Effect of dietary macronutrient composition on tissue-specific lipoprotein lipase activity and insulin action in normal-weight subjects[1–3]

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ABSTRACT The effects of macronutrient composition on fasting and postprandial activities of adipose tissue lipoprotein lipase (ATLPL) and skeletal muscle LPL (SMLPL) and on insulin sensitivity (S_I) were studied in 25 normal-weight subjects. Each subject was fed a high-carbohydrate (HC) diet for 16 d and a high-fat (HF) diet for 16 d, in randomized order. On day 15 of each diet, biopsies for ATLPL and SMLPL were done in the fasted state and 6 h postprandially. On day 16 of each diet, a euglycemic clamp was used to measure S_I. There was no effect of diet composition on fasting ATLPL or SMLPL. With both diets and in both tissues, LPL increased significantly from fasting to 6 h postprandially. In adipose tissue only there was a significant difference between the 2 diets in LPL meal response (HC >HF, $P = 0.024$). There was no effect of diet composition on S_I. After the HC diet only, there were significant correlations between fasting SMLPL and S_I, but not ATLPL. After the HF diet, associations between insulin action and LPL were evident only in the postprandial state. In summary, 16 d of HC compared with HF feeding in normal-weight subjects increased the responsiveness of ATLPL to an HC compared with an HF meal. However, the same diets had no effect on fasting ATLPL or SMLPL, the responsiveness of SMLPL to a meal, or S_I. These data suggest that in normal-weight subjects habitual dietary carbohydrate intake may have a stronger effect on subcutaneous fat storage than does dietary fat intake. *Am J Clin Nutr* 1998;68:296–302.

INTRODUCTION

The macronutrient content of the human diet affects lipoprotein lipase (LPL) activity (1, 2) and most often these effects are tissue specific. In humans, when adipose tissue LPL (ATLPL) is measured after an overnight fast, diets high in carbohydrate are usually associated with increases in ATLPL (3, 4) but with no changes or decreases in skeletal muscle LPL (SMLPL) (4). The effect of high-fat diets on tissue-specific regulation of LPL in humans has not been tested. Overall, these relations may be explained by diet-dependent changes in insulin secretion, insulin sensitivity (S_I), or both. We showed previously in normal-weight

subjects that insulin infusion, under conditions of euglycemia, has a stimulatory effect on ATLPL (5, 6) but an inhibitory effect on SMLPL (6, 7). Moreover, the ingestion of fat has been shown to markedly blunt the insulin- and glucose-stimulated response of LPL in adipose tissue (8).

Several studies in humans have shown that the macronutrient content of the diet can also alter insulin action (9–12). Some studies have shown that isoenergetic diets containing a higher percentage of energy as fat produce insulin resistance (9, 12), whereas other studies found no difference in S_I after high-fat compared with high-carbohydrate feeding (13, 14). If an increased percentage of energy consumed as fat is truly associated with insulin resistance, that behavior may have long-term adverse consequences, specifically, the development of type 2 diabetes, dyslipidemia, and hypertension and increased risk of atherosclerotic cardiovascular disease (9, 12, 15). Although high-fat diets also appear to lead to obesity (16), insulin resistance may well be a better predictor of weight stabilization than of weight gain (17).

The purpose of this study was to determine the effect of dietary macronutrient composition on fasting ATLPL and SMLPL activities and on postprandial enzyme changes after a single meal. The relation between dietary induced changes in S_I and tissue-specific LPL response was also examined.

SUBJECTS AND METHODS

Subject procedures

After approval of the protocol by the Colorado Multiple Institutional Review Board and the Scientific Advisory Committee of

the General Clinical Research Center at the University of Colorado Health Sciences Center, 25 normal-weight individuals (15 men, 10 women) gave their informed consent and were enrolled in the study. Subjects were between 25 and 34 y of age ($\bar{x} \pm$ SEM: 29 ± 1 y), were at their maximum body mass index (22.1 ± 0.3, in kg/m²), and had been weight stable for 3 3 mo before enrollment. Each potential subject was given the Diet Habit Survey (18) to determine his or her current free-living diet composition.

Results of screening laboratory tests, including liver and renal panels, complete blood count, urinalysis, and measurement of serum electrolytes, fasting glucose, and thyroid-stimulating hormone, were within normal limits for each individual. Fasting lipid concentrations, including concentrations of triacylglycerol, cholesterol, HDL cholesterol, and LDL cholesterol, were also within the normal range. None of the subjects were taking medication that affected lipid or carbohydrate metabolism.

On day 0 of the study protocol, each subject was admitted to the General Clinical Research Center for fasting measurements of serum glucose and insulin concentrations and plasma triacylglycerol, total cholesterol, HDL-cholesterol, and HDL_2-cholesterol concentrations. At breakfast on day 0, each subject was started on either a high-carbohydrate (HC) or high-fat (HF) mixed-food diet. Diet order was randomized at the time subjects entered the study. The first diet (diet phase 1) was administered for 16 d, followed by metabolic studies on days 15 and 16. The subject was then discharged for a 4–6-wk washout phase under free-living conditions, during which time he or she returned to the outpatient clinic once a week to be weighed and for weight stability to be documented. The washout phase was immediately followed by the alternate experimental diet. The second diet (diet phase 2) also lasted for 16 d, with metabolic studies performed on days 15 and 16. Each diet was provided as 3 meals/d plus snacks by the metabolic kitchen of the General Clinical Research Center. Breakfast had to be eaten every morning at the General Clinical Research Center after confirmation of body weight. The other 2 daily meals were prepared by the kitchen personnel and were eaten elsewhere by the subject ad libitum. Scheduling was designed to optimize the chances of studying each woman in the same phase of her menstrual cycle.

The macronutrient composition of the HC diet was 55% carbohydrate, 25% fat, and 20% protein; the HF diet was 30% carbohydrate, 50% fat, and 20% protein. Energy intake (kJ/d) was calculated for each subject to allow the subject's admission weight to be maintained on the basis of approximated basal energy expenditure and self-reported physical activity level. Body weight was monitored daily and the energy prescription was changed as necessary to maintain weight stability over each diet period. Body weight on day 0 of diet phase 1 was used as the goal weight for both diet phases. Diets were nutritionally adequate and matched for ratio of polyunsaturated to saturated fat (≈0.3:1.0), total fiber content (14–18 g), and total cholesterol content (≈35.9 µg/kJ). An alcohol intake of 40 g/wk was mandatory and was factored into the energy prescription.

On the morning of day 15 (of each diet phase) after subjects had fasted overnight for 12 h, blood work and biopsies of gluteal adipose tissue and skeletal muscle (vastus lateralis) (6) were performed. A meal of the same composition as the experimental diet and consisting of 40% of the subject's total daily energy prescription was then ingested ($t = 0$) as the breakfast meal, with postprandial blood samples drawn hourly for 6 h for measurement of triacylglycerol, fatty acids, glycerol, glucose, and

insulin. Adipose tissue and skeletal muscle biopsies were repeated on the contralateral side of the body at 6 h.

On day 16 of each diet phase, a 3-h euglycemic clamp study was performed to determine S_I (19). Insulin was infused intravenously at a rate of 258 pmol·min⁻¹·m⁻² and 20% dextrose was given intravenously at a variable rate to maintain the euglycemic goal (determined as the fasting blood glucose concentration measured on the morning of day 16 of diet phase 1). Individual S_I with each diet was then determined as the mean glucose infusion rate (GIR, mmol·min⁻¹·m⁻²) over the last hour of the infusion study (120–180 min). Fasting serum insulin concentrations were also considered to be an indicator of relative S_I (20).

Independent assessment of meal effect

An additional 8 normal-weight subjects (5 men, 3 women) were also studied to test whether changes seen in ATLPL in the larger cohort were due to the diet or the meal. The mean age for these subjects was 30 ± 3 y and their mean body mass index was stable at 22.8 ± 0.8. Each of the 8 subjects was fed a single meal of either HC or HF composition (in randomized order) on 2 separate days, a minimum of 1 wk apart. Gluteal ATLPL was measured in the fasted state and again 6 h postprandially and the meal-induced change in ATLPL calculated.

Laboratory procedures

LPL activity was measured in 40–45-mg pieces of tissue as described previously (21). Heparin-releasable lipase activity was measured against an emulsified serum-containing [¹⁴C]triolein substrate. Adipocyte size was determined by the method of DiGirolamo et al (22) after cells were isolated from tissue pieces in 2 g collagenase/L by the method of Rodbell (23).

Serum insulin concentrations were measured by radioimmunoassay (24). Serum fatty acid concentrations were measured enzymatically, with a colorimetric endpoint (25). Plasma triacylglycerol concentrations were measured enzymatically (26), as were serum glycerol concentrations (27). HDL cholesterol was separated from total cholesterol by use of the two-step dextran sulfate–magnesium chloride precipitation method of Warnick et al (28).

Data analysis

Variables of normal distribution were analyzed by using Student's t test and Pearson correlation analyses (r). Variables that were not normally distributed were analyzed by using the Wilcoxon rank-sum test and Spearman rank analysis (r_s). Analysis of covariance was used to compare the results of the preprandial serum and plasma measurements on day 15, ie, concentrations of glucose, insulin, triacylglycerol, cholesterol, HDL cholesterol, and HDL_2 cholesterol. Incremental area under the curve (IAUC) was calculated from the postprandial concentrations of glucose, insulin, triacylglycerol, fatty acids, and glycerol and was used as a summarizing measurement for each variable as described by Wolever and Jenkins (29). Analyses that yielded probabilities ≤0.05 were considered to be statistically significant. Results are presented as means ± SEMs.

RESULTS

The study group of 25 normal-weight subjects consisted of 10 women and 15 men between 25 and 34 y of age ($\bar{x} \pm$ SEM: 29 ± 1 y). At the screening visit, while the subjects were consuming a

TABLE 1
Fasting serum or plasma values of normal-weight subjects on day 0 and day 15 of a high-carbohydrate (HC) or high-fat (HF) diet[1]

	HC diet		HF diet	
	Day 0	Day 15	Day 0	Day 15
Glucose (mmol/L)	4.9 ± 0.1	4.6 ± 0.1	4.8 ± 0.1	4.7 ± 0.1
Insulin (pmol/L)	43 ± 7	38 ± 3	50 ± 7	39 ± 2
Triacylglycerol (mmol/L)	1.13 ± 0.11	1.14 ± 0.11	1.20 ± 0.11	0.88 ± 0.08^2
Fatty acids (g/L)	—	0.15 ± 0.01	—	0.14 ± 0.01
Glycerol (g/L)	—	8.93 ± 0.83	—	8.57 ± 0.83
Cholesterol (mmol/L)	3.98 ± 0.16	3.90 ± 0.16	3.85 ± 0.16	3.93 ± 0.16
HDL cholesterol (mmol/L)	1.27 ± 0.08	1.22 ± 0.06	1.27 ± 0.08	1.30 ± 0.06^3
HDL_2 cholesterol (mmol/L)	0.23 ± 0.05	0.19 ± 0.04	0.21 ± 0.05	0.25 ± 0.04^3

[1] $\overline{x} \pm$ SEM; $n = 25$. Study design was as follows: diet phase 1 for 16 d (either HC or HF diet), followed by 4–6-wk washout, followed by diet phase 2 for 16 d (either HC or HF diet).
[2,3] Significantly different from day 15 of HC diet: [2] $P<0.01$, [3] $P<0.05$.

free-living diet, the percentage dietary fat intake for the group was estimated to be $28 \pm 1\%$ with use of the Diet Habit Survey (18). The weight to be maintained for the women was 57.4 ± 1.7 kg and for the men was 76.7 ± 2.4 kg. The maintenance body mass index by sex was 20.5 ± 0.5 for women and 22.8 ± 0.6 for men. Weight stability was achieved over each diet phase (phase 1: women, 57.8 ± 1.6 kg, men, 77.1 ± 2.3 kg; phase 2: women, 58.2 ± 1.6 kg, men, 77.8 ± 2.3 kg).

There were no differences in any of the blood values measured in the fasted state on day 0 of each phase (**Table 1**). As expected, concentrations of HDL cholesterol were higher in women than in men (1.50 ± 0.10 compared with 1.09 ± 0.05 mmol/L, respectively, $P<0.001$), as were concentrations of HDL_2 cholesterol (0.34 ± 0.08 compared with 0.10 ± 0.03 mmol/L, respectively, $P = 0.001$). The daily energy intake required for weight stability was not significantly affected by macronutrient composition (HC compared with HF: $14\,067 \pm 862$ compared with $13\,711 \pm 841$ kJ/d), although both sexes tended to require slightly more energy while consuming the HC diet to maintain their weight (men consuming the HC compared with the HF diet: $16\,610 \pm 950$ compared with $16\,129 \pm 950$ kJ/d; women consuming the HC compared with the HF diet: $10\,255 \pm 397$ compared with $10\,075 \pm 385$ kJ/d).

The results of fasting blood analyses done on the morning of day 15 of both the HC and HF diets (Table 1) indicated that there was an effect of dietary macronutrient composition on fasting triacylglycerol (significantly lower with the HF diet, $P = 0.002$), fasting HDL cholesterol (significantly higher with the HF diet, $P = 0.013$), and HDL_2 cholesterol (significantly higher with the HF diet, $P = 0.035$). Shown in **Table 2** is the response of plasma and serum variables to meals of both compositions as analyzed by determining IAUCs for the value of each variable measured hourly from 0 to 6 h after the meal. There were significant differences between the 2 meals for plasma glucose IAUC (HC>HF, $P = 0.005$), serum insulin IAUC (HC>HF, $P = 0.026$), and plasma triacylglycerol IAUC (HC<HF, $P = 0.039$).

The LPL results from the adipose tissue biopsies performed on each subject on day 15 of each diet are shown in **Figure 1**. There was no effect of diet composition on fasting (0 h) ATLPL activity or fasting SMLPL activity. However, ATLPL activity increased significantly from fasting to 6 h postprandially with both diets (HC: from 1.4 ± 0.5 to 3.6 ± 0.8 nmol fatty acids$\cdot$min$^{-1}\cdot10^{-6}$ cells, $P = 0.002$; HF: from 1.1 ± 0.3 to 2.1 ± 0.4 nmol fatty acids$\cdot$min$^{-1}\cdot10^{-6}$ cells, $P = 0.002$). There was a significant difference in the 0–6-h change in ATLPL activ-

ity after ingestion of the HC meal compared with the HF meal (2.2 ± 0.6 compared with 1.0 ± 0.3 nmol fatty acids$\cdot$min$^{-1}\cdot10^{-6}$ cells, respectively, $P = 0.024$). There was no differential effect of macronutrient diet on gluteal fat cell size (HC compared with HF: 368 ± 25 compared with 353 ± 21 pL; NS). No diet differences in ATLPL meal response were seen when enzyme activity was expressed per gram tissue ($P = 0.168$).

The LPL results from the skeletal muscle biopsies performed on each subject on day 15 of each diet are also shown in Figure 1. There was no effect of diet composition on fasting (0 h) SMLPL activity. After both diets, SMLPL activity increased significantly from fasting to 6 h postprandially (HC: from 1.0 ± 0.3 to 1.9 ± 0.4 nmol fatty acids$\cdot$min$^{-1}\cdot$g^{-1}, $P = 0.015$; HF: from 0.8 ± 0.3 to 1.5 ± 0.2 nmol fatty acids$\cdot$min$^{-1}\cdot$g^{-1}, $P = 0.0001$). However, the 0–6-h change in skeletal muscle LPL activity was not significantly different between the 2 diets.

To test whether the relations of the aforementioned metabolic variables with ATLPL after HC and HF feeding were due to the diet or the meal, gluteal ATLPL was measured in 8 subjects in the fasted state (under free-living diet conditions) and then 6 h postprandially and the meal-induced change in ATLPL calculated. The results shown in **Figure 2** indicate that there was no difference in the 0–6-h ATLPL meal response after the HC meal compared with after the HF meal (1.2 ± 0.5 compared with 1.5 ± 0.9 nmol fatty acids$\cdot$min$^{-1}\cdot10^{-6}$ cells, $P = $ NS). Moreover, the 0–6-h ATLPL meal responses in these 8 subjects were similar to those in the 25 subjects. Therefore, the effects on LPL seen after 15 d of an HC compared with an HF diet in the present study of 25 subjects appear to have been due to the macronutrient composition of the diet rather than of the meal. The independent test group ($n = 8$) also showed significant differences between the 2 meals in the calculated postprandial IAUC for

TABLE 2
Incremental areas under the curve (IAUCs) for normal-weight subjects on day 15 of a high-carbohydrate (HC) or high-fat (HF) diet[1]

	HC Diet	HF Diet
Glucose IAUC (mmol $\cdot$ h/L)	$35\,711 \pm 755$	$34\,226 \pm 867^2$
Insulin IAUC (pmol $\cdot$ h/L)	$12\,185 \pm 1459$	9056 ± 952^3
Triacylglycerol IAUC (mmol $\cdot$ h/L)	$52\,438 \pm 4039$	$52\,736 \pm 5082^3$
Fatty acid IAUC (g $\cdot$ h/L)	$94\,067 \pm 6252$	$144\,660 \pm 7232$
Glycerol IAUC (g $\cdot$ h/L)	$28\,042 \pm 1605$	$36\,318 \pm 2723$

[1] $\overline{x} \pm$ SEM; $n = 25$.
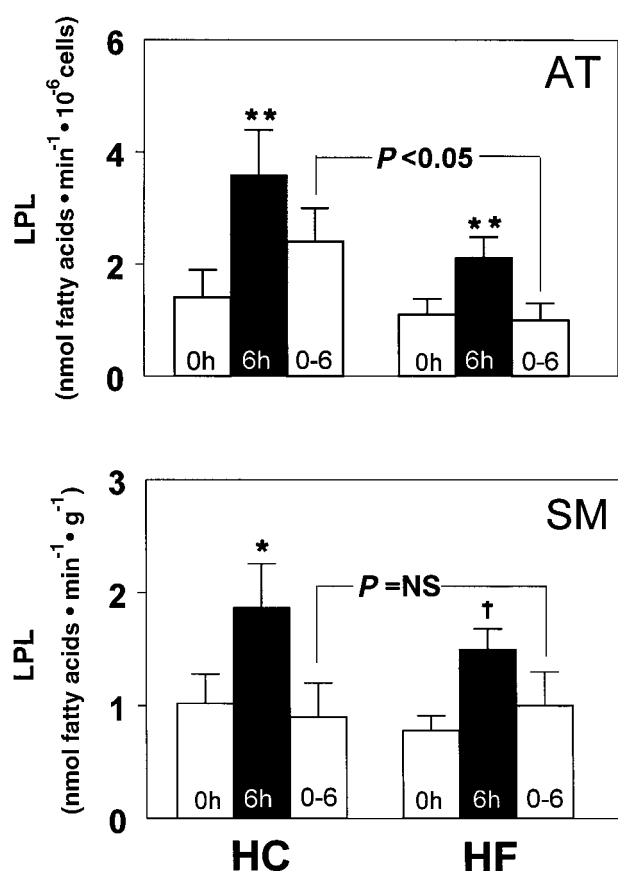[2,3] Significantly different from HC diet: [2] $P<0.01$, [3] $P<0.05$.

FIGURE 1. Mean (± SEM) gluteal adipose tissue (AT) and vastus lateralis skeletal muscle (SM) lipoprotein lipase (LPL) activity, measured in 25 normal-weight subjects on day 15 of a high-carbohydrate (HC) or a high-fat (HF) diet both in the fasted state (0 h) and postprandially (6 h) after the ingestion of a meal making up 40% of the individual's prescribed daily energy intake and having the same composition as the diet (HC or HF). Also shown is the 0–6-h meal response. **ATLPL at 6 h significantly different from fasting ATLPL, $P < 0.01$ (both HC and HF diets). There was a significant increase in the 0–6-h meal response of ATLPL after the HC diet and meal compared with the HF diet and meal ($P < 0.05$). *,†SMLPL at 6 h significantly different from fasting SMLPL: *$P < 0.05$ (HC diet), †$P = 0.0001$ (HF diet). There was no difference in the 0–6-h meal response of SMLPL after the HC compared with the HF diet and meal.

FIGURE 2. Independent assessment of mean (± SEM) gluteal adipose tissue (AT) lipoprotein lipase (LPL) activity in 8 normal-weight subjects in the fasted state (0 h) after consuming a free-living diet and then postprandially (6 h) after a high-carbohydrate (HC) or a high-fat (HF) meal (meal testing done a minimum of 1 wk apart). Fasting ATLPL on 2 separate testing days was not different. *After the HC meal only, ATLPL at 6 h was significantly different from fasting ATLPL, $P < 0.05$. There was no difference in the 0–6-h meal response of ATLPL between the HC and HF diets.

serum insulin (HC >HF, $P = 0.008$) and for plasma triacylglycerol (HC <HF, $P = 0.011$), in agreement with the results for the group of 25 subjects. However, in the smaller group of subjects, postprandial glucose IAUC values were not different between the 2 meals.

Measurement of the GIR during a euglycemic insulin-glucose clamp is considered to be the best quantitative method for assessing S_I (30, 31). The GIR was unaffected by the macronutrient composition of the diet in these normal-weight subjects (HC compared with HF: 1.97 ± 0.14 compared with 1.83 ± 0.13 mmol·min^{-1}·m^{-2}; **Figure 3**). In fact, whereas 13 of the subjects had greater GIRs after 16 d of the HC diet than after 16 d of the HF diet, 12 subjects responded in the opposite manner (ie, GIRs greater after the HF diet than after the HC diet). Moreover, both sexes showed similar variability in the effect of macronutrient intake on the GIR. There was no differential effect of diet com-
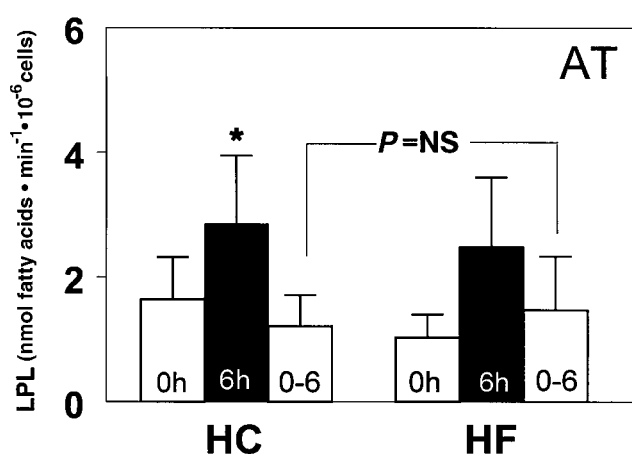
position on steady state insulin concentrations (HC compared with HF: 369 ± 21 compared with 371 ± 29 pmol/L) nor on steady state glucose concentrations (HC compared with HF: 4.4 ± 0.1 compared with 4.4 ± 0.1 mmol/L) achieved over the course of the euglycemic clamp. With the HC diet, the CVs for steady state insulin and steady state glucose were 28.4% and 7.3%, respectively. With the HF diet, the CV for steady state insulin was 38.9% and that for steady state glucose was 6.2%.

Overall, the tissue-specific changes in LPL activity after HC compared with HF diets and meals were variable. Activity levels of SMLPL were seemingly affected by relative S_I after 15–16 d of exposure to a diet high in carbohydrate. There was a significant inverse correlation between fasting serum insulin concentrations and the level of activity of SMLPL measured in the fasted state on day 15 with the HC diet ($r_s = -0.433$, $P = 0.030$). The relation between SMLPL activity and S_I after HC feeding was also shown by a positive correlation between fasting SMLPL activity and the GIR ($r = 0.434$, $P = 0.030$). These SMLPL correlations did not exist after HF feeding. Moreover, fasting ATLPL activity was not correlated with either the fasting serum insulin concentration nor the GIR after HC feeding.

Although the above relations of fasting SMLPL activity with S_I were not evident after 15–16 d of the HF diet, there were several relations between metabolic variables that were unique to the HF macronutrient perturbation. The significant SMLPL correlations discovered under the HF feeding conditions, however, became evident only at the 6-h postprandial time point after the HF meal. On day 15, the response of SMLPL to the HF meal was inversely correlated with fasting serum glucose ($r_s = -0.440$, $P = 0.028$). Also, after the HF diet and HF meal, the 0–6-h change in SMLPL activity was positively correlated with S_I as measured by the GIR during the clamp study ($r_s = 0.438$, $P = 0.028$). In adipose tissue, a strong inverse correlation was found between the response of ATLPL to the HF meal and fasting glucose the morning of day 15 ($r_s = -0.630$, $P = 0.0007$).
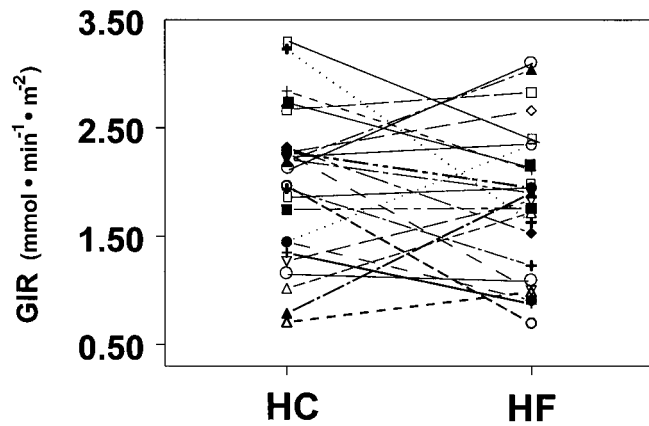
FIGURE 3. Mean glucose infusion rate (GIR, by individual) measured in 25 normal-weight subjects over the last hour of a 3-h insulin and glucose infusion performed on day 16 of a high-carbohydrate (HC) diet and on day 16 of a high-fat (HF) diet (in randomized order). Insulin was infused at a rate of 258 pmol·min^{-1}·m^{-2} with a variable infusion of 20% dextrose to maintain the euglycemic goal. There was no significant difference in the GIR between the HC and HF diets.

This relation was not seen in adipose tissue after the HC diet or meal. In the smaller group of 8 subjects in whom there was no prior diet preparation, no correlation was found between fasting serum glucose and the 0–6-h postprandial change in ATLPL after either the HF or the HC meal.

DISCUSSION

Fasting ATLPL activity has been shown to be similar in the same individual when measured on separate occasions and among individuals of the same body type (ie, lean or obese) (6, 8, 32). However, few studies have examined the effect of dietary macronutrient manipulation on the fasting enzyme activity in adipose tissue in humans. One study done by Pykalisto et al (3) documented a 47% increase in fasting ATLPL in obese control subjects and obese subjects with type 2 diabetes after 2 wk of an 85%-carbohydrate diet compared with a control diet of normal composition (45% carbohydrate). An 85%-carbohydrate diet, however, is beyond the composition seen in the most extreme nutritional intake patterns in the population. In the present study of normal-weight, nondiabetic subjects in which both diets were substantially altered but still realistic in composition, there was no difference in fasting ATLPL activity after 15 d of the HC compared with the HF diet.

Many studies have supported the idea that ATLPL is an insulin- and glucose-responsive enzyme under conditions of both parenteral and oral energy stimulation (3, 5, 6, 33–36). Taskinen and Nikkila (35) had 14 moderately obese women consume an isoenergetic diet (45% carbohydrate, 35% fat, and 20% protein) for 10 d and then fed each a standard breakfast (≈3347 kJ) and a standard lunch (≈2092 kJ) after a 10-h fast the night before. Gluteal ATLPL activity increased on average 21% when measured 1 h after ingestion of the standard lunch. The data presented here in 25 normal-weight subjects also showed a significant increase in ATLPL, but 6 h postprandially compared with fasting after both diets. The postprandial responsiveness of ATLPL was lower after the HF meal than after the HC meal. However, an independent examination of 8 normal-weight subjects who received HC and

HF meals only (without prior diet preparation) showed no effect of the meal composition on the ATLPL postprandial response. Therefore, the difference seen in the postprandial ATLPL response to an HC compared with an HF meal in the present study was apparently dependent on the macronutrient composition of the preceding diet, rather than on the composition of the meal. As noted previously, there was no difference between the 2 diets in S_I.

Skeletal muscle LPL activity is responsive to various physiologic changes, among them starvation and feeding. In humans, it has been shown that the consumption of higher quantities of carbohydrate results in lower SMLPL activity (4, 37). Lithell et al (4) had 7 normal-weight men take part in a study in which the diet was changed from an ordinary mixed diet to an HF diet (>70% of energy) for 3 d, followed by an HC diet (>70% of energy) for 3 d. Fasting serum insulin concentrations were higher after the HC diet than after the HF diet, as were serum triacylglycerol concentrations. Fasting SMLPL activity, however, was lower after the HC diet than after the HF exposure. The authors suggested that the increased insulin concentration during the short-term HC diet may have down-regulated SMLPL activity. It is important to note that the diets in the study by Lithell et al were extreme in composition and given only for 3 d, the diet order was not randomized, nor was there a washout period between the 2 experimental diets. A similar study done by Jacobs et al (37) showed similar results but with the same study limitations.

The effects of more long-term dietary manipulation on fasting SMLPL activity have been examined in humans. Kiens et al (38) studied 19 physically trained men and found that 4 wk of an HF diet (54% of energy) significantly increased ($P<0.05$) fasting SMLPL activity compared with that when the individuals consumed a "control" diet (≈43% of energy as fat). When dietary macronutrient composition was then changed to 51% of energy as carbohydrate for 4 wk, fasting SMLPL activity returned to control levels and was again significantly less ($P<0.05$) than after the HF feeding.

In contrast with the above studies, the data presented here indicate that fasting SMLPL activity was unaffected by 15 d of HC compared with HF feeding. Length of diet exposure was much longer in the present study than in the studies by Lithell et al (4) and Jacobs et al (37), and the 4–6-wk washout phase between diets precluded any carryover effect of the previous diet. Randomization of diet order and the less extreme macronutrient composition of the experimental diets are also factors that lend strength to the results presented here.

Several investigators have observed in in vivo studies in humans (6) and animals (39) that LPL activity often changes in opposite directions (ATLPL increases, SMLPL decreases) when stimulated by insulin or glucose. However, some animal studies have also shown that SMLPL remains unchanged in response to insulin (40–42). In rats, HF diets have been shown to result in either no change or in increased SMLPL activity (2). In the data presented here for 25 normal-weight subjects, although SMLPL response to a meal was not different between the 2 diets, SMLPL activity did increase significantly after both HC and HF meals.

In the 25 normal-weight subjects studied, whole-body S_I was variably affected by the macronutrient composition of the diet, with the group data showing no significant effect. The variability of S_I was expressed equally in both sexes. These results agree with those reported by Borkman et al (14), who found that S_I was not enhanced after 3 wk of an HC diet compared with S_I meas-

ured after 3 wk of an HF diet. In fact, as seen in the data presented here, insulin-stimulated peripheral glucose uptake (measured during a euglycemic clamp) was the same with both diets in the study by Borkman et al. One difference in that study is that the 2 diets were fed consecutively with no washout phase separating them and a carryover effect was not assessed. Because both diets were artificially manipulated to include compositions beyond the normal range, it is hazardous to assume that there was no carryover effect. Swinburn et al (13) did a study in which 12 Caucasians and 12 Pima Indians (58.4–213.5 kg) were each fed an HC and an HF diet for 2 wk (randomized, crossover design). A frequent-sampling intravenous-glucose-tolerance test was carried out for determination of S_I. No difference was found in S_I between the 2 diets, regardless of race, sex, or body weight (ie, obese compared with lean). Again, the administration of the 2 diets consecutively may have introduced a carryover effect.

As noted earlier, many investigators have shown that insulin action in humans can be altered by changes in the macronutrient content of the diet. In contrast with the results presented here, Lovejoy and DiGirolamo (9) found that S_I (measured with a frequent-sampling intravenous-glucose-tolerance test) was inversely correlated with percentage fat intake in 38 lean and obese subjects, suggesting that as individuals increased the proportion of fat in their diet, relative insulin resistance resulted. The results of the food-frequency questionnaire administered indicated that the habitual dietary composition of the lean subjects ($n = 22$) was 36 ± 1% fat and 45 ± 1% carbohydrate, whereas that of the obese group was significantly different (42 ± 1% fat and 40 ± 1% carbohydrate, $P < 0.01$). The data were not controlled for weight as a covariant. However, the fact that the correlation was not maintained when the lean subjects were examined separately tends to support the data from the present study, in which no effect of macronutrient composition on S_I was found in normal-weight individuals. The issue of the composition of the subjects' diets before the study must also be considered when comparing the results of the study by Lovejoy et al (9) with those of the present study.

Interesting relations between insulin action and SMLPL activity after HC feeding were uncovered. An inverse correlation was seen between fasting glucose concentrations and SMLPL after 15 d of HC feeding, whereas the correlation between S_I measured by the euglycemic clamp technique and fasting SMLPL was positive. Thus, both an indirect and direct assessment of S_I was related to fasting SMLPL after 15 d of HC feeding. These SMLPL correlations were not seen after HF feeding, nor was fasting insulin or insulin-mediated glucose disposal correlated with fasting ATLPL after HC feeding. This relation between S_I after HC feeding in normal-weight subjects was similar to that seen by Pollare et al (43); however, in that study, correlations between S_I measured by the euglycemic clamp technique and fasting SMLPL were quantified across a wide range of insulin sensitivities (normal-weight and obese subjects with and without hyperinsulinemia and subjects with type 2 diabetes). A significant relation between insulin sensitivity and SMLPL was not seen in normal-weight subjects alone, nor was the previous dietary period controlled. Unlike after the HC diet, after the HF diet it was the change in SMLPL with the HF meal that was correlated with S_I, not the fasting enzyme activity. The explanation for the difference between the effect of an HC compared with an HF diet on fasting SMLPL activity compared with the change in the enzyme after an HF meal is not readily apparent, but could

relate to the effect of an HF meal after HF feeding on lipolysis in adipose tissue. In adipose tissue, HF feeding followed by an HF meal may permit sufficient fatty acids to be available locally to inhibit the heparin-releasable enzyme activity or displace it from endothelial binding sites (44, 45), thus, the decrease in the meal response. In muscle, these fatty acids may independently enhance SMLPL (46) and overcome the expected inhibitory effect of insulin (6).

The results of this study indicate that the tissue-specific regulation of LPL in adipose tissue and skeletal muscle is affected primarily by diet macronutrient composition rather than meal composition. The effects of both HC and HF diets and meals on postprandial changes in ATLPL activity were dependent on the preceding 15-d diet exposure, indicative of a necessary preparation period of the adipose tissue by the substrate mix. Although this was true after both diets, the HC diet allowed greater postprandial LPL response. Diet macronutrient composition was shown to affect the relations of LPL to measures of insulin action. Although basal activity levels of SMLPL were not directly affected by diet composition, fasting SMLPL after the HC diet was clearly affected by the degree of whole-body S_I after sustained HC intake. After the HF diet or meal, the associations between SMLPL and insulin action were evident only postprandially, perhaps indicating that the addition of an HF meal to an HF diet was required to saturate the fat fuel compartment before fuel metabolism could be forced in the direction of fat oxidation.

In summary, several weeks of HC compared with HF feeding in normal-weight subjects increased the responsiveness of ATLPL to an HC compared with an HF meal. However, the same diet exposure had no effect on fasting activity levels of ATLPL or SMLPL, the responsiveness of SMLPL to a meal, or S_I. These data suggest that habitual dietary carbohydrate intake may have a stronger effect on subcutaneous fat storage than does the amount of dietary fat. ❧

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