A high-carbohydrate diet lowers the rate of adipose tissue mitochondrial respiration

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The hormone insulin plays a fundamental role in cellular nutrient signaling, including mitochondrial function. Joslin and Benedict observed in 1912 [1] that the metabolic rate in insulin-deficient states (i.e., type 1 diabetes) is significantly higher than expected—a phenomenon subsequently shown to be correctable with administration of insulin [2]. A component of this change in energy expenditure may involve insulin-induced changes in adipocyte mitochondrial bioenergetics. Dallon et al. [3] found that insulin produces a more tightly coupled state between electron transport and oxidative phosphorylation in rodent brown adipose mitochondria, lowering mitochondrial respiration rate and energy expenditure. Moreover, evidence from rodent models suggests that insulin has a suppressive effect on adipose tissue mitochondrial respiration, thereby lowering adipocyte energy expenditure [4]. While little is known about hormonal and nutrient effects on mitochondrial respiration in human adipose tissue, a recent meta-analysis found that higher versus lower-carbohydrate diets (which increase insulin secretion) reduce total energy expenditure in humans after 2.5 weeks, with control for energy intake or body weight [5]. The aim of this exploratory study is to test the hypothesis that a high-carbohydrate diet would lower measures of mitochondrial respiration in adipose tissue, consistent with the carbohydrate-insulin model of obesity [6].

Materials and Methods

Study design

The analyses presented here comprise an ancillary study of the Framingham State Food Study, in which the primary outcome was total energy expenditure [7]. Briefly, adults with BMI ≥ 25 were provided prepared diets at 60% of estimated energy requirements to produce a 10–14% weight loss during the run-in phase. Individuals achieving the target weight loss were weight-stabilized by adjustment of dietary energy and randomly assigned to low- (20%), moderate- (40%), or high- (60%) carbohydrate diets for a 20-week test diet phase. All diets had 20% protein, derived from similar sources across diets, with the remainder of energy derived from fat. Energy provision during the weight-loss maintenance test diet phase was adjusted periodically, targeting ±2 kg of the pre-randomization (post-weight loss) level. Additional detail on diets and experimental design were previously described [7]. A subset of participants in the parent trial was recruited into this ancillary study prior to randomization, protecting against selection bias. Adipose biopsies were obtained following weight stabilization and again after 10–15 weeks on test diets, in conjunction with another ancillary study focused on metabolic fuels [8].

Participants

Participants in the first two of three cohorts in the parent trial were invited to opt-in, by completing a telephone interview (n = 51) followed by an in-person informational session (n = 43). After considering additional exclusion criteria (history of allergy or prior reaction to lidocaine, conditions or medications associated with increased risk for bleeding, infection or skin reaction following adipose tissue biopsy, inadequate weight loss in the parent study, and scheduling conflicts with the ancillary study visits), 30 participants opted-in, were eligible for the ancillary study, and were among those randomized to the test diets in the parent trial (see Table 1 for baseline characteristics and weight data). One participant in the low-carbohydrate group did not complete the study.

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Subcutaneous abdominal fat was sampled by needle biopsy before and after the dietary intervention and following an overnight fast. Briefly, a sterile field was prepared, and an area 3 to 5 cm lateral to the umbilicus was isolated, cleaned, and anesthetized with 1% lidocaine. A liposuction cannula (Unitech 1 or 3-hole cannula, 3 mm × 12 cm) was connected to a 60-mL syringe via a 24–3-hole cannula, 3 mm × 12 cm) was connected to a 60-mL syringe. Following biopsy, the tissue was frozen at −80 °C until assay. Samples were thawed in ice-cold buffer (60 mM K-MES, 35 mM KCl, 7.23 mM K₂EGTA, 2.77 mM CaK₂EGTA, 20 mM imidazole, 20 mM taurine, 5.7 mM ATP, 15 mM PCCR, 636 mM MgCl₂·6H₂O, pH 7.1) at 4 °C for at least 60 min and then washed (105 mM K-MES, 30 mM KCl, 10 mM KH₂PO₄, 5 mM MgCl₂·6H₂O, 0.5 mg/ml BSA, pH 7.1) at 4 °C for at least 15 min. Samples were stored at −70 °C until assay.

Mitochondrial respiration assay

Before addition of sample into respiration chambers (Oroboros O2K, Innsbruck, Austria), a baseline respiration rate was determined. After addition of sample, the chambers were hyperoxygenated to ~250 nmol/ml, as previously validated for quantification of mitochondrial respiration rates in human adipose [9]. Respiration was determined by a substrate-uncoupler-inhibitor-titration protocol. Electron flow through complex I was supported by glutamate + malate (10 and 2 mM, respectively) to determine leak oxygen consumption (GM). Following stabilization, ADP (2.5 mM) was added to determine oxidative phosphorylation capacity (GMD). Succinate (2.5 mM) was added to determine complex I + II electron flow into the Q-junction (GMDS). Finally, the chemical uncoupler carbonyl cyanide 4-(trifluoromethoxy) phenylhydrazone (0.05 μM) was added to determine full electron transport system capacity in cells over oxidative phosphorylation (FCCP). Mitochondrial membrane integrity was confirmed in all experiments by observing the effect of adding cytochrome c (not shown; 10 μM). Following the protocol, samples were lysed for protein quantification (BCA protein assay; Pierce). Respiration rates are shown relative to total sample protein. Assays were batched by diet group assignments (designated by numerical codes), with laboratory staff masked to codes.

RESULTS AND DISCUSSION

In the initial condition (GM), an assessment of mitochondrial respiration in a basal state of complex I activation (via glutamate + malate), no differences in mitochondrial respiration were found by diet group. With addition of ADP (GMD) to engage oxidative phosphorylation (via complex V), the high-carbohydrate group had lower respiration compared with the moderate-carbohydrate (p = 0.021) but not with the low-carbohydrate group. When complex II-supported respiration was tested with the addition of succinate (GMDS), the high-carbohydrate group had lower respiration versus moderate- (p = 0.035) or low- (p = 0.038) carbohydrate groups. Finally, the addition of FCCP, which elicits a maximal respiratory response (i.e., mitochondrial uncoupling), produced a robust difference between the high- versus moderate- (p = 0.039) or low- (p = 0.005) carbohydrate groups (Fig. 1).

Our study suggests that a high-carbohydrate diet, possibly through an increase in insulin secretion [8], lowers mitochondrial respiratory function—a metabolic state that would favor deposition rather than oxidation of fat and predispose to weight gain. This finding is consistent with longer-term feeding trials examining the effects of dietary carbohydrate, as a proportion of total energy intake, on total energy expenditure.
Additional research is needed to replicate these findings, conduct quantitative energetic studies (e.g., ATP generation), examine generalizability to other populations and experimental conditions, and explore translation to the prevention and treatment of obesity.

DATA AVAILABILITY
The protocol and dataset are available at Open Science Framework (https://osf.io/rvbuj/).

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AUTHOR CONTRIBUTIONS
BTB supervised the mitochondrial studies and co-wrote the first draft of the manuscript. KJS helped design and conduct the trial and revised the manuscript. CMA helped conduct the trial and revised the manuscript. SY conducted the statistical analysis and revised the manuscript. ERS and CMW helped conduct the mitochondrial assays. CBE helped design the trial and revised the manuscript. DSL helped design the trial, co-wrote the first draft of the manuscript, and provided supervision. All authors approved the final manuscript.

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COMPETING INTERESTS
BTB received royalties for a book about insulin resistance. CMA. received royalties for books that recommend a low-carbohydrate diet. DSL received royalties for books that recommend a low-glycemic load diet; his spouse owns a nutrition education and consulting business. No other author has relevant disclosures.

ADDITIONAL INFORMATION
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