Original Article
CLINICAL TRIALS AND INVESTIGATIONS

Obesity

Weight Loss on Low-Fat vs. Low-Carbohydrate Diets by Insulin Resistance Status Among Overweight Adults and Adults With Obesity: A Randomized Pilot Trial

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Objective: To test for differential weight loss response to low-fat (LF) vs. low-carbohydrate (LC) diets by insulin resistance status with emphasis on overall quality of both diets.
Methods: Sixty-one adults, BMI 28-40 kg/m^2, were randomized in a 2 × 2 design to LF or LC by insulin resistance status in this pilot study. Primary outcome was 6-month weight change. Participants were characterized as more insulin resistant (IR) or more insulin sensitive (IS) by median split of baseline insulin-area-under-the-curve from an oral glucose tolerance test. Intervention consisted of 14 one-hour class-based educational sessions.
Results: Baseline % carbohydrate:% fat:% protein was 44:38:18. At 6 months, the LF group reported 57:21:22 and the LC group reported 22:53:25 (IR and IS combined). Six-month weight loss (kg) was 7.4 ± 6.0 (LF-IR), 10.4 ± 7.8 (LF-IS), 9.6 ± 6.6 (LC-IR), and 8.6 ± 5.6 (LC-IS). No significant main effects were detected for weight loss by diet group or IR status; there was no significant diet × IR interaction. Significant differences in several secondary outcomes were observed.
Conclusions: Substantial weight loss was achieved overall, but a significant diet × IR status interaction was not observed. Opportunity to detect differential response may have been limited by the focus on high diet quality for both diet groups and sample size.

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Introduction

Obesity is related to increased risk of heart disease, stroke, type 2 diabetes, and some cancers (1). Individuals with moderate obesity with insulin resistance have a greater metabolic risk profile for these chronic diseases than those with greater insulin sensitivity, even at the same weight (2). Weight loss improves insulin sensitivity and lowers cardiovascular risk (3). However, most people find successful weight loss challenging. The weight loss diet traditionally recommended by health professionals has been a low-fat (LF), calorie-restricted diet (4), which may be particularly inappropriate for insulin resistant (IR) individuals, and has been challenged by proponents of alternative dietary strategies, particularly low-carbohydrate (LC) (5-7).

Several weight loss diet studies have examined whether differences in glucose and insulin dynamics (e.g., differential insulin secretion or insulin resistance status) are a mediating factor for successful weight loss on LF vs. LC diets (8-12). These studies have consistently observed that overweight adults with higher insulin secretion or insulin resistance lose more weight on LC than LF diets. In contrast, lower insulin secretion or the more insulin sensitive (IS) individuals in these trials had more or comparable success with an LF diet. McClain et al. (10) observed that participants with higher baseline fasting insulin concentrations had lower adherence than participants with lower fasting insulin concentrations when assigned LF, even when unaware of their baseline fasting insulin status. Several proposed mechanisms support the plausibility of greater weight loss on an LC diet among IR individuals, including increased fatty acid uptake, inhibition of lipolysis, and effects on hunger, snacking, and energy intake (12-18).

The study objective was to conduct a pilot study continuing the research on the potential mediating effects of insulin resistance

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status on weight loss responses to LF vs. LC diets. Particular emphasis was placed on maximizing the fat vs. carbohydrate differentials on the two diets and on overall nutritional quality.

Methods

Participants

Participants were recruited from the local community primarily through media advertisements. Premenopausal women and men aged 18-50 years were invited to enroll if BMI was 28-40 kg/m^2, body weight was stable over the previous 2 months, and medications were stable for $\geq$3 months. Potential participants were excluded if they self-reported: hypertension (except for those stable on antihypertension medications), type 1 or 2 diabetes mellitus, heart, renal, or liver disease, cancer or active neoplasms, hyperthyroidism unless treated and under control, taking any medications known to affect weight/energy expenditure or blood lipids, smoking, alcohol intake $\geq$3 drinks/day, pregnancy, lactation, no menstruation for the previous 12 months, or plans to become pregnant within the next year. Race/ethnicity data were collected by self-report. All study participants provided written informed consent. The study was approved by the Stanford University Human Subjects Committee.

Study design

The study employed a 2 $\times$ 2 design: LF vs. LC diets and more IR vs. more IS. We suggest the terms "insulin resistance" and "insulin sensitivity" here be interpreted cautiously as we used a proxy measure for this, rather than a direct measure (expanded discussion in Section 1 of Supporting Information). The method of determining relative insulin resistance was to calculate an area under the curve of insulin concentrations (AUC-INS) from four blood samples taken during an oral glucose tolerance test (OGTT) (time 0, 30, 60, and 120 min) conducted prior to randomization. Median AUC-INS was determined separately for women and men. Those above the median were considered to be relatively more IR, and those below were considered relatively more IS.

A random number generator (Microsoft Excel) was used to stratify the randomization to LF vs. LC by insulin resistance status and gender. The duration of the intervention was 6 months, and the primary outcome was 6-month weight change.

Intervention

The intervention was a class-based education program led by a single health educator (RC). Participants were assigned to groups of 14-16 per class to follow either an LF or an LC diet. There were 14 one-hour classes over 6 months; once every week for 8 weeks, then once every other week for 8 weeks, and then once every month for 8 weeks.

Dietary strategy. There were four central components to the dietary strategy. The first was "how low can you go" (Limbo). LF participants were instructed to cut back to 20 g/day of total fat, and for LC to 20 g/day of digestible carbohydrate. The goal was to achieve the lowest level of fat or carbohydrate intake within the first 8 weeks. The second stage (Titrate) was to slowly add fat or carbohydrate back to the diet in increments of 5 g/day (e.g., from 20 to 25 g/day) and then hold it at that amount for 1-4 weeks before adding another 5 g/day. The third component was to identify the lowest level of fat or carbohydrate intake participants felt could be maintained long term, potentially for the rest of their lives. The fourth strategy was to promote high nutrient density (Quality). Other Quality concepts included "real food," "minimally processed," "seasonal," "organic," "grass-fed," "whole grain," and "pasture-raised," depending on diet assignment. Both diet groups received similar instructions to drink water, maximize vegetable intake, and to minimize added sugars, refined white flour products, and sources of trans fats. Participants on the LC diet were asked to consume half an avocado each day (approximately 160 kcal), as well choosing other sources of plant-based fats, including olive oil, nuts and seeds, and nut butters. Hass avocados were provided by the Hass Avocado board and were distributed to the participants. All participants were encouraged to take an active role in making food choices; by preparing their own foods at home, reading labels, and asking for appropriate modifications for restaurant menu items.

In summary, the diet strategy for both LF and LC was a "Limbo-Titrate-Quality" approach designed to motivate participants to achieve the lowest possible level of fat or carbohydrate intake i.e., equally ambitious with maximal overall nutritional quality and a dietary pattern that could be continued for a lifetime.

Beyond fat and carbohydrate lowering. Notably, there were no calorie restriction targets in the intervention. Participants were encouraged to track their intake using daily food journals and computer tracking programs. Although the first 8 weeks of classes focused specifically on separate strategies to lower fat or carbohydrate intake, the subsequent 4 months of classes addressed more global topics for both diet groups, similarly, such as mindful eating, adequate sleep, body acceptance, and sugar addiction.

Physical activity. All participants were encouraged to be physically active. Participants who were already physically active at baseline were encouraged to maintain or increase their activity. Those who were sedentary at baseline were encouraged to begin moderate exercise. All participants were given pedometers (Omron HJ-112 Digital Pocket Pedometer).

Data collection

All data were collected at baseline and at 3 and 6 months. Clinic and laboratory staff members were blinded to treatment assignment. Participants were blinded as to their baseline OGTT results.

Diet and physical activity data. Three telephone-administered 24-h recall interviews were conducted at each time point using Nutrition Data System for Research (NDS-R) software [Nutrition Coordinating Center (NCC), University of Minnesota, versions 4.05.33 (2011) and 4.06.34 (2012)]. Interviews were conducted on two weekdays and one weekend day, nonconsecutive whenever possible, unannounced, during a 2-week window. Average daily energy expenditure was assessed using the Stanford 7-day physical activity recall (19).

Anthropometric data. Height was measured to the nearest millimeter using a standard wall-mounted stadiometer. Body weight was measured to the nearest 0.1 kg on a calibrated clinical scale. Waist circumference was measured to the nearest millimeter at the umbilicus.

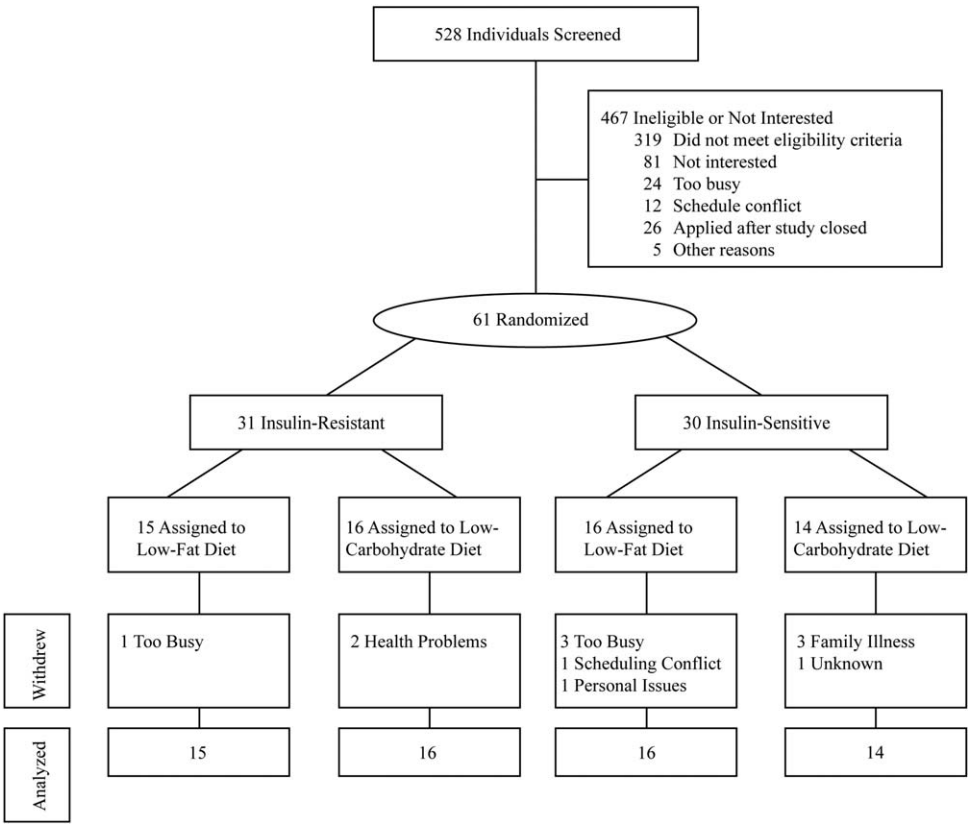

Figure 1 Participant flow through the trial.

Metabolic measures. Blood samples were collected after ≥10 h fast. Plasma total cholesterol and triglycerides (free glycerol blank subtracted) were measured enzymatically using established clinical chemistry laboratory methods (Northwest Lipid Laboratory, Seattle, WA) (20,21). High-density lipoprotein cholesterol (HDL-C) was measured by liquid selective detergent followed by enzymatic determination of cholesterol (22). Low-density lipoprotein cholesterol (LDL-C) was calculated according to Friedewald et al. (23). Total plasma insulin in serum was measured by radioimmunoassay (24), and blood glucose was measured using a modification of the glucose oxidase/peroxidase method (25,26) (Diabetes Research Center, Washington University, St Louis, MO). Resting blood pressure was assessed three times at 2-min intervals as described elsewhere (27); the initial reading was discarded, and the last two readings were averaged.

Statistical methods

The primary objective was to test whether there was a significant interaction in weight loss on LF vs. LC diets by insulin resistance status as estimated by AUC-INS. Dietary composition data (energy, % carbohydrate, fat, and protein, and grams of fiber, added sugars, and saturated fat) are presented as raw, unadjusted mean (±SD) (i.e., no imputation for missing data).

For the main analysis, data were multiply imputed with the MICE package in R 3.0 using five imputation steps and five imputed data sets. Each imputed data set was fit to a linear regression model using change in weight at 6 months as the outcome and with subject height, diet, insulin resistance-insulin sensitivity (IR-IS) status, and an interaction term between diet and IR-IS status as predictors. Resulting variance estimates were pooled to account for the additional variability induced by the imputation process. In a sensitivity analysis, we repeated these models after replacing the dichotomous IR-IS status with continuous baseline insulin AUC. Other exploratory analyses included the use of INS-30, INS-120, and Glu-$AUC_{0\text{-}30}$ × Ins-$AUC_{0\text{-}30}$. We also fit models where, instead of adjusting for baseline height, we adjusted for baseline BMI.

We also explored longitudinal differences between risk factors across the four diet × IR-IS groups (e.g., LF-IR, LC-IR, LF-IS, and LC-IS). For each risk factor, a mixed effect model was fit with the corresponding risk factor as the outcome and group, time point and group time point interaction as predictors. Models were either linear or logistic, depending on the nature of the risk factor. For risk factors with significant interaction term *P*-values, additional pairwise comparisons among the four groups were made using Tukey's HSD test. All statistical tests were two-tailed using a significance level of 0.05.

Results

Participants were enrolled from February to April, 2012. Sixty-one eligible participants were randomized into four groups—two classes

TABLE 1 Baseline participant characteristics[a]

Variable	Insulin resistant		Insulin sensitive	
	Low-fat ($n = 15$)	Low-carbohydrate ($n = 16$)	Low-fat ($n = 16$)	Low-carbohydrate ($n = 14$)
Percent women	60	63	63	64
Age (years)	44 ± 5	42 ± 6	41 ± 6	43 ± 7
Education (years)	16.0 ± 2.0	16.4 ± 1.8	15.9 ± 3.3	16.1 ± 1.9
Percent non-white	13	13	25	0
Anthropometrics				
BMI (kg/m^2)	35.0 ± 2.4	34.2 ± 3.8	32.6 ± 2.9	31.2 ± 1.9
Waist circumference (cm)	108.8 ± 10.4	110.0 ± 10.7	105.1 ± 9.5	98.8 ± 9.3
Cardiovascular disease risk factors				
LDL-C (mg/dl)	118 ± 20	108 ± 19	111 ± 30	113 ± 34
HDL-C (mg/dl)	43 ± 10	44 ± 13	51 ±17	49 ± 14
Triglycerides (mg/dl)	146 ± 58	156 ± 68	117 ± 59	136 ± 99
Fasting insulin (μU/ml)	21 ± 5.9	27.7 ± 11.4	13.4 ± 3.1	13.4 ± 3.1
Fasting glucose (mg/dl)	100.9 ± 11.2	102.5 ± 11.8	102.7 ± 11.6	99.1 ± 7.5
Insulin AUC (μU min/ml)	130.9 ± 54	144 ± 49.5	65 ± 19	58 ± 18.4
Blood pressure (mm Hg)				
Systolic	122 ± 12	125 ± 10	116 ± 13	117 ± 12
Diastolic	81 ± 8	83 ± 8	77 ± 9	78 ± 7
Percent metabolic syndrome	46.67	56.25	18.75	35.71
Physical activity (kcal/kg/day)	33.2 ± 0.9	32.7 ± 1.2	34.0 ± 1.7	33.6 ± 1.4

HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol.
[a]Data are expressed as mean ± SD unless otherwise indicated.

of LF and two classes of LC, with approximately 50% IR and 50% IS in each class (Figure 1). Baseline characteristics are presented in Table 1. By design, INS-AUC (and the highly correlated fasting insulin) was higher for IR vs. IS. As expected, BMI was higher among the more IR vs. IS participants, with a trend for higher triglycerides and blood pressure, lower HDL-C, and a higher percentage of metabolic syndrome in the IR group.

Average class attendance was 81% ± 13% (mean ± SD) for the LF classes and 85% ± 11% for the LC classes. Of the 61 participants enrolled, 49 (80%) completed the 6-month protocol; data were missing at 6 months from six participants in each diet group.

Dietary adherence and physical activity

Participants in both LF and LC made substantial dietary changes as assessed at 3- and 6-months, relative to baseline (Figure 2). With average baseline energy intake percentages of 44:38:18 from carbohydrate:fat:protein, the two diet groups shifted to an average ratio of approximately 58:22 carbohydrate:fat for LF, and 21:53 for LC (average at 6 months), with protein being relatively similar, particularly at 6 months. Between the 3- and 6-month time points, there was modest recidivism in the LC group whereas macronutrient ratios were more stable for LF during this phase. Average energy intake from alcohol ranged from 1% to 4% of energy in the four LF and LC classes (energy intake from alcohol excluded from Figure 2 data). Reported energy intake suggested an average ~600 kcal/day decrease at 3 and 6 months relative to baseline (~30% energy). An expanded presentation of macronutrient distribution for all four subgroups at all three time points is available in the Supporting Information Section 2 and Supporting Information Table S1.

On average, the LF group decreased absolute amounts (grams) of added sugar intake by ~50% and saturated fat by ~66% while increasing fiber intake by ~25% relative to baseline; the LC group decreased added sugar intake by ~70%, fiber by ~40%, and increased saturated fat by ~10% (Table 2). These were changes of absolute intake amounts in the context of a general ~30% reduction of overall energy intake.

Energy expenditure increased modestly and similarly for both diet groups. Baseline energy expenditure for the LF group was 33.7 ± 1.4 kcal/kg/day, which increased at 3 and 6 months to 34.2 ± 1.6 and 34.6 ± 2.6 kcal/kg/day, respectively. In parallel, baseline energy expenditure for the LC group was 32.7 ± 0.9 kcal/kg/day, which increased at 3 and 6 months to 33.5 ± 1.3 and 33.8 ± 1.9 kcal/kg/day, respectively.

Six-month weight loss for four groups

Average weight loss after 6 months for the $n = 49$ that completed the protocol was 9.0 ± 6.5 kg (19.8 ± 14.3 lbs), which represented 8.9 ± 5.7% of baseline weight. The 6-month weight loss results by diet type and IR-IS status group were 7.5 ± 6.0 kg for LF-IR,

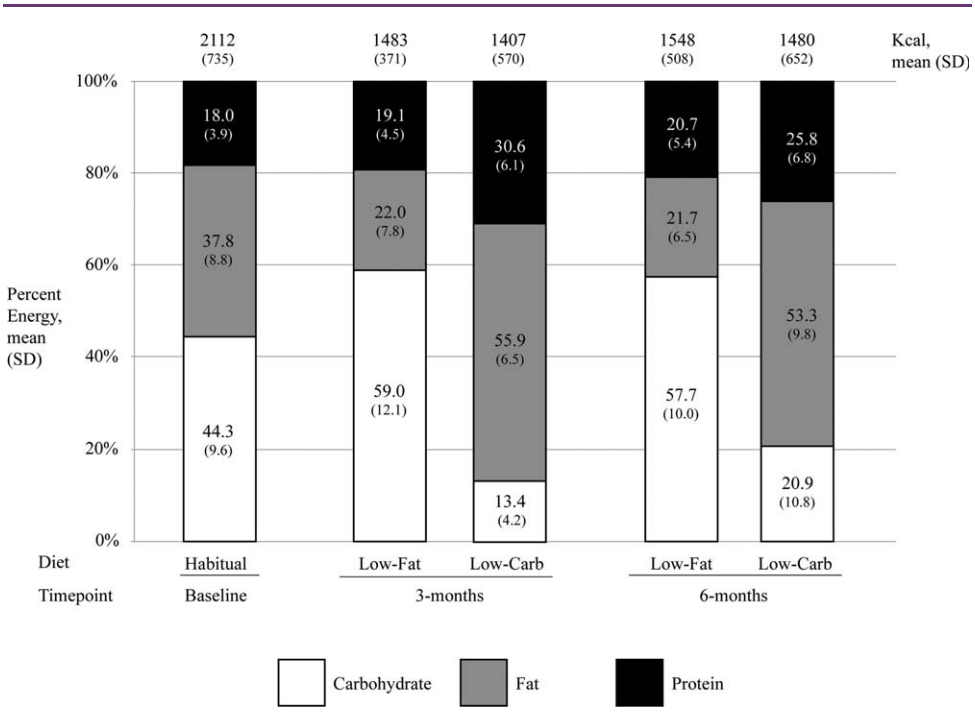

Figure 2 Dietary assessment: proportions of carbohydrates, fats, and proteins for each diet at baseline, 3 months, and 6 months.

10.4 ± 7.8 kg for LF-IS, 9.6 ± 6.6 kg for LC-IR, and 8.6 ± 5.6 kg for LC-IS (Figure 3). A significant interaction between diet assignment and IR-IS status was not detected, and there were no significant main effect differences in weight loss detected by diet group or by IR-IS status. We found no meaningful differences in estimate direction or significance between the models where baseline height was a confounder and models where baseline BMI was a confounder or when using INS-30, INS-120, or Glu-$AUC_{0\text{-}30}$ × Ins-$AUC_{0\text{-}30}$ in the models.

TABLE 2 Selected dietary components (mean ± SD)[a]

	Low-fat	Low-carbohydrate
Total fiber (g/1000 kcal)		
Baseline	9 ± 4	12 ± 5
3 months	18 ± 8	9 ± 3
6 months	16 ± 7	10 ± 4
Added sugars (g/1000 kcal)		
Baseline	20 ± 10	19 ± 10
3 months	16 ± 11	4 ± 4
6 months	16 ± 13	7 ± 6
Saturated fat (g/1000 kcal)		
Baseline	15 ± 4	13 ± 4
3 months	7 ± 3	20 ± 5
6 months	7 ± 3	19 ± 6

[a]Sample sizes: baseline low-fat $n = 31$ and low-carbohydrate $n = 30$; 3 months $n = 26$ for each diet group; 6 months $n = 25$ for each group.

Risk factor changes

With few exceptions, risk factors changed in a beneficial way across all groups (Table 3). Triglyceride concentrations dropped by ~25% across the four groups combined. Both diastolic and systolic blood pressure decreased for all four groups. HDL-C concentrations

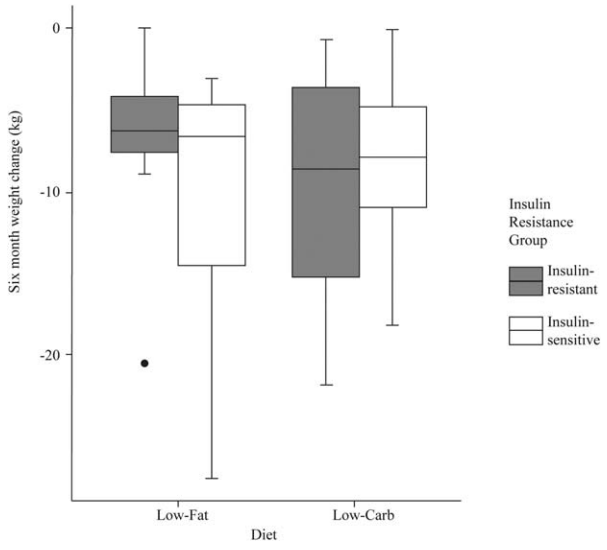

Figure 3 Six-month weight change by diet and insulin resistance group, $n = 49$. Six-month weight loss (kg) was 7.4 ± 6.0 (LF-IR), 10.4 ± 7.8 (LF-IS), 9.6 ± 6.6 (LC-IR), and 8.6 ± 5.6 (LC-IS).

TABLE 3 Risk factor changes and metabolic syndrome prevalence by diet group-insulin resistance status

	Insulin resistant		Insulin sensitive		
	Low-fat ($n = 15$*)	Low-carbohydrate ($n = 16$*)	Low-fat ($n = 16$*)	Low-carbohydrate ($n = 14$*)	*P*-value (time × treatment group)†
Risk factor changes					
LDL-C (mg/dl)					
3 months	−13.2 (20.1)	12.6 (21.0)	−16.2 (27.8)	23.9 (46.6)	
6 months	−14.4 (23.8)[a,b]	6.5 (25.7)[a,b]	−20.8 (26.1)[a]	17.8 (40.8)[b]	0.006
HDL-C (mg/dl)					
3 months	−2.6 (6.0)	0.1 (4.8)	−3.5 (6.5)	2.1 (6.6)	
6 months	3.7 (5.9)	4.3 (3.7)	0.6 (5.8)	4.4 (7.0)	0.273
Triglycerides (mg/dl)					
3 months	−8.1 (39.5)	−40.1 (77.9)	−12.8 (23.0)	−20.9 (33.9)	
6 months	−34.1 (30.1)	−35.1 (67.4)	−9.6 (27.9)	−32.2 (41.4)	0.588
Fasting insulin (μU/mL)					
6 months	−8.2 (5.6)[a,b]	−12.7 (8.1)[a]	−4.5 (4.4)[b]	−3.8 (3.8)[b]	0.003
Fasting glucose (mg/dl)					
3 months	−5.0 (10.3)	−4.6 (12.9)	−8.1 (10.7)	0.8 (8.7)	
6 months	−2.8 (10.0)	−5.7 (13.6)	−3.6 (16.5)	3.8 (7.8)	0.125
Insulin AUC (μU min/ml)					
3 months	−65.8 (4.9)	−68.3 (50.1)	−27.5 (25.4)	−11.2 (23.6)	
6 months	−57.6 (38.4)[a,c]	−67.5 (49.3)[a]	−15.1 (32.7)[b]	−18.3 (20.5)[b,c]	<0.0001
Blood pressure systolic (mm Hg)					
3 months	−1.1 (5.2)	−6.7 (11.9)	−3.8 (6.3)	−6.0 (7.4)	
6 months	−2.2 (9.0)	−8.8 (10.1)	−6.9 (10.2)	−4.4 (8.1)	0.165
Blood pressure diastolic (mm Hg)					
3 months	−0.7 (3.8)	−3.8 (7.3)	−3.7 (4.3)	−3.8 (4.3)	
6 months	−2.4 (6.8)	−5.3 (7.5)	−6.4 (7.1)	−4.3 (5.6)	0.311
Prevalence					
Metabolic syndrome (%)					
3 months	46.7	18.8	6.3	14.3	
6 months	20.0	12.5	12.5	7.1	0.508

Data are expressed as mean (SD), except for metabolic syndrome (%).
*Table column headings indicate sample size used in statistical testing. Values reflect actual data from those who completed the 6-month protocol: $n = 14$, $n = 14$, $n = 11$, and $n = 10$, respectively.
†Intention-to-treat analysis, with baseline data carried forward for missing values. *P*-value for time × diet group interaction for 6-month change determined using mixed-model and autoregressive covariance structure.
[a,b,c]For 6-month risk factors with time × group $P < 0.05$, pair-wise differences are indicated by superscripts; pairs with a shared superscript are not different.

increased by almost 10% in three of the four groups, with a negligible overall change in the LF-IS group. Fasting glucose decreased modestly, on average, in three of the four groups, with a negligible 6-month change in the LC-IS group. At baseline 40% of participants met metabolic syndrome criteria, which was down to 15% overall at 6 months. No significant 6-month change differences were detected among groups for any of the above risk factors.

Six-month changes in LDL-C concentrations were statistically different among groups, with decreases for LF and increases for LC, regardless of IR-IS status. Fasting insulin concentrations dropped significantly more for the two IR groups than the two IS groups, although by definition the IR groups had higher baseline insulin levels and thus greater capacity for improvement. Overall average fasting insulin concentrations decreased for all four groups. This same pattern was observed for INS-AUC.

Discussion

In this pilot study, we investigated whether there was a differential weight loss response to LF vs. LC diets by baseline IR-IS status, using INS-AUC as a proxy measure, among nondiabetic overweight adults and adults with obesity who were otherwise in general good health. Overall, participants experienced substantial weight loss: an average of 9.0 ± 6.5 kg, which represented 8.9 ± 5.7% of baseline weight. However, a significant interaction between diet assignment and IR-IS status was not detected for weight loss. Dietary assessment indicated substantial diet differentiation between the LF and LC groups, which was supported by observed changes in secondary metabolic outcomes, including fasting insulin, LDL-C, HDL-C, and triglycerides. In addition, the dietary assessment data indicate that the substantial dietary changes achieved by mid-study were largely maintained to the end of the study at 6 months.

Several other studies have reported a statistically significant interaction in weight loss between diet type and IR-IS status, including a previous investigation by our own research group (8-10,12). Two of the studies were feeding studies, of 4-6 month duration with small sample sizes of four to eight per treatment arm (8,12). These studies, perhaps because of the more rigorous control of diet, and the 30% restriction of energy intake, achieved greater weight loss overall than the two free-living studies which used an *ad libitum* approach (9,10). However, the free-living studies had larger sample sizes and longer durations than the feeding studies. Notably, the four previous studies used three different methods to assess insulin and glucose dynamics. Compared with this set of four previous studies, in this free-living study, the magnitude of overall weight loss was comparable to the feeding studies and substantially higher than the other two free-living studies while using an *ad libitum* approach to energy intake. The INS-AUC method used in this study to differentiate greater IR from greater IS individuals was different than all of the other studies, and was more a measure of hyperinsulinemia than a direct measure of insulin resistance. In absolute numbers, the average weight loss results in this study paralleled the findings from the other studies—the more IR group lost slightly more weight on LC, and the IS group lost slightly more weight on LF, but the differences were not statistically or clinically significant. With so many differences among the previous four studies and this study, which all address the same general research question, we are not able to determine whether we failed to detect a true effect that the other studies correctly identified, or if we truly and accurately identified no effect in our study population using the design described.

There are multiple mechanisms that could be responsible for a potential differential weight loss response to LF vs. LC diets by variability in insulin and glucose dynamics, including differential hunger/satiety, energy expenditure, fatty-acid metabolism, lipolysis, and adipogenesis. Several groups of investigators have observed one or more factors along a continuum that suggest LF relative to LC diets cause greater excursions in postprandial glucose and insulin metabolism, may increase 24-h hunger, and may subsequently increase overall energy intake due to their higher glycemic load (11,28-31). Related research suggests that diets with a higher glycemic index can affect hormones regulating metabolism (13-15). Under these conditions, IR individuals may feel less satiated and experience stronger physiologically driven urges to consume more food after consuming a lower fat/higher carbohydrate meal compared with IS individuals.

In separate experiments with humans, one a parallel design and another a cross-over, the lab group of Ludwig and coworkers found that substantial weight loss achieved by or followed by isocaloric diets differing in glycemic load led to differential changes in resting energy expenditure and total energy expenditure; the observed results favored greater energy expenditure on the lower glycemic load/lower carbohydrate diets (17,32). Although IR-IS status was not addressed as a potential covariate in these analyses, it is plausible that the more IR individuals who were on higher glycemic load/higher carbohydrate diets would experience an even greater decrease in energy expenditure than the more IS individuals on the same diet, making it more difficult to achieve or maintain weight loss.

Further discussion of the observed changes and lack of changes in some of the risk factors in Table 3 is presented in the Supporting Information Section 3.

The study design and conduct included several important strengths. One was the high degree of dietary differentiation achieved for those assigned to LF vs. LC. In many weight loss diet studies, the combination of modest dietary goals and substantial recidivism over time (i.e., weak treatment fidelity) can lead to a lack of physiologically meaningful dietary differences between treatment arms. The differences in proportions of energy intake from fat vs. carbohydrate achieved and maintained out to 6 months in the two diet groups of this study involved a substantial shift of approximately 25% of energy intake. The use of three unannounced 24-h recalls and NDS-R for dietary assessment at three time points, and the high rate of completion of these assessments was an important methodological strength. Other strengths included the relatively high retention rate of 80% and the identical drop-out rates in both diets. Stratifying the randomization by IR-IS status was an important design component, and the use of INS-AUC from OGTTs to identify and differentiate participants who were more IR vs. more IS was superior to fasting measures that could have been used (e.g., fasting insulin or TG/HDL-C ratio).

The major limitations of this pilot study were the duration and sample size. Given a consistent pattern of maximal weight loss at 6 months followed by weight stabilization and often regain across a range of published studies, it is more optimal to include follow-ups of a year or more in weight loss studies. Also, given the substantial heterogeneity of intergroup weight loss typical of these types of trials, large sample sizes are a preferred design component; the null finding for an interaction between IR-IS status and diet assignment for weight loss difference in this study may have been attributable to a lack of adequate statistical power. However, a primary objective of this pilot study was to test the approach undertaken to achieve greater differentiation of diets and treatment fidelity for the purpose of incorporating this approach in a future, larger, longer trial; that follow-up trial, with a sample size of 600 and duration of 1 year is currently underway. An expanded discussion of study limitations is presented in the Supporting Information Section 4. Despite the limitations of the pilot study, we believe the high degree of apparent treatment diet differentiation, the relatively high average weight loss across both treatment arms, and the interesting findings of risk factor changes at 3 and 6 months are results worthy of dissemination.

In conclusion, our pilot study achieved substantial differentiation of LF vs. LC study diets in a free-living population that led to an average weight loss of 9% body weight over 6 months in overweight adults and adults with obesity. Our findings did not detect differential effects by diet, by IR-IS status, or the interaction of these conditions. Further research on a larger study population for a longer period of time is warranted using the novel dietary intervention approach developed here. O

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References

1. Centers for Disease Control and Prevention. Overweight and obesity (Webpage). 2014 [updated September 9, 2014]. Available at: http://www.cdc.gov/obesity/data/adult.html
2. McLaughlin T, Abbasi F, Lamendola C, Reaven G. Heterogeneity in the prevalence of risk factors for cardiovascular disease and type 2 diabetes mellitus in obese individuals: effect of differences in insulin sensitivity. *Arch Intern Med* 2007;167:642-648.
3. Reaven GM. The insulin resistance syndrome: definition and dietary approaches to treatment. *Annu Rev Nutr* 2005;25:391-406.
4. Clinical guidelines on the identification, evaluation, and treatment of overweight and obesity in adults–the evidence report. National Institutes of Health. *Obes Res* 1998;6(Suppl 2): 51S-209S.
5. Agatston A. *The South Beach Diet*. New York: St. Martin's Griffin; 2005.
6. Atkins RC. *Dr. Atkins' New Diet Revolution*. New York: Harper Collins; 2009.
7. Sears B. *The Zone: A Dietary Road Map*. New York: Regan Books; 1995.
8. Cornier MA, Donahoo WT, Pereira R, et al. Insulin sensitivity determines the effectiveness of dietary macronutrient composition on weight loss in obese women. *Obes Res* 2005;13:703-709.
9. Ebbeling CB, Leidig MM, Feldman HA, Lovesky MM, Ludwig DS. Effects of a low-glycemic load vs. low-fat diet in obese young adults: a randomized trial. *JAMA* 2007;297:2092-2102.
10. McClain AD, Otten JJ, Hekler EB, Gardner CD. Adherence to a low-fat vs. low-carbohydrate diet differs by insulin resistance status. *Diabetes Obes Metab* 2013;15:87-90.
11. McLaughlin T, Carter S, Lamendola C, et al. Effects of moderate variations in macronutrient composition on weight loss and reduction in cardiovascular disease risk in obese, insulin-resistant adults. *Am J Clin Nutr* 2006;84:813-821.
12. Pittas AG, Das SK, Hajduk CL, et al. A low-glycemic load diet facilitates greater weight loss in overweight adults with high insulin secretion but not in overweight adults with low insulin secretion in the CALERIE trial. *Diabetes Care* 2005;28:2939-2941.
13. Boden G, Sargrad K, Homko C, Mozzoli M, Stein TP. Effect of a low-carbohydrate diet on appetite, blood glucose levels, and insulin resistance in obese patients with type 2 diabetes. *Ann Intern Med* 2005;142:403-411.
14. Ludwig DS. The glycemic index: physiological mechanisms relating to obesity, diabetes, and cardiovascular disease. *JAMA* 2002;287:2414-2423.
15. Ludwig DS, Majzoub JA, Al-Zahrani A, Dallal GE, Blanco I, Roberts SB. High glycemic index foods, overeating, and obesity. *Pediatrics* 1999;103:E26.
16. Pawlak DB, Kushner JA, Ludwig DS. Effects of dietary glycaemic index on adiposity, glucose homoeostasis, and plasma lipids in animals. *Lancet* 2004;364:778-785.
17. Pereira MA, Swain J, Goldfine AB, Rifai N, Ludwig DS. Effects of a low-glycemic load diet on resting energy expenditure and heart disease risk factors during weight loss. *JAMA* 2004;292:2482-2490.
18. Rodin J. Insulin levels, hunger, and food intake: an example of feedback loops in body weight regulation. *Health Psychol* 1985;4:1-24.
19. Sallis JF, Haskell WL, Wood PD, et al. Physical activity assessment methodology in the Five-City Project. *Am J Epidemiol* 1985;121:91-106.
20. Allain CC, Poon LS, Chan CS, Richmond W, Fu PC. Enzymatic determination of total serum cholesterol. *Clin Chem* 1974;20:470-475.
21. Sampson EJ, Demers LM, Krieg AF. Faster enzymatic procedure for serum triglycerides. *Clin Chem* 1975;21:1983-1985.
22. Warnick GR, Albers JJ. A comprehensive evaluation of the heparin-manganese precipitation procedure for estimating high density lipoprotein cholesterol. *J Lipid Res* 1978;19:65-76.
23. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem* 1972;18:499-502.
24. Morgan CR, Lazarow A. Immunoassay of insulin: two antibody system: plasma insulin levels in normal, sub diabetic, and diabetic rats. *Diabetes* 1963;12:115-126.
25. Lott JA, Turner K. Evaluation of Trinder's glucose oxidase method for measuring glucose in serum and urine. *Clin Chem* 1975;21:1754-1760.
26. Trinder P. Determination of blood glucose using an oxidase-peroxidase system with a non-carcinogenic chromogen. *J Clin Pathol* 1969;22:158-161.
27. King AC, Sallis JF, Dunn AL, et al. Overview of the Activity Counseling Trial (ACT) intervention for promoting physical activity in primary health care settings. Activity Counseling Trial Research Group. *Med Sci Sports Exerc* 1998;30:1086-1096.
28. Arumugam V, Lee JS, Nowak JK, et al. A high-glycemic meal pattern elicited increased subjective appetite sensations in overweight and obese women. *Appetite* 2008;50:215-222.
29. Lennerz BS, Alsop DC, Holsen LM, et al. Effects of dietary glycemic index on brain regions related to reward and craving in men. *Am J Clin Nutr* 2013;98:641-647.
30. Liu AG, Most MM, Brashear MM, Johnson WD, Cefalu WT, Greenway FL. Reducing the glycemic index or carbohydrate content of mixed meals reduces postprandial glycemia and insulinemia over the entire day but does not affect satiety. *Diabetes Care* 2012;35:1633-1637.
31. Shikany JM, Margolis KL, Pettinger M, et al. Effects of a low-fat dietary intervention on glucose, insulin, and insulin resistance in the Women's Health Initiative (WHI) Dietary Modification trial. *Am J Clin Nutr* 2011;94:75-85.
32. Ebbeling CB, Swain JF, Feldman HA, et al. Effects of dietary composition on energy expenditure during weight-loss maintenance. *JAMA* 2012;307:2627-2634.