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Diets High in Protein or Saturated Fat Do Not Affect Insulin Sensitivity or Plasma Concentrations of Lipids and Lipoproteins in Overweight and Obese Adults[1–3]

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Abstract

Background: Previous human studies reported inconsistent effects of dietary protein and branched-chain amino acids (BCAAs) on insulin action and glucose metabolism. Similarly, it is unclear whether saturated fat (SF) intake influences these metabolic variables.

Objective: The objective of this study was to test the effects of high [30% of energy (%E)] vs. moderate (20%E) intakes of protein (primarily whey) on insulin action and lipid and lipoprotein concentrations in the context of both high (15%E) and low (7%E) SF diets.

Methods: The study was conducted as a randomized controlled trial in 158 overweight and obese men and women. After a 4-wk baseline diet [55%E carbohydrate, 15%E protein, 30%E fat (7%E SF)], participants were randomly assigned to 4 wk of either the baseline diet or 1 of 4 test diets containing 35%E carbohydrate and either 20%E or 30%E protein and either 7%E or 15%E SF. Frequently sampled i.v. glucose tolerance tests were administered after each dietary period.

Results: Other than significantly higher fasting glucose concentrations for high vs. moderate protein intakes with a low-fat diet (difference ± SE: 0.47 ± 0.14 mmol/L; $P = 0.001$), there were no significant effects of dietary protein or SF on glucose metabolism, plasma insulin, or concentrations of lipids and lipoproteins. Changes in plasma BCAAs across all diets were negatively correlated with changes in the metabolic clearance rate of insulin ($\rho = -0.18$, $P = 0.03$) and positively correlated with changes in the acute insulin response to glucose ($\rho = 0.15$, $P = 0.05$).

Conclusions: These findings suggest that short-term intake of BCAAs can influence insulin dynamics. However, in this group of overweight and obese individuals, neither high protein nor SF intake affected insulin sensitivity or plasma concentrations of lipids and lipoproteins. This trial was registered at clinicaltrials.gov as NCT00508937. J. Nutr. 144: 1753–1759, 2014.

Introduction

Insulin resistance increases the risk of developing type 2 diabetes. High-protein diets have been hypothesized to improve insulin resistance, but such diets may also promote weight loss, and weight loss, even if modest, can improve insulin sensitivity. Whether high-protein diets can improve glucose tolerance and insulin sensitivity independent of weight loss remains unclear. Although some studies showed improved glucose homeostasis with high-protein vs. conventional hypocaloric diets with similar weight loss (1–3), others showed that high protein intake produced either no improvement in (4–7) or worsening of (8,9) glucose homeostasis. The few trials that directly tested the effects of higher protein diets on insulin sensitivity in the absence of weight loss also produced mixed results (9–11).

These discrepancies may be due in part to different protein sources used in the studies, which can differ in their effects on glucose homeostasis (12). Higher intakes of dairy products have been associated with lower diabetes risk (13–15). Although it is not known which component of dairy confers reduced risk, there is evidence that dairy protein, especially whey, may improve glucose homeostasis by stimulating insulin secretion (16,17). On

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[3] Supplemental Figure 1 and Supplemental Tables 1–3 are available from the "Online Supporting Material" link in the online posting of the article and from the same link in the online table of contents at http://jn.nutrition.org.

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the other hand, metabolomic studies found that high plasma concentrations of BCAAs, which are abundant in whey, are associated with insulin resistance and obesity (18–20); and in mice, BCAA feeding induces insulin resistance (19).

High-protein diets, particularly those enriched with animal protein, typically are high in saturated fat (SF)[8]. There is, however, no compelling evidence that dietary SF influences insulin sensitivity in humans. Specifically, several large intervention trials produced no difference in insulin sensitivity with isocaloric replacement of SF with monounsaturated fat when total fat remained high [>37% of energy (%E)] (21–23).

We therefore conducted a randomized controlled trial to determine whether a high-protein weight-maintenance diet altered insulin sensitivity and/or cardiovascular disease risk factors compared with a lower protein weight-maintenance diet at 2 amounts of SF intake (7% vs. 15%E). We also tested whether plasma biomarkers of protein and SF intake were associated with measures of insulin sensitivity and dynamics.

Participants and Methods

Study population. Participants were recruited through Internet and direct mail solicitations. Eligibility criteria were as follows: age ≥18 y, no present use of tobacco products, BMI of 25–40 kg/m^2, HOMA-IR (glucose × insulin/22.5) ≥2.5, fasting blood glucose <7.0 mmol/L, plasma TGs <5.65 mmol/L, and total and LDL cholesterol (LDL-C) ≤95th percentile for age and sex. Exclusion criteria were history of diabetes, cardiovascular disease, or other chronic disease or taking drugs known to affect glucose or lipid metabolism, blood thinning agents, dietary supplements, or hormones. Participants' characteristics at screening are presented in **Supplemental Table 1**.

All participants provided written informed consent. The protocol was reviewed and approved by the institutional review boards of Children's Hospital and Research Center Oakland and the University of California, San Francisco.

Study design. This outpatient study in free-living participants was carried out at 2 research clinics: the Cholesterol Research Center (Berkeley, CA) and San Francisco General Hospital (San Francisco, CA). All participants consumed a baseline run-in diet for 4 wk, after which they were randomly assigned to the baseline diet (control) or 1 of the following 4 diets for 4 wk: *1*) high protein, high SF; *2*) high protein, low SF; *3*) moderate protein, high SF; or *4*) moderate protein, low SF (**Fig. 1**). They were monitored weekly to maintain a relatively stable weight throughout the study [±3% of initial weight up to ±5 pounds (2.3 kg)] and their usual level of physical activity as monitored by a pedometer and activity log.

After each 4-wk dietary period, body weight was measured and percentage body fat was assessed by bioimpedance (TBF 551 body weight scale; Tanita) and a blood sample was collected after a 12- to 14-h overnight fast. A frequently sampled i.v. glucose tolerance test was performed as previously described (24). Briefly, an i.v. catheter was inserted into each arm, and after 45 min of rest 2 baseline blood samples were drawn (−15 and −5 min). At time 0, a 0.3 g/kg bolus of 50% dextrose was given; and blood samples were drawn at 2, 4, 6, 8, 14, and 19 min. At 20 min, an i.v. bolus of human insulin (0.03 U/kg) was administered. Blood sampling continued at 22, 24, 30, 40, 60, 80, 100, 120, 140, 160, 180, 210, and 240 min. The insulin sensitivity index (S_I) was calculated by using minimal model analysis (25). The acute insulin response to glucose (AIRg) was calculated as the AUC in the first 8 min after infusing the dextrose. The disposition index (DI) was calculated as the product of S_I and AIRg, and the metabolic clearance rate of insulin (MCRi) was calculated by dividing the dose of insulin normalized for body weight by the AUC of insulin above basal, the latter estimated by fitting an exponential decay curve to the insulin profile between t = 20 and 120 min (MLAB; Civilized Software) (26).

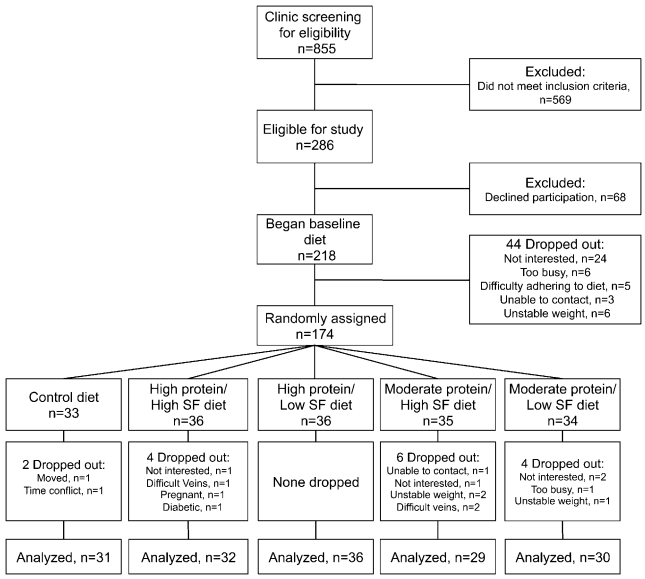

FIGURE 1 Participant enrollment. SF, saturated fat.

Dietary interventions. **Table 1** presents the nutritional composition of the prescribed study diets over their 7-d rotating menus. Participants were provided with frozen entrées (Lifespring Home Nutrition) for lunch and dinner and were required to purchase foods and prepare breakfast and snacks according to menu instructions and shopping lists. Whey protein isolate (Provon 290; Glanbia Nutritionals) was used to partially meet the increased protein content of the moderate- and high-protein diets. High-, low-, and nonfat dairy products (milk, cheese, yogurt, butter) were primarily used to achieve differences in SF between the high- and low-SF diets. Body weight was measured weekly and, if needed, energy intake was adjusted to achieve stable weight. All diets met the RDA for vitamins and minerals (27). A 5-point compliance score was assigned by the dietitian using weekly interviews, menu checklists, and grocery receipts.

Laboratory measurements. Insulin was measured by ELISA (Millipore). Total cholesterol, HDL cholesterol (HDL-C), TGs, and glucose were measured by enzymatic endpoint measurements by using enzyme reagent kits (Ciba Corning Diagnostics) on a Ciba Corning Express

TABLE 1 Composition of baseline and experimental diets[1]

		High protein		Moderate protein	
	Baseline control	High SF	Low SF	High SF	Low SF
Carbohydrate, *%E*	55	35	35	35	35
GI	55	61	61	58	57
GL	213	146	144	138	136
Protein, *%E*	15	30	30	20	20
Whey isolate, *g*	0	51	51	15	9
Total fat, *%E*	30	35	35	45	45
SFAs	7	15	7	15	7
MUFAs	13	10	18	20	29
PUFAs	7	7	8	7	7
Cholesterol, *mg*	357	355	314	358	356

[1] Values shown are for the 12,540-kJ menus. GI, glycemic index; GL, glycemic load; SF, saturated fat; %E, percentage of energy.

[8] Abbreviations used: AIRg, acute insulin response to glucose; DI, disposition index; HDL-C, HDL cholesterol; LDL-C, LDL cholesterol; MCRi, metabolic clearance rate of insulin; SF, saturated fat; S_I, insulin sensitivity index; %E, percentage of energy.

Plus 550 analyzer. LDL-C was calculated by using the Friedewald equation (28). apoB and apoAI were measured by immunoturbidimetric assays (Bacton Assay Systems and Express Plus 550 analyzer) (29,30). Lipoprotein(a) was measured by immunoassay (Myriad RBM). Lipoprotein subclass concentrations were measured by ion mobility, which uses gas-phase differential electrophoretic macromolecular mobility to directly measure lipoprotein particle concentration (31). Plasma concentrations of SFAs (10:0, 12:0, 14:0, 15:0, 16:0, and 18:0) and BCAAs (isoleucine, leucine, and valine) were quantified by Lipomics, as previously described (32). FAs are expressed as mole percentages.

Statistical procedures. ANCOVA was used to compare the baseline means when adjusted for sex, with **Supplemental Table 2** presenting the baseline means ± SDs for each diet. ANCOVA was also used to test whether the sex-adjusted mean 4-wk changes from baseline differed across diets. Those results are presented in **Table 2** and **Supplemental Table 3**, which include the adjusted means ± SEs for each diet. To assess the effect of protein and SF, linear contrasts were used to estimate the mean (95% CI) difference in 4-wk changes from baseline for the high- vs. moderate-protein diets when averaged over the 2 amounts of SF to assess the effect of protein and for the high- and low-SF diets when averaged over the moderate- and high-protein diets to assess the effects of SF. Interactions between SF and protein intake were compared by using linear contrast. Differences between individual diets were tested by using the Tukey-Kramer honestly significant difference test. The distributions of the variables after the baseline diet and their changes after the experimental diets were examined for departures from normality and log-transformed as required. As expected, the differences during the dietary interventions were more normally distributed than the cross-sectional measurements and required fewer transformations. Spearman's correlation coefficients (ρ) were used to evaluate the relations between changes in BCAAs and glucose metabolism. Baseline values in the text are presented as means ± SDs, and changes in text and figures are presented as means ± SEs. A P value <0.05 was considered significant. All statistical procedures were performed by using JMP 9.0 (SAS Institute).

Results

Study participants. One hundred fifty-nine participants completed the study. One participant who became diabetic during the study was excluded from analysis. Figure 1 displays details of participant recruitment and withdrawal. Age and BMI of participants at baseline were 38 ± 12 y and 33.9 ± 3.8 kg/m^2, respectively, and did not differ between diet groups. Ninety-five percent of participants had a dietary compliance score of at least 4 of 5 and the inclusion of compliance score as a covariate did not significantly alter results.

Effects of the diets on glucose metabolism and plasma lipid and lipoprotein concentrations. Other than a small difference in total cholesterol, there were no significant differences in mean plasma glucose, lipid, or lipoprotein measurements between the experimental groups after the 4-wk baseline run-in diet (Supplemental Table 2). We used linear contrasts to assess the effects of dietary protein and SF on outcome measures (see Methods). Table 2 shows that fasting plasma glucose concentrations increased significantly after consuming the high-protein diets relative to the moderate-protein diets, with a significant interaction between protein and SF intake (P = 0.001) such that there was a significant increase in fasting glucose with the high- vs. moderate-protein diet when SF intake was low. There were no other significant effects of either protein or SF on changes in glucose metabolism or plasma lipids (Table 2), apolipoproteins, or lipoprotein subclass concentrations from the baseline diet (Supplemental Table 3).

Verification of dietary compliance on the basis of plasma amino and FA concentrations. Plasma BCAAs (sum of leucine, isoleucine, and valine) were measured as biomarkers of protein intake. Higher protein intake was associated with a greater increase in plasma BCAAs from the baseline diet (**Fig. 2***A*).

Plasma concentrations of a panel of FAs were used as biomarkers of dietary fat intake. Compared with participants consuming the low-SF diets, participants consuming the high-SF diets had significantly greater increases from the baseline run-in diet in mean plasma concentrations of lauric (12:0), myristic

TABLE 2 Changes in body weight, waist circumference, glucose metabolism, and plasma lipid and lipoprotein concentrations in overweight and obese adults after 4 wk of consuming diets containing different amounts of protein and SF[1]

		High protein		Moderate protein		Mean (95% CI) difference for protein and SF effects[2]	
	Control (n = 31)	High SF (n = 32)	Low SF (n = 36)	High SF (n = 29)	Low SF (n = 30)	High–moderate protein	High–low SF
Weight, *kg*	−0.6 ± 0.5	−0.7 ± 0.4	−0.3 ± 0.4	−0.1 ± 0.5	0.3 ± 0.5	−0.6 (−1.4, 0.3)	−0.4 (−1.3, 0.5)
BMI, *kg/m^2*	−0.1 ± 0.1	−0.1 ± 0.1	−0.0 ± 0.1	−0.0 ± 0.1	0.1 ± 0.1	−0.1 (−0.2, 0.1)	−0.1 (−0.2, 0.1)
Body fat, *%*	−0.6 ± 0.3	−0.2 ± 0.3	0.4 ± 0.3	−0.4 ± 0.3	−0.3 ± 0.3	0.3 (−0.2, 0.9)	−0.4 (−0.9, 0.2)
Waist circumference, *cm*	0 ± 1	−2 ± 1	−2 ± 1	−2 ± 1	0 ± 1	−1 (−3, 1)	−1 (−2, 1)
Glucose, *mmol/L*	0.07 ± 0.10^{a,b}	−0.17 ± 0.10^{b,c}	0.14 ± 0.09^{a}	0.01 ± 0.10^{a,b}	−0.32 ± 0.10^{c}	0.14 (−0.05, 0.34)	0.01 (−0.18, 0.21)
Insulin, *pmol/L*	−1.6 ± 1.1	−0.2 ± 1.1	1.3 ± 1.0	−0.0 ± 1.1	−2.1 ± 1.1	1.6 (−0.5, 3.7)	0.3 (−1.8, 2.5)
S_I,[3] $\times 10^{-5}$ *min^{-1} per pmol/L*	0.2 ± 0.3	−0.2 ± 0.3	−0.1 ± 0.5	−0.5 ± 0.3	−0.7 ± 0.4	0.5 (−0.3, 1.1)	0.1 (−0.5, 0.6)
AIRg,[3] *pmol/L × 10 min*	−62 ± 60	49 ± 32	56 ± 46	−15 ± 21	35 ± 43	47 (−55, 149)	−36 (−140, 67)
DI[3]	4 ± 193	−72 ± 190	227 ± 181	−95 ± 201	−76 ± 196	163 (−213, 539)	−159 (−539, 222)
MCRi, *L/min*	0.5 ± 0.6	−0.2 ± 0.6	0.3 ± 0.6	0.3 ± 0.6	0.6 ± 0.6	−0.4 (−1.6, 0.8)	−0.4 (−1.6, 0.8)
TGs,[3] *mmol/L*	−0.11 ± 0.10	−0.12 ± 0.10	−0.31 ± 0.10	−0.26 ± 0.11	−0.21 ± 0.10	0.02 (−0.18, 0.22)	0.07 (−0.13, 0.27)
TC, *mmol/L*	−0.04 ± 0.08	−0.18 ± 0.08	−0.20 ± 0.08	−0.09 ± 0.09	−0.08 ± 0.09	−0.11 (−0.27, 0.06)	0.00 (−0.17, 0.17)
LDL-C, *mmol/L*	0.01 ± 0.08	−0.11 ± 0.08	−0.09 ± 0.07	0.01 ± 0.08	−0.01 ± 0.08	−0.10 (−0.25, 0.05)	0.00 (−0.15, 0.15)
HDL-C, *mmol/L*	0.00 ± 0.02	−0.01 ± 0.02	0.00 ± 0.02	0.02 ± 0.02	0.01 ± 0.02	−0.02 (−0.06, 0.05)	−0.01 (−0.04, 0.03)

[1] Values for diets are means ± SEs, adjusted for sex. Means within a row without a common letter are significantly different, $P < 0.05$. AIRg, acute insulin response to glucose; DI, disposition index; HDL-C, HDL cholesterol; LDL-C, LDL cholesterol; MCRi, metabolic clearance rate of insulin; SF, saturated fat; S_I, insulin sensitivity index; TC, total cholesterol.
[2] For comparison of high- vs. moderate-protein and comparison of high- vs. low-SF diets.
[3] ANCOVAs were repeated with the use of log-transformed data, and levels of significance only negligibly improved.

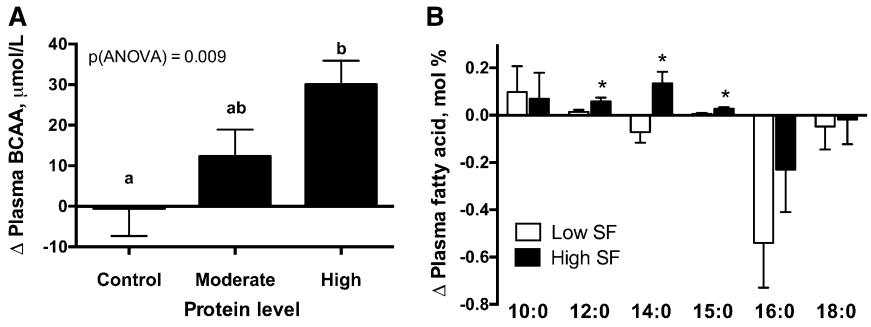

FIGURE 2 Changes in plasma concentrations of BCAAs (*A*) and SFAs (*B*) in overweight and obese adults after 4 wk of consuming diets containing different amounts of protein and SF. Data from high-SF and low-SF diets were combined for each protein group (control: $n = 31$; moderate: $n = 59$; high: $n = 68$), and data from moderate- and high-protein diets were combined for each SF group (low SF: $n = 66$; high SF: $n = 61$). Values are means ± SEs. Groups without a common letter are significantly different, $P < 0.05$ (*A*). *Different from low-SF diet, $P < 0.05$ (*B*). SF, saturated fat.

(14:0), and pentadecanoic (15:0) acids, which are enriched in dairy fat (Fig. 2*B*). Among these, 15:0 is a specific marker for dairy fat intake (33). There was no significant effect of SF on changes in other measured plasma SFAs (10:0, 16:0, and 18:0) from the baseline diet. SFAs 16:0 and 18:0 were reported to have weaker correlations with dietary intake, in part due to endogenous production (34,35). Mean ± SE plasma total MUFAs decreased after consumption of the high-SF diet in comparison with the low-SF diet (high-SF diet: −0.91 ± 0.28 mol%; low-SF diet: 0.23 ± 0.32 mol%; $P = 0.009$). There were no significant differences in changes in plasma total PUFA concentrations from the baseline diet between the high-SF and low-SF groups.

Relations of plasma BCAAs to measures of glucose metabolism. Despite overall increases in mean plasma BCAA concentrations from the baseline diet with increasing protein amount in the experimental diets, there was considerable individual variation in the response. We used this variation to explore the relation of changes in plasma BCAAs and changes in measures of glucose metabolism independent of the experimental diets. In cross-sectional analysis, BCAAs measured after the baseline diet were positively correlated with fasting plasma concentrations of insulin ($\rho = 0.26$, $P = 0.001$) and glucose ($\rho = 0.26$, $P = 0.001$) and with HOMA-IR ($\rho = 0.30$, $P = 0.0001$) and were negatively correlated with AIRg ($\rho = -0.17$, $P = 0.03$) and DI ($\rho = -0.25$, $P = 0.002$) but not S_I ($\rho = -0.06$, $P = 0.42$) or MCRi ($\rho = -0.1$, $P = 0.19$). Changes in BCAAs were inversely correlated with changes in MCRi ($\rho = -0.18$, $P = 0.03$) and positively correlated with changes in AIRg ($\rho = 0.15$, $P = 0.05$) (**Supplemental Fig. 1**). Notably, the inverse correlation between changes in MCRi and plasma BCAAs was driven by the individuals who consumed the high-SF diet (P–interaction = 0.05). BCAA response was not significantly correlated with changes in fasting glucose ($\rho = 0.08$, $P = 0.32$), insulin ($\rho = 0.05$, $P = 0.52$), S_I ($\rho = -0.10$, $P = 0.20$), or DI ($\rho = -0.06$, $P = 0.45$).

Discussion

Epidemiologic studies suggest that high protein intake may be associated with increased risk of developing type 2 diabetes, a condition usually characterized by insulin resistance (36,37). Further studies indicated that the protein source may be an important determinant, with red and processed meat conferring greater risk (38–40). Although a high intake of dairy products has been associated with reduced risk of diabetes (13–15), and dairy products are a major source of protein, less is known about the relation of dairy protein to diabetes risk.

There have been few intervention studies that tested the effects of isocaloric substitution of protein for carbohydrate, or protein for fat, on measurements of glucose homeostasis in nondiabetic individuals. Long-term consumers of high-protein diets were reported to have greater glucose-stimulated insulin secretion and slightly lower insulin sensitivity (8) than that observed in individuals with lower protein intake. In a study comparing an isoenergetic high-protein diet (25–30%E) with a conventional-protein (15%E) control diet and 2 high-cereal-fiber diets with conventional (15%E) or moderate protein (20–25%E), there was a decrease in insulin sensitivity as measured by euglycemic hyperinsulinemic clamp when compared with either baseline or the high-cereal-fiber diets. However, changes in insulin sensitivity from baseline in the high-protein diet vs. the conventional-protein control diet were not different, suggesting that protein content per se was not the causative factor (9).

In the present study we found no significant effects on insulin sensitivity of short-term increases in intake of protein, in conjunction with reduced dietary carbohydrate, in nondiabetic overweight and obese individuals. We used whey protein isolate to partially meet the increased protein content of the moderate- and high-protein diets. Acutely, milk proteins, specifically whey, consumed with glucose or standardized meals increase postprandial insulin response, resulting in improved glucose excursion (12,16,41,42), with reported effects on both markers of insulin secretion (12) and hepatic extraction of insulin (42). Whey protein is rich in BCAAs, which appear in the plasma postprandially (12). Recently, metabolomic profiling showed that elevated basal concentrations of BCAAs are associated with obesity and surrogate measures of insulin resistance such as HOMA-IR (19,20). In cross-sectional analysis, a cluster of amino acids including BCAAs was inversely related to more specific measures of insulin action, including S_I and DI (18). In prospective studies, baseline BCAA concentrations predicted ~6-y HOMA-IR (43) and 2-h glucose (44) values and diabetes incidence (45). Moreover, skeletal insulin resistance can be induced by acute amino acid infusion in humans (46) and by BCAA feeding in mice (19). Although we observed cross-sectional relations of BCAAs with fasting glucose, insulin, HOMA-IR, AIRg, and DI, changes in BCAA concentrations were not associated with changes in fasting glucose, insulin sensitivity (S_I), or DI. Rather, we found that increases in BCAAs over the 4-wk diet intervention were correlated with decreased insulin clearance (MCRi) and increased secretion (AIRg). Notably, reduced insulin clearance was reported to predict the incidence of type 2 diabetes (47) and was associated with glucose intolerance, abdominal obesity, and nonalcoholic fatty liver disease. Thus, it is possible that BCAA effects on insulin dynamics may precede the development of insulin resistance and type 2 diabetes. We also observed that the relation between plasma BCAAs and insulin clearance was attenuated with the

low-SF diet, suggesting that either low SF or high monounsaturated fat intake blunts the association of BCAAs and insulin action. In this regard, Newgard et al. (19) found that in mice dietary BCAAs reduced insulin sensitivity only on the background of a diet high in total and SF and not when fed a standard chow diet. Greater BCAA flux and catabolism in muscle and liver are postulated to contribute to incomplete FA oxidation (48), which is associated with reduced insulin action (49). This may be exacerbated by the increase in FA oxidation with a high-fat diet (50); however, it unknown whether SF may preferentially promote this process.

Studies in animal models indicated that insulin sensitivity is impaired by diets high in SF [reviewed in (51–53)], and some human observational studies reported positive associations between SF intake and hyperinsulinemia, independent of body fat (54–58). However, in the majority of human intervention studies, changes in dietary fat quality had no effects on insulin sensitivity (52,53,59), including several large trials comparing replacement of monounsaturated fat for SF in the context of a higher fat diet. Our data support the evidence that high SF intake does not have a major impact on insulin sensitivity.

Measures of biomarkers of SF and protein intake suggest that our results are not due to poor dietary compliance. Plasma concentrations of pentadecanoic acid (15:0), a specific marker of intake of dairy fat (33,60), the primary source of added SF in the high-SF diet, were significantly higher in the high-SF group; and plasma concentrations of BCAAs were correlated with protein content of the prescribed diets.

Replacement of monounsaturated fat by SF is known to increase plasma LDL-C and often HDL-C (61,62). However, we observed no difference in these measurements between the high-SF and low-SF diets. It is possible that this is related to the selection of overweight and obese individuals for this study. There are reports that individuals with higher BMI values exhibit smaller than expected reductions in LDL-C in response to reductions in dietary SF compared with those with lower BMI values (63–66), as well as those with evidence for insulin resistance (67). Attenuated LDL-C lowering with reduction in dietary SF is particularly evident in women (65,66), who made up the majority of our population. Moreover, in the recent LIPGENE study, an ~7%E substitution of monounsaturated fat for SF in individuals with the metabolic syndrome (mean BMI: ~32 kg/m^2) resulted in no significant changes in total cholesterol, LDL-C, or HDL-C, despite evidence for dietary compliance as assessed by plasma FAs (22). Although there is no known basis for reduced responsiveness of obese individuals to changes in dietary SF, it may be that very high tissue concentrations of SFAs or cholesterol dampen the effect of exogenous FAs on hepatic cholesterol content (63,65,67).

Strengths of our study include a comprehensive design for testing effects of changes in both protein and SF intake, lack of potential confounding by weight loss, detailed measurements of insulin action, and demonstration of dietary compliance by plasma biomarkers of both protein and SF intake. Limitations include the short-term dietary intervention and the restriction of the study population to overweight and obese individuals. In addition, because higher protein intake was achieved with the addition of whey protein to a mixed-protein diet, and higher SF intake was achieved primarily by using whole-fat dairy products, it is possible that the present findings would not apply to comparable dietary amounts of protein and SF from other food sources. Finally, physiologically meaningful effects of the diets may have been smaller than those that the study was statistically powered to detect.

In conclusion, our results show that, in the absence of weight loss, increased consumption of protein or SF primarily from dairy sources does not significantly alter insulin sensitivity or insulin action in nondiabetic overweight and obese individuals. However, we found that diet-induced increases in plasma BCAAs correlate with increased insulin secretion and reduced insulin clearance, with the latter relation being influenced by the type of fat. Additional studies are warranted to better understand how BCAA metabolism influences insulin dynamics in humans.

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