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The effect of dietary carbohydrate on genes for fatty acid synthase and inflammatory cytokines in adipose tissues from lean and obese subjects

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Abstract

Background: Hepatic de novo lipogenesis (DNL) is markedly stimulated in humans by low-fat diets enriched in simple sugars. However, the dietary responsiveness of the key enzyme controlling DNL in human adipose tissue, fatty acid synthase (FAS), is uncertain.
Hypothesis: Adipose tissue mRNA for FAS is increased in lean and obese subjects when hepatic DNL is elevated by a eucaloric, low-fat, high-sugar diet.
Design: Twelve lean and seven obese volunteers were given two eucaloric diets (10% vs. 30% fat; 75% vs. 55% carbohydrate; sugar/starch 60/40) each for 2 weeks by a random-order cross-over design. FAS mRNA in abdominal and gluteal adipose tissues was compared to hepatic DNL measured in serum by isotopic and nonisotopic methods. Adipose tissue mRNA for tumor necrosis factor-α and IL-6, which are inflammatory cytokines that modulate DNL, was also assayed.
Results: The low-fat high-sugar diet induced a 4-fold increase in maximum hepatic DNL ($P<.001$) but only a 1.3-fold increase in adipose tissue FAS mRNA ($P=.029$) and no change in cytokine mRNA. There was a borderline significant positive correlation between changes in FAS mRNA and hepatic DNL ($P=.039$). Compared to lean subjects, obese subjects had lower levels of FAS mRNA and higher levels of cytokine mRNA ($P<.001$).
Conclusions: The results suggest that key elements of human adipose tissue DNL are less responsive to dietary carbohydrate than is hepatic DNL and may be regulated by diet-independent factors. Irrespective of diet, there is reduced expression of the *FAS* gene and increased expression of cytokine genes in adipose tissues of obese subjects.
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1. Introduction

A clear understanding of the metabolic effects of low-fat high-carbohydrate diets is needed as the public consumption of sugar climbs [1–3]. Sugar is implicated as an important nutritional factor in the obesity and diabetes epidemics [2,3], as well as in metabolic syndrome and atherogenic dyslipidemia [4]. Using both stable isotopic and nonisotopic methods, a marked increase in hepatic de novo lipogenesis (DNL) by dietary carbohydrate was demonstrated in vivo from measurements of serum very-low-density lipoprotein (VLDL) triacylglycerols (TGs) in weight-stable humans [4–9]. Lean and obese subjects had similar large increases in DNL on eucaloric very-low-fat solid food diets enriched in simple sugars, and these increases did not correlate with fasting or 24-h serum insulin, glucagon or glucose concentrations [5]. The increases in DNL did not cause weight gain but positively correlated with increases in serum TG concentrations and the ratio of saturated to unsaturated fatty

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acids in VLDL-TG, which are both associated with adverse effects on the cardiovascular system. The importance of the type as well as the amount of dietary carbohydrate was demonstrated by the lack of an increase in hepatic DNL after low-fat diets rich in complex carbohydrates [8,10].

Although animal studies clearly show that both adipose tissue and the liver are major sites of DNL on a high-carbohydrate diet [11–13], the lipogenic responsiveness of human adipose tissue to dietary carbohydrate is uncertain. Previous in vitro studies in adipose tissues of lean subjects overfed carbohydrate showed large increases in DNL [14–16] and mRNA levels of fatty acid synthase (FAS), acetyl CoA carboxylase (ACC1) and sterol response element-binding protein 1c (SREBP-1c), a key transcription factor controlling the expression of lipogenic genes [17]. In addition, in vivo measurements of hepatic and total body DNL with [^{13}C]acetate labeling and indirect calorimetry in lean subjects after massive intravenous carbohydrate overfeeding suggested that the major site of DNL under these conditions is the adipose tissue, rather than the liver [18]. In contrast, others have measured little increase in adipose tissue DNL or mRNA levels for lipogenic enzymes in lean subjects after isocaloric or hypercaloric high-carbohydrate diets [19,20]. Less is known about the dietary responsiveness of DNL in adipose tissues from obese subjects. Although several rodent models of obesity show increased DNL in adipose tissue [21] and the development of drugs that inhibit DNL as a treatment for human obesity is underway [22,23], similar DNL [14,15,24,25] and lower levels of FAS, ACC1 and SREBP-1c mRNA in subcutaneous and visceral adipose tissues have been reported in overweight versus lean subjects after ad libitum [26,27] or hypercaloric high-carbohydrate [15,17] diets.

The objective of the present study was to determine the dietary responsiveness of the gene for the key enzyme controlling rates of DNL (*FAS*) in adipose tissues from weight-stable lean and obese subjects. We evaluated the effect of the isocaloric substitution of dietary carbohydrate for fat on adipose tissue levels of FAS mRNA and compared it to large changes in hepatic DNL measured in plasma by an isotopic and a nonisotopic method, as previously reported [5]. We also determined whether the adipose tissue expression of the *FAS* gene varied with the expression of the genes for tumor necrosis factor-α (TNF-α) and IL-6 — inflammatory cytokines that are produced and secreted by adipose tissue and that suppress the transcription of the *FAS* gene [28–31].

2. Materials and methods

2.1. Subjects

As previously described [5], 12 lean and 7 obese otherwise healthy volunteers were studied as inpatients at The Rockefeller University Clinical Research Center. Studies were approved by The Rockefeller University Institutional Review Board, and written informed consent was obtained from each participating subject. To be eligible, the following criteria were met: screening fasting LDL cholesterol <4.14 mmol/L, TG <2.27 mmol/L and HDL cholesterol >0.91 mmol/L; lipid profiles were similar in lean and obese subjects. Participants were no less than 10% below their maximum weights and were weight-stable within 10% for the previous 6 months. All subjects had normal 3-h oral glucose tolerance tests.

2.2. Diets

The details of the diet have been previously reported [5]. Two solid food diets that differed in the proportion of carbohydrate and fat were given for 2 weeks each according to a random-order cross-over design. One diet, called the "high-sugar" diet, had 10% of energy as fat and 75% as carbohydrate; the other diet, called the "low-sugar" diet, had 30% of energy as fat and 55% as carbohydrate. Both diets had identical carbohydrate compositions and ratios of sugar to starch (60:40). The total fructose intake (including fructose in sucrose) was 18% energy (or 113 g/10,520 kJ) on the high-sugar diet and 13% energy (or 81 g/10,520 kJ) on the low-sugar diet. For both diets, the total fiber was 6.5 g/100 g carbohydrate, and the soluble to insoluble fiber ratio was 0.25.

The initial total calories were 5723 kJ/m^2 and required minimal adjustment across diets to keep weight constant. Each diet had a single-day menu (same food items each day) with three meals of similar energy and macronutrient composition. The fatty acid compositions of the two diets were identical and matched each subject's adipose tissue composition in order to measure fatty acid synthesis by the linoleate dilution (LD) method [7].

2.3. Real-time reverse transcription–polymerase chain reaction (RT-PCR) of adipose tissue mRNA

At the end of each dietary period, after a 12-h fast, adipose biopsies were performed at the abdominal and gluteal sites after anesthetizing each region with 10–20 ml of 1% lidocaine. At least 500 mg of subcutaneous fat was removed through a small incision with multiple passes of a 2.5-mm blunt-end liposuction needle attached to a 60-ml syringe partially filled with saline. Within 1/2 h, the adipose tissue was rinsed with normal saline, blotted dry, weighed and snap frozen in liquid nitrogen for storage at −70°C.

RNA was extracted from adipose tissue using RNeasy mini kits as recommended by the manufacturer (Qiagen, Valencia, CA). One-hundred-milligram aliquots were thawed, briefly homogenized in lysis buffer using a Tissue Tearor (Biospec Products, Inc., Bartlesville, OK) and centrifuged for 3 min at room temperature at 8000×*g*. Then 500 μl of the supernatant below the fat layer was removed and diluted with 500 μl of 70% ethanol, and 500 μl was passed over an RNeasy silica-gel-based mini column that was centrifuged for 15 s at 8000×*g*. The eluted solution

was discarded, and the remaining 500 μl was passed over the column, which was centrifuged again and rinsed with RPE and RWI buffers. RNA was eluted from the column by adding 30 μl of RNAase-free water and centrifuging for 1 min at 8000×*g*.

Extracted RNA (~5 μg/100 mg tissue) was converted to cDNA in a Gene Amp PCR 9700 thermocycler (Applied Biosystems, Inc., Foster City, CA) by first denaturing at 70°C for 10 min in the presence of random hexamers (Invitrogen, Carlsbad, CA); chilling for 5 min; leaving at room temperature for 10 min; incubating at 37°C for 90 min with first-strand buffer, dNTP, DTT and reverse transcriptase (Invitrogen); and then denaturing at 95°C for 5 min.

cDNA was amplified using *TaqMan* Master Mix (Applied BioSystems, Inc.), forward and reverse oligonucleotide primers and a *TaqMan* probe. For FAS mRNA, primers and probe were designed from a published gene sequence (GenBank accession no. U26644.1) using Primer Express, Version 1.0 (Applied Biosystems, Inc.): forward sequence, 5′ ACTTGCAGGAGTTCTGGGACAA 3′; reverse sequence, 5′ GAAGGAGGCATCAAACCTAGACA 3′; and probe sequence, 5′ CGTAGAGCCCAGCCTTCCAGCGA 3′. The probe was labeled at the 5′ end with the reporter dye 6-carboxy-fluorescein and at the 3′ end with the quencher 6-carboxy-tetramethyl-rhodamine. Oligonucleotide primers and *TaqMan* probes for human TNF, IL-6 and β-actin mRNA were purchased from Applied Biosystems, Inc. For each assay, β-actin mRNA was amplified separately and simultaneously with the target gene(s) to normalize for total RNA. The β-actin signal per 100-mg adipose tissue was similar irrespective of obesity, anatomical site and diet.

Real-time RT-PCR was carried out in triplicate for each sample on an Applied Biosystems, Inc., 7700 (FAS) or 7900 (TNF and IL-6) sequence detection system. Each reaction well contained cDNA, *TaqMan* PCR Master Mix, 300 nmol/L of each forward and reverse primer, and 100 nmol/L of *TaqMan* probe. Cycling parameters were 50°C for 2 min, 95°C for 10 min, then 40 cycles of 95°C for 15 s and 60°C for 1 min. For every run, serial dilutions of a stock cDNA sample from human adipose tissue were used to create standard curves for each gene by plotting C_t (threshold cycle number at which the fluorescence signal is linearly increasing above background) versus log cDNA. C_t readings for unknown samples were used to calculate the amount of each target gene relative to β-actin. Nontemplate controls without cDNA never showed significant DNA contamination.

2.4. In vivo measurements of fatty acid synthesis

Two independent methods were used simultaneously to measure DNL in vivo: the LD method and the mass isotopomer distribution analysis (MIDA) technique, both described in detail elsewhere [7,32,33]. Using the LD method, the fraction of total synthesized fatty acids in VLDL-TG was calculated from the decrease (or dilution) of linoleate relative to the concentration in the diet and adipose tissue (made to be identical) by synthesized fatty acids. Using the MIDA method, the fraction of synthesized palmitate in VLDL-TG was measured after the intravenous infusion of [1-^{13}C]acetate by calculating the ^{13}C enrichment of the biosynthetic true precursor pool (hepatic acetyl-CoA), based on the ratio of double-labeled to single-labeled mass isotopomers in VLDL-TG palmitate. The intravenous infusion of sodium [1-^{13}C]acetate (Cambridge Isotope Laboratories, Andover, MA) was at 4.5 mmol/h, beginning 15 h before breakfast and continuing in most subjects for at least an additional 24 h until the following morning, for a total of 39 h, in order to allow newly labeled palmitate in VLDL-TG and hepatic TG storage pools to reach plateau values [7,32,33].

As previously described, at the end of each diet period, blood was sampled at 0800 h before [^{13}C]acetate infusion and beginning on the morning after the start of the infusion, at 0900, 1500, 2100, 1200, 0300 and 0900 h. Meals were provided at 0900, 1300 and 1700 h. Chylomicrons were removed, and VLDL was isolated by density gradient ultracentrifugation. TGs were isolated after chloroform/methanol extraction and separated from other lipids by thin-layer chromatography, and the fatty acid composition of TG was analyzed by capillary gas chromatography (model 5890; Hewlett Packard, Palo Alto, CA). The fatty acid compositions of the diets and subcutaneous adipose tissue sampled from abdominal and gluteal sites were similarly analyzed. The ^{13}C enrichment of palmitate from VLDL-TG was measured by gas chromatography/mass spectrometry, as described [33]. Fasting and maximum-fed values of DNL were used for statistical analysis.

2.5. Analysis of serum glucose, insulin, glucagon and cytokines

At the end of each 2-week diet period, serum concentrations of glucose, insulin and glucagon were measured at 0, 0.5, 1, 1.5, 2 and 3 h after breakfast, then every 2–3 h until the following morning. Glucose was measured by glucose oxidase assay, and insulin was analyzed by EIA (Abbott Laboratories, Santa Clara, CA). Glucagon was measured in duplicate by double antibody radioimmunoassay of EDTA/aprotinin-treated plasma (Diagnostic Products Corp., Los Angeles, CA). Serum concentrations of IL-6, TNF and high-sensitivity C-reactive protein (hsCRP) were measured with chemiluminescent EIA on the Immulite 2000 immunoassay system (Diagnostic Products Corp.) in fasting samples taken just before the adipose biopsy. The intrasample coefficient of variation was <6% for IL-6, TNF and hsCRP. The serum IL-6 results below the detection limit of 0.14 IU/ml were assigned the value of 0.07 (i.e., halfway between 0 and 0.14).

2.6. Statistical methods

Three-way analysis of variance (ANOVA) was used to identify factors contributing to the ratio of adipose tissue

target mRNA (FAS, TNF or IL-6) to β-actin mRNA. The dependent variable was the logarithm of the ratio (arbitrary units); the fixed factors were diet (high-sugar and low-sugar), site (abdomen, gluteus) and group (lean and obese); and the random factor was subjects within a group. Pearson correlation analysis and simple linear regression were used to assess relationships among continuous variables; because of skewing of the distribution, the logarithm of hsCRP was tested. Scatter plots were drawn to check for nonlinear relationships and outliers. To compare the direction of change between diets for FAS mRNA and hepatic DNL, the asymptotic two-sided sign test for continuous variables was used [34]. There was no evidence for period or carryover effects. Data analysis was performed using Excel XP/2000 (Microsoft, Inc., Redmond, WA), SigmaStat 3.00 (SPSS, Inc., Chicago, IL) and S-PLUS 7 (Insightful, Inc., Seattle, WA) statistical software. Data are shown as mean±S.D., except in Fig. 3, where S.E.M. is displayed. $P<.01$ was considered statistically significant.

3. Results

3.1. Effect of diet

Table 1 shows the subject characteristics and average effects of the substitution of dietary carbohydrate enriched in sugar for fat on markers of DNL in the adipose tissues and liver of lean and obese subjects. Obese subjects were similar in age and sex compared to lean subjects but had significantly higher waist/hip circumference ratios. Fig. 1 shows the effects in individual subjects. The high-sugar diet marginally increased the expression of the *FAS* gene by 1.3-fold in adipose tissues of lean and obese subjects ($P=.029$ by ANOVA). In contrast, as we reported previously [5], the same high-sugar diet dramatically increased hepatic DNL to an extent that was not significantly different in lean and obese subjects. By the LD method, the maximum percent DNL after meals was fourfold higher during the high-sugar than the low-sugar diet (41±13% vs. 10±11%, $P<.001$, between diets). All subjects had higher percent DNL while on the high-sugar compared to the low-sugar diet. The MIDA method gave qualitatively similar results that were highly correlated with the results obtained by the LD method ($R^2=.63$, $P<.001$).

In Fig. 2, the diet-induced changes in FAS mRNA, expressed as the mean of abdominal and gluteal values, are plotted against the changes in maximum percent hepatic DNL by the LD (left panel) and MIDA (right panel) methods. The responses of each subject on the two diets are connected. With both methods, in 12 subjects, both FAS mRNA and hepatic DNL increased during the high-sugar diet compared to the low-sugar diet, but in five subjects, FAS mRNA decreased or did not change when hepatic DNL increased. The sign test for the direction of change between diets for the LD and MIDA methods yielded borderline positive trends (P values of .039 and .066, respectively). On the high-sugar diet, there was a significant positive correlation between mean FAS mRNA and maximum hepatic DNL by MIDA ($R^2=.36$, $P=.007$), but not by LD ($R^2=.06$, $P=.301$). This relationship was less strong but in a similar direction for hepatic DNL measured after an overnight fast by either method (data not shown).

Table 1
Changes in adipose tissue, liver and plasma in response to dietary carbohydrate in lean and obese subjects

	Lean		Obese	
Sex (female:male)	6:6		4:3	
Age (years)	31 (18–62)		42 (22–61)	
Body mass index (kg/m^2)	23 (20–27)		34 (31–38) *	
Waist/hip circumference	0.87 (0.76–1.02)		0.99 (0.80–1.10) *	
	High sugar	Low sugar	High sugar	Low sugar
Adipose tissue (fasting)				
Abdominal mRNA FAS/actin	2.13±1.21	1.57±0.78	0.81±0.45 *	0.53±0.23 *
Gluteal mRNA FAS/actin	1.58±0.73	1.23±0.61	0.56±0.22 *	0.47±0.20 *
Liver (fed)				
Maximum % DNL (LD method)	43±13	12±13 **	37±15	6±6 **
Maximum % DNL (MIDA method)	49±7	28±12 **	36±10	24±10 **
Liver (fasting)				
% DNL (LD method)	31±22	1±3 **	21±21	1±2 **
% DNL (MIDA method)	37±15	19±13 **	27±7	16±9 **
Plasma (fasting)				
TG (mmol/L)	1.58±0.59	1.02±0.37 **	2.08±1.15	1.28±0.43 **
Glucose (mmol/L)	4.7±0.3	4.6±0.3	4.7±0.3	4.8±0.2
Insulin (μU/ml)	6±2	6±1	13±6 *	10±2 *
Glucagon (pmol/ml)	71±18	69±20	56±8	56±10

* $P<.01$, lean versus obese.
** $P<.01$, high-sugar diet versus low-sugar diet.

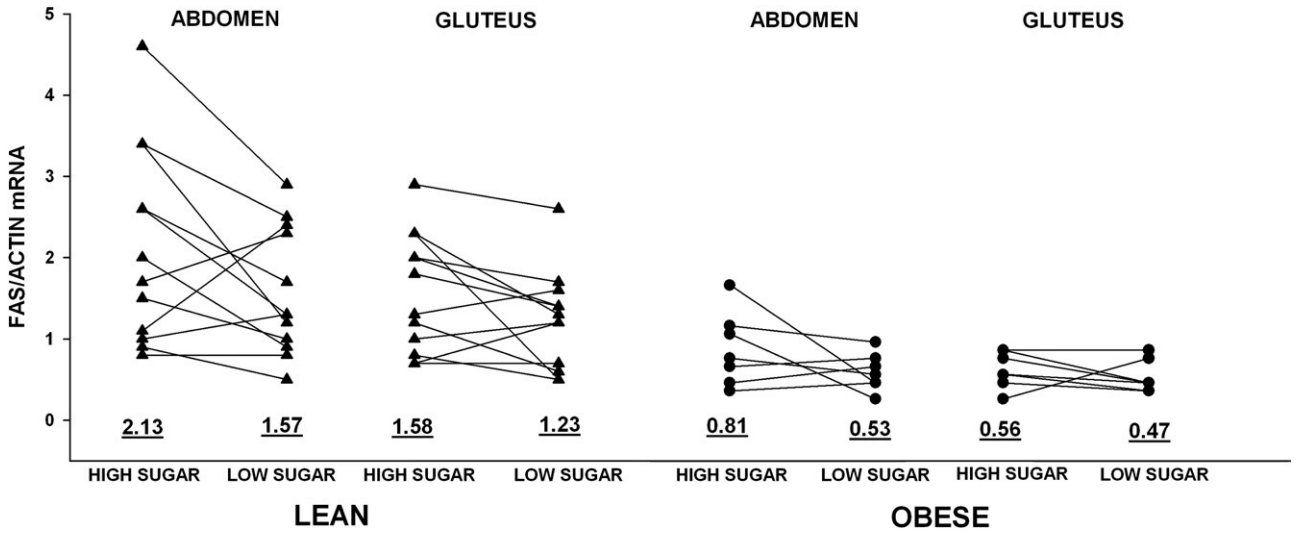

Fig. 1. Ratios of mRNA for FAS to actin in abdominal and gluteal adipose tissues from lean (n=12) and obese (n=7) subjects after a high-sugar diet or a low-sugar diet. Mean values are given below the individual results. Three-way ANOVA: lean>obese, P<.001; abdomen>gluteus, P=.005; high-sugar diet>low-sugar diet, P=.029. There were no significant interactions.

Similar to the minimal effect of dietary carbohydrate on FAS mRNA, there was no statistically significant effect of diet on the adipose tissue mRNA levels of TNF and IL-6 (Fig. 3). Serum levels of TNF, IL-6 and hsCRP were not also affected by diet (data not shown).

3.2. Effect of anatomical site

Subcutaneous adipose tissue at the abdominal site has a higher percentage of palmitate than the gluteal site [35] and may synthesize more palmitate in response to dietary carbohydrate. Adipose tissue levels of FAS mRNA were slightly but significantly higher at abdominal sites than at gluteal sites on both diets (Fig. 1). For both diets, the levels of FAS mRNA in abdominal and gluteal sites were strongly positively correlated with each other (R^2=.72, P<.001). There were no significant site-specific differences in the expressions of the genes for TNF and IL-6.

3.3. Effect of obesity

Adipose tissue levels of FAS mRNA were significantly reduced by twofold to threefold in obese subjects compared to lean subjects. This was true on both diets at both abdominal and gluteal sites (Fig. 1).

In contrast to the reduced *FAS* gene expression, expressions of the genes for TNF and IL-6 were significantly elevated in obese adipose tissue compared to lean adipose tissue (Fig. 3). Adipose tissue mRNA levels of TNF and IL-6 were strongly correlated with each other. Serum concentrations of hsCRP

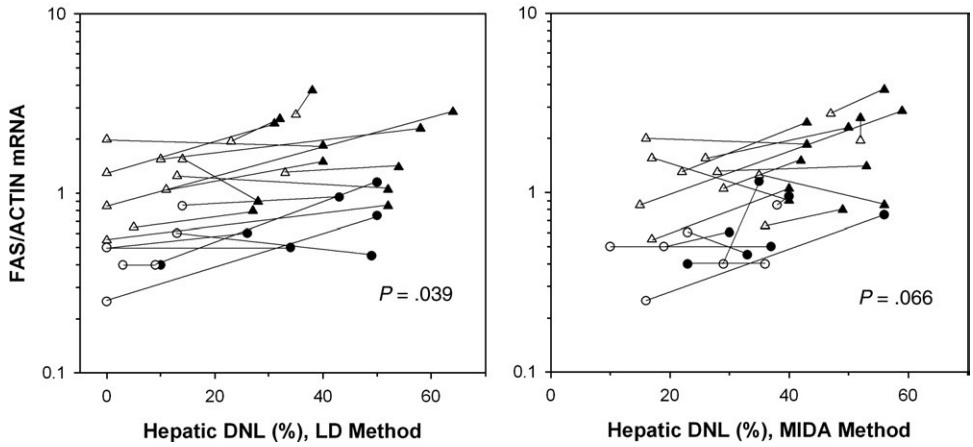

Fig. 2. Mean values for FAS/actin mRNA in abdominal and gluteal adipose tissues are plotted against hepatic DNL measured by LD (left panel) and MIDA (right panel). Twelve lean and seven obese subjects; black=high-sugar diet; white=low-sugar diet; lean=triangles; obese=circles. The responses to the two diets per subject are connected. For each plot, the positive relationship between changes in markers of DNL in adipose tissue and in the liver is of borderline statistical significance (sign test for continuous variables).

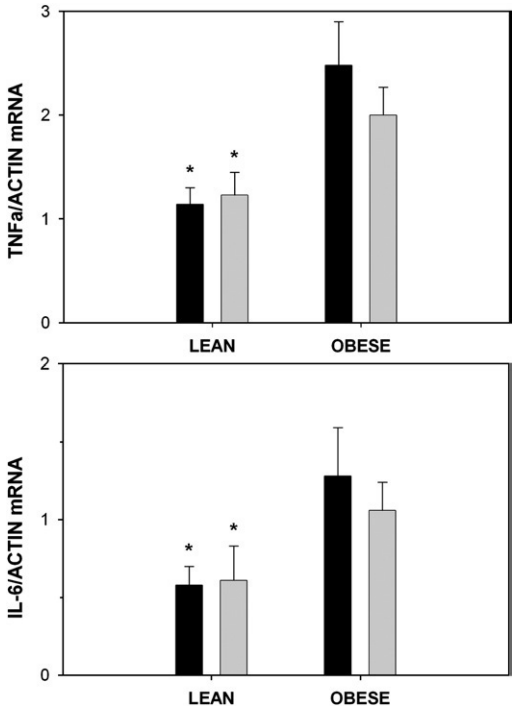

Fig. 3. Ratios of mRNA for TNF-α to actin (top panel) and IL-6 to actin (bottom panel) in adipose tissues from lean (n=12) and obese (n=7) subjects after a high-sugar diet (dark bar) or a low-sugar diet (light bar). Mean±S.E.M. Three-way ANOVA: obese>lean for both cytokines (TNF, P=.004; IL-6, P=.008; as shown by asterisk). High-sugar diet not different from low-sugar diet (P=.72 and .88 for TNF and IL-6, respectively). The values did not differ by site; thus, the average of abdominal and gluteal values per subject was used. There were no significant interactions.

(but not serum TNF and IL-6) were higher in obese subjects than in lean subjects (high-sugar diet: 8.4±6.3 vs. 1.3±1.6 IU/L, P=.02; low-sugar diet: 7.0±5.3 vs. 0.8±0.7 IU/L, P=.02) and were significantly positively correlated with body mass index, weight/height ratio and serum insulin (data not shown). Linear regression analysis, however, did not show significant inverse relations between FAS mRNA and serum hsCRP or serum or mRNA levels of TNF IL-6. Hepatic DNL and serum triglycerides did not vary with these markers of inflammation.

3.4. Relationships with serum insulin, glucose and glucagon

Other mediators known to directly modulate the expression of the *FAS* gene are insulin, glucose and glucagon. The fasting levels of insulin, glucose and glucagon in each diet in lean and obese subjects are shown in Table 1. As previously reported [5], meal-stimulated and 24-h (but not fasting) serum concentrations of insulin were significantly higher in the high-sugar diet than in the low-sugar diet. Compared to lean subjects, obese subjects had significantly higher fasting, meal-stimulated and 24-h serum concentrations of insulin, reflecting mild insulin resistance. Glucose levels were higher and glucagon levels were lower in obese subjects than in lean subjects (P=.03 for both comparisons), but were not different between diets. However, as previously reported for hepatic DNL [5], linear regression analysis did not show a significant relation between FAS mRNA and fasting, meal-stimulated or 24-h serum insulin, glucose or glucagon concentrations.

4. Discussion

It has been established that an increase in dietary carbohydrate can dramatically increase DNL in the human liver, but the dietary responsiveness of DNL in human adipose tissue is more controversial. In our study, a eucaloric, very-low-fat, high-carbohydrate diet enriched in sugar markedly induced DNL in the liver but had a blunted effect on the expression of *FAS* gene in adipose tissues of lean and obese subjects. Thus, in both lean and obese subjects, there are likely large tissue-specific differences in DNL in response to dietary carbohydrate. The borderline positive relationship between hepatic DNL and levels of FAS mRNA in adipose tissue suggests that DNL is, to some extent, coordinated in human adipose tissue and liver, but is modified in adipose tissue by factors other than dietary carbohydrate. The large difference in the expression of the gene for FAS in adipose tissues from lean and obese subjects, regardless of diet, also supports the existence of important diet-independent mediators.

Table 2 summarizes the conflicting literature of studies that measured changes in markers of DNL in adipose tissue after changes in dietary carbohydrate. The results are difficult to compare since the studies vary in the duration and mode of feeding, absolute amount and quality of carbohydrate, and markers of adipose tissue DNL. The only previous study that simultaneously measured the effect of an isocaloric substitution of dietary carbohydrate for fat on DNL in the liver and FAS mRNA in adipose tissue was performed in lean volunteers who consumed two diets each for 21 days as outpatients [20]. The authors concluded that dietary carbohydrate stimulated an increase in DNL in the liver, but not in adipose tissue. The high-carbohydrate diet in our study was lower in fat, higher in carbohydrate and higher in the ratio of sugar to starch, and stimulated hepatic DNL to a greater extent (41% vs. 13%). Even after more marked stimulation of DNL in the liver, our results support their findings that adipose tissue is less diet responsive than the liver in lean subjects and, for the first time, extend the finding to obese subjects.

In both adipose tissue and liver, changes in DNL are thought to occur mainly after changes in the transcription rate of the gene for FAS via a variety of mediators. We evaluated the potential autocrine, paracrine and endocrine effects of inflammatory cytokines on the adipose tissue and serum. In vitro and in obese animal models, TNF and IL-6, secreted locally and into the circulation by adipocytes and macrophages that infiltrate adipose tissue depots [28,36], stimulate lipolysis but inhibit DNL directly [28–31] or indirectly by decreasing insulin sensitivity and glucose

Table 2
Review of the literature: response of adipose tissue DNL to dietary carbohydrate

Reference	Subjects	Design	Diets	Method	Results
Current study	12 lean, 7 obese; male and female	14 days, random order, cross-over, inpatient	Isocaloric high-carbohydrate vs. low-carbohydrate meals (10% fat, 75% carbohydrate vs. 30% fat, 55% carbohydrate, both 60/40 sugar/starch)	ABD and GLT tissue mRNA	Borderline 1.3-fold increase in FAS mRNA (fasting) in lean and obese at both sites
				Hepatic DNL by [^{13}C]acetate/MIDA and LD methods	Fourfold increase in lean and obese (~40% DNL in VLDL-TG by both methods)
				Indirect calorimetry	No net total DNL
No change					
Letexier et al. [20]	5 lean; male and female	21 days, random order, cross-over, 4-month washout, outpatient	Isocaloric high-carbohydrate vs. low-carbohydrate meals (30% fat, 55% carbohydrate vs. 45% fat, 40% carbohydrate, both 40/60 sugar/starch)	ABD tissue mRNA	No change in FAS mRNA (fasting)
				Hepatic DNL by $^{2}H_2O$	Twofold increase (12.9% DNL in TG)
Diraison et al. [19]	10 lean; male and female	14 days, single order, parallel (five per group), outpatient	Ad libitum diet, then hypercaloric meals (35% fat, 50% carbohydrate then 23% fat, 61% carbohydrate, both 40/60 sugar/starch)	ABD tissue mRNA	No change in FAS mRNA (fasting)
				ABD tissue DNL by $^{2}H_2O$	No change
				Hepatic DNL by $^{2}H_2O$	Threefold increase (10.6% DNL in TG)
				Indirect calorimetry	Low net total (and adipose tissue) DNL (1.5 g/day)
Increase					
Minehira et al. [17]	11 lean, 8 overweight; male and female	4 days, random order, cross-over, 2- to 4-week washout, outpatient	Hypercaloric high-carbohydrate vs. isocaloric low-carbohydrate meals (20% fat, 70% carbohydrate, 60/40 sugar/starch vs. 35% fat, 50% carbohydrate, 40/60 sugar/starch) then oral glucose over 5 h	GLT tissue mRNA	~1.8-Fold increase in FAS mRNA (fed) in lean and overweight
				Indirect calorimetry	4.5-Fold increase in net total DNL in lean; 1.3-fold increase in overweight
Chascione et al. [16]	6 lean; male and female; undernourished	6–10 days, single order, cross-over, inpatient	Hypocaloric low-carbohydrate total parenteral nutrition intravenously (2 days), then hypercaloric glucose intravenously	ABD, GLT or thorax tissue incubation with [^{14}C]glucose +insulin	79-Fold increase in DNL (fed, geometric mean)
				Indirect calorimetry	Estimated low percentage of net total body DNL (~7%)
Sjostrom [14]	3 lean, 9 obese; female	23 days, single order, cross-over, outpatient	Ad libitum, then hypocaloric high-carbohydrate formula meals (1% fat, 90% glucose)	ABD tissue incubation with [^{14}C]glucose+insulin	11-Fold increase in DNL (fasting) in lean and obese
				Subcellular incubation with [^{14}C]acetate and [^{14}C]citrate	Threefold increase in DNL (fasting) in lean and obese
Sjostrom [15]	6 obese; female	6 days, single order cross-over, inpatient	Ad libitum, then hypercaloric high-carbohydrate formula meals plus insulin (0.1% fat, 90% carbohydrate, mostly glucose)	ABD tissue incubation with [^{14}C]glucose+insulin	Ninefold increase in DNL (NS, variable; fed=fasting)
				Subcellular incubation with [^{14}C]citrate	Threefold increase in DNL (fed=fasting)

ABD=abdominal; GLT=gluteal.

transport [37–39]. DNL may also be inhibited by leptin, another cytokine-like hormone elevated in obese adipose tissue, directly or after up-regulation of inflammatory cytokines [40,41]. In addition, recent epidemiological findings suggest that an increase in dietary glycemic load may increase serum or adipose tissue markers of inflammation and contribute to dyslipidemia and insulin resistance [42]. In the current study, the high levels of cytokine mRNA and low levels of FAS mRNA in the adipose tissues of obese versus lean subjects irrespective of diet hint at a diet-independent role for inflammatory cytokines in the suppression of FAS in obese adipose tissue. However, an accurate measurement of the activity [43], as well as mRNA levels of FAS and other lipogenic enzymes in small amounts of adipose tissue of more subjects, is required to better understand the regulation of DNL by cytokines.

An increase in dietary carbohydrate and glycemic load is known to produce an increase in the serum levels of insulin and glucose and, in turn, could directly increase the transcription of the *FAS* gene and rates of DNL [44–46]. Increases in serum insulin and glucose in response to dietary carbohydrate are exaggerated in the obese, but could increase or decrease FAS in

adipose tissue, depending on tissue sensitivity to these mediators. However, as was true for hepatic DNL, we did not find clear evidence for insulin-mediated or glucose-mediated changes in the expression of the *FAS* gene in adipose tissue. A relationship between FAS mRNA and serum glucagon, a direct inhibitor, was also not apparent. It is possible that different results may be obtained in more insulin-resistant obese subjects with higher insulin levels.

The low levels of FAS mRNA in obese compared to lean subjects are consistent with other reports [20,26,27] and may be an adaptation to higher TG turnover, adipocyte lipid accumulation and total fat stores. Adult obese rodents also have tissue-specific differences in DNL with high activity in the adipose tissue of young obese rodents [21,47] that then decline with age to low levels [21,40,47,48] but remain high in the liver irrespective of diet [49]. A higher adipose tissue DNL has also been found in human infants and children compared to adults on ad libitum diets [47]. The results of this study were obtained after weight maintenance in adults and do not exclude the possibility of significant carbohydrate-induced adipose tissue DNL during weight gain. As shown in Table 2, three of the four hypercaloric high-carbohydrate feeding regimens significantly increased markers of adipose tissue DNL, albeit to a similar or lower extent in obese compared to lean subjects. Whether less extreme manipulations in dietary carbohydrate and sugar result in an increase in adipose tissue DNL that contributes to obesity requires further study.

In conclusion, very-low-fat high-sugar diets that maintain body weight increase hepatic DNL and serum concentrations of TG but have a slight effect on the gene expression of FAS and presumably DNL in lean and obese human adipose tissues. Further studies are required to determine the importance of carbohydrate-induced DNL in the lipid homeostasis of adipocytes and to define the mediators that regulate the lipogenic pathway in this key tissue.

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