institutional Review Board for determination of whether they are eligible to have access to study data. Upon approval, a limited data set necessary for replication would be provided to the investigator.

RESULTS

Unlike other SCFAs, the BSCFAs exhibited a bimodal distribution. Twenty-five participants had high concentrations of BSCFAs (isovalerate, isobutyrate, and methylbutyrate) and constituted the H-BSCFA group (Fig. 1).

Table 1 displays the demographic and anthropometric characteristics, lifestyle features, lipid profile, and levels of SCFAs between the H-BSCFA and L-BSCFA groups. No differences were observed in age, sex, race, BMI, waist circumference, or lipid levels. Lifestyle factors (i.e., substance use, physical activity, diet) were similar between the groups. AST levels were lower in the H-BSCFA group. The H-BSCFA group had higher levels of acetic acid, butyric acid, hexanoic acid, propionic acid, and a trend toward increased valeric acid ($P = 0.055$) (Table 1).

Of the H-BSCFA group, 12% had prediabetes and 4% had diabetes, whereas in the L-BSCFA group, 41% had prediabetes and 8% had diabetes ($P = 0.006$). In other words, the odds ratio (OR) of having dysglycemia was reduced in the H-BSCFA group versus the L-BSCFA group

(OR 0.20; 95% CI 0.066–0.59; $P = 0.0036$). This remained significant after adjusting for age, sex, BMI, and race (adjusted OR 0.17; 95% CI 0.053–0.51; $P = 0.0019$). Given the association with five SCFA levels, we further adjusted for these along with age, sex, BMI and race, again finding the association of H-BSCFA with lower prevalence of dysglycemia remaining significant (adjusted OR 0.15; 95% CI 0.038–0.61; $P = 0.0076$).

We analyzed glucose homeostasis via OGTT. The H-BSCFA group had lower fasting glucose levels, lower glucose level at 120 min, and lower glucose AUC (Table 2). The two groups did not differ in fasting insulin levels or total insulin AUC (Table 2). The H-BSCFA group had lower fasting Cpep levels and lower overall AUC of Cpep (Table 2).

We found no differences in insulin sensitivity (as indicated by the ISI) or insulin secretion (AUC-Ins$_{30}$/AUC-Glu$_{30}$) values between the H-BSCFA and L-BSCFA groups (Table 2). The H-BSCFA group had higher disposition index versus L-BSCFA. Furthermore, insulin clearance as Cpep to insulin ratio was lower in the H-BSCFA group (Table 2).

DISCUSSION

Understanding the link between the gut and metabolic regulation is a crucial step in identifying potential therapeutic targets for diabetes. Recent studies have shown a strong relationship between gut metabolites, such as SCFAs and BSCFAs, and glucose homeostasis (2). We found that 25 study participants with high levels of plasma BSCFAs had lower prevalence of dysglycemia compared with the L-BSCFA group. Consistent with this finding, the H-BSCFA group also had lower fasting and postprandial glucose levels and higher disposition index. Higher disposition index is well established as protective against diabetes (21). Additional findings in the H-BSCFA group versus the L-BSCFA group included lower Cpep levels and lower insulin clearance without differences in fasting insulin, AUC insulin, insulin sensitivity, or insulin secretion values.

The finding that the H-BSCFA group had no difference in total insulin levels but had lower Cpep and insulin clearance values is intriguing. Lower Cpep levels in the H-BSCFA
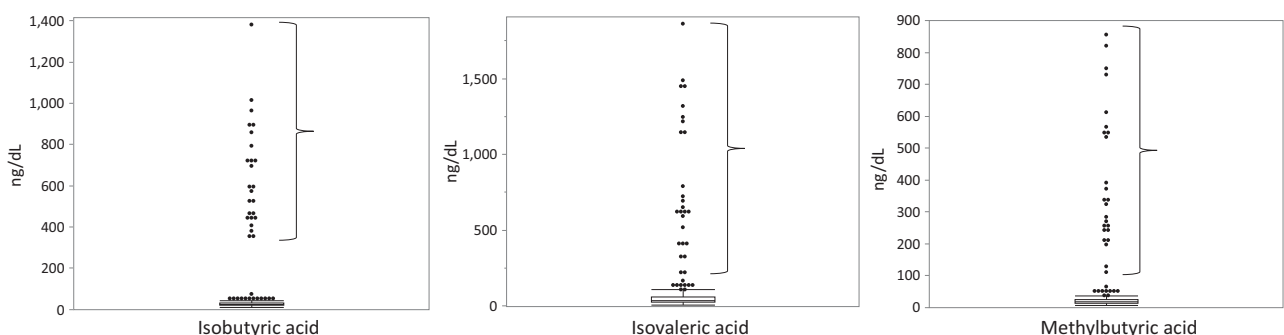
Figure 1—Bimodal distribution of BSCFAs. The brackets indicate 25 individuals with higher levels of isobutyric acid, isovaleric acid, and methylbutyric acid.

Table 1—Demographic, anthropometric, lifestyle, lipid, and SCFA variables in the high- and low-BSCFA groups

	H-BSCFA ($n = 25$)	L-BSCFA ($n = 320$)	*P* value
Age (years)	60 (12.5)	60 (14.0)	0.55
Sex (% male)	44.0	38.8	0.60
Race (% African American)	44.0	35.9	0.42
BMI (kg/m^2)	28.6 (10.4)	28.0 (7.7)	0.46
Waist circumference (cm)	100.5 (31.2)	97.0 (21.5)	0.54
Alcohol use (% current)	80.0	73.4	0.77
Cigarette use (% current)	28.0	13.1	0.12
Exercise (% yes)	92.0	84.8	0.33
METs (total in 1 week)	8,707.5 (10,755.0)	8,332.5 (6,581.3)	0.66
DASH score	23 (4.25)	24 (6.00)	0.69
HEI-15 diet score	70.9 (14.9)	69.07 (15.35)	0.79
A-MED diet score	4 (3)	4 (3)	0.45
Total protein (g/day)	72.0 (62.2)	59.3 (41.0)	0.35
Animal protein (g/day)	41.7 (45.2)	36.7 (28.4)	0.24
Vegetable protein (g/day)	26.5 (24.7)	23.3 (17.1)	0.40
AST (units/L)	16.0 (7.0)	20.0 (7.0)	0.045
ALT (units/L)	11.5 (6.3)	11.5 (6.6)	0.34
Total cholesterol (mmol/L)	4.58 (1.06)	4.65 (1.18)	0.29
HDL cholesterol (mmol/L)	1.29 (0.47)	1.37 (0.57)	0.11
LDL cholesterol (mmol/L)	2.80 (1.08)	2.72 (1.17)	0.82
Triglycerides (mmol/L)	0.79 (0.42)	0.91 (0.56)	0.29
Isobutyric acid (ng/mL)	590.4 (380.9)	22.7 (9.5)	<0.0001
Isovaleric acid (ng/mL)	652.0 (762.4)	34.9 (27.3)	<0.0001
Methylbutyric acid (ng/mL)	335.9 (313.2)	17.4 (8.1)	<0.0001
Acetic acid (ng/mL)	6,237.1 (2,354.0)	2,335.8 (1,761.9)	<0.0001
Butyric acid (ng/mL)	38.1 (52.9)	28.2 (26.9)	0.0027
Hexanoic acid (ng/mL)	107.1 (95.1)	32.9 (21.2)	<0.0001
Propionic acid (ng/mL)	277.7 (166.1)	76.7 (63.1)	<0.0001
Valeric acid (ng/mL)	7.6 (6.94)	6.4 (5.9)	0.055

Data are reported as median (interquartile range) for quantitative traits and percentage for sex, race, alcohol use, cigarette use, and exercise. Quantitative traits were compared with *t* tests and qualitative traits were compared with χ^2 tests. A-MED, Alternative Mediterranean Diet; DASH, Dietary Approaches to Stop Hypertension; HEI-15, Healthy Eating Index–2015.

group does not indicate that β-cell function was compromised; in fact, the disposition index was increased. Because disposition index is widely used as a measure of the β-cell's ability to compensate for changes in insulin sensitivity, we postulate that the H-BSCFA group's β-cell function is not diminished, but rather, it is functionally preserved from this group's lower insulin clearance. If the effect of BSCFA is primarily on β-cell efficiency, however, it is also possible that the lower insulin clearance we observed in our study is a spurious finding, driven by the effect of lower Cpep on the AUC Cpep to AUC insulin ratio.

Alternatively, it is possible that BSCFA can act either directly or indirectly on insulin clearance without exacerbating insulin resistance. We found no difference in insulin sensitivity between the two groups. Additionally, our study revealed that the H-BSCFA group had both lower insulin clearance and lower rates of dysglycemia. This is seemingly contrary to the widely known positive relationship between insulin clearance and sensitivity (22), and to the inverse association of insulin clearance and incidence of diabetes (12). Given the finding of lower AST levels in the H-BSCFA group, it is possible that BSCFA may affect insulin clearance at the level of the liver, because other SCFAs can activate peroxisome proliferator–activated receptor α (PPAR-α), which decreases hepatic insulin clearance (23,24). Longer branched-chain fatty acids can increase hepatic PPAR-α expression,

Table 2—Glucose and insulin homeostasis traits in the high- and low-BSCFA groups

	H-BSCFA (n = 25)	L-BSCFA (n = 320)	P value
Fasting glucose (mmol/L)	5.11 (0.56)	5.38 (0.72)	0.0006
Glucose at 120 min (mmol/L)	5.27 (2.53)	6.33 (2.68)	0.014
AUC glucose (mmol/L × min)	780.2 (149.0)	885.1 (241.5)	0.0025
Fasting insulin (pmol/L)	47.8 (41.5)	56.5 (52.4)	0.96
AUC insulin (pmol/L × min)	40,547.7 (32,271.3)	50,171.9 (44,858.5)	0.35
Fasting Cpep (nmol/L)	0.45 (0.29)	0.70 (0.41)	<0.0001
AUC Cpep (nmol/L × min)	167.3 (95.7)	300.2 (130.0)	<0.0001
Insulin sensitivity (ISI)	5.16 (3.55)	4.03 (3.62)	0.20
Insulin secretion (AUC-Ins$_{30}$/AUC-Glu$_{30}$)	0.37 (0.27)	0.35 (0.31)	0.42
Disposition index (ISI × AUC-Ins$_{30}$/AUC-Glu$_{30}$)	1.85 (1.40)	1.48 (1.06)	0.037
Insulin clearance (AUC-Cpep/AUC-Ins)	0.068 (0.030)	0.11 (0.051)	<0.0001

Data are reported as median (interquartile range). Traits were compared with *t* tests.

improving hepatic lipid metabolism (25). Perhaps like SCFAs and branched-chain fatty acids, BSCFAs may activate PPAR-α receptors, causing decreased insulin clearance and a compensatory decrease in insulin secretion to maintain insulin sensitivity. Direct studies of BSCFAs in hepatocytes are needed.

BSCFAs are derived from fermentation of BCAAs in the gut. Of note, several studies have found that higher circulating levels of BCAAs are associated with insulin resistance, dysglycemia, and risk of T2D (5). A possible explanation of our findings is that higher BSCFA levels reflect a higher rate of metabolism of BCAAs, resulting in lower bioavailability of BCAAs and improved glycemia in the H-BSCFA group. This hypothesis remains to be tested.

Our findings suggest that lifestyle factors do not affect levels of plasma BSCFAs. Perhaps plasma BSCFA level is not dependent on diet or physical activity, but rather on differences in gut-to-bloodstream bioavailability, hepatic clearance of BSCFAs, gut transit time, or human genetic variation. The H-BSCFA group also had increased levels of the five other SCFAs compared with the L-BSCFA group. The observation that having high BSCFA levels was associated with lower odds of dysglycemia even when adjusting for the other five SCFAs suggests a primary effect of BSCFAs rather than a confounding effect of the other SCFAs.

The cross-sectional nature of our study limits our ability to assess whether higher levels of BSCFAs are causal for improving dysglycemia. Another limitation is sample size; trends for lower fasting and postprandial insulin levels and higher insulin sensitivity in the H-BSCFA group (Table 2) may have achieved significance in a larger sample size. Also, the diet scores were based on a validated questionnaire; however, there is a possibility of under-reporting intake. Another limitation of our study is the use of OGTT-derived measures rather than using direct measures of β-cell function and insulin sensitivity that can be obtained with clamp studies.

To our knowledge, this is the first study to report a beneficial association between high plasma BSCFA levels and decreased prevalence of dysglycemia in humans. Although more studies are required to investigate this association, this is a promising first step in identifying novel therapeutic targets for the prevention and treatment of T2D.

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