Differences in the regulation of adipose tissue and liver lipogenesis by carbohydrates in humans

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Abstract **We assessed the contributions of human liver and adipose tissue de novo lipogenesis (DNL) to triacylglycerol (TAG) synthesis. Volunteers were fed a high-energy, high-carbohydrate diet (HC, n = 5) or a normocaloric diet (NC, n = 10). NC subjects remained in the fasting state (Study 1, n = 5) or received oral glucose (Study 2, n = 5) throughout the test (12 h). HC subjects remained in the fasting state (Study 3). They ingested deuterated water and [U-^{13}C]acetate to trace lipogenesis. Adipose tissue fatty-acid (FA) synthase (FAS), acetyl-CoA carboxylase 1 (ACC1), and SREBP-1c mRNA were measured. Plasma TAG-FA was labeled by ^{13}C and deuterium showing active liver lipogenesis, which was stimulated ($P < 0.05$) by oral glucose and HC diet. Adipose tissue TAG had no detectable ^{13}C enrichment in any test, showing no significant incorporation of TAG-FA provided by liver lipogenesis, but were labeled by deuterium in all tests, showing active DNL in situ; however, rough quantitative estimates showed that adipose DNL was minimal (<1 g), and poorly stimulated by oral glucose or HC diet. mRNA levels were not increased by the HC diet. Adipose DNL is active in humans, but contributes little to TAG stores and is less responsive than liver DNL to stimulation by carbohydrates.**—Diraison, F., V. Yankah, D. Letexier, E. Dusserre, P. Jones, and M. Beylot. **Differences in the regulation of adipose tissue and liver lipogenesis by carbohydrates in humans.** ***J. Lipid Res.*** **2003.** 44: **846–853.**

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The appearance of obesity results from a long-term imbalance between storage and mobilization of triacylglycerols (TAGs) in adipose tissue. Mobilization of TAG stores could be impaired in some obese patients through genetic variations or abnormal regulation of adrenoceptors (1) or hormone sensitive lipase (1, 2), resulting in a decreased lipolytic activity of adipocytes. A decreased ability to oxidize long-chain fatty acids (FAs) (3) could also promote the storage of orally ingested TAG and contribute to the development of obesity. Less is known about the possible role of an increase of lipogenesis in promoting excessive fat storage. De novo lipogenesis (DNL), which occurs in liver and adipose tissue, is considered in humans to be a minor pathway unlikely to play a significant role in the regulation of fat stores (4, 5); however, hepatic lipogenesis can be largely modified in lean subjects by modifications of total energy intake, dietary fat-carbohydrate ratio, or glucose and/or insulin concentration (6–8). Hepatic lipogenesis was not found increased in obese subjects receiving the normocaloric diet for 2 weeks (9), but was increased in ad libitum-fed obese subjects (10, 11); it could thus contribute on a long-term basis to the development of excessive fat stores. In vitro, insulin stimulates the transcription (12) and the activity (13) of FA synthase (FAS) in cultured human adipocytes. Whether human adipose tissue lipogenesis can also be modified in vivo by metabolic and dietary factors and could contribute to the development of obesity remains debatable. This issue has important implications given the increasing prevalence of obesity on one hand and the present recommendation of high carbohydrate-to-fat ratio in the diet on the other hand (6, 14). Studies measuring the incorporation in adipose tissue of radioactivity from ^{14}C-labeled glucose suggest that adipose tissue lipogenesis contributed little to the metabolic fate of an oral glucose load (15). Measuring lipogenic activity in adipose tissue samples obtained from obese subjects fed a high-carbohydrate (HC) diet, Sjöström concluded that DNL in adipose tissue was of little quantitative importance (16). On the other hand, recent studies of overfed control subjects showed that net fat synthesis determined by indirect calorimetry was largely increased in these subjects (17) and that the stimulation of hepatic lipogenesis explained only a small part of this increase in lipid synthesis (8). These findings strongly suggested that lipogenesis was also stimulated in other tissue, presumably adipose tissue, and thus that adipose tissue DNL could be

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involved in the development of excessive fat stores; however, the evidence obtained in these studies for an increase in adipose tissue lipogenesis was only indirect. Moreover, the situation investigated massive overfeeding with very high carbohydrate-to-fat ratio was extreme, and thus the relevance of the results obtained to clinical nutrition was limited; therefore, we tested in the present study whether less drastic variations of dietary factors can modulate in human beings lipogenesis in adipose tissue as in liver. To achieve this aim, we compared the responses of human liver and adipose tissue lipogenesis to short-term oral ingestion of glucose and to a 2 week moderate increase in both total energy and carbohydrate dietary intake. This was performed by using a dual tracer method allowing a separate labeling of hepatic and adipose tissue lipogenesis. In addition, the response of the expression of the lipogenic pathway in adipose tissue was determined by measuring the concentrations in subcutaneous adipose tissue of mRNAs for FAS, acetyl-CoA carboxylase 1 (ACC1), the ACC isoform implicated in lipogenesis, and sterol-regulatory element-binding protein-1c (SREBP-1c), the main transcription factor controlling FAS and ACC1 expression (18).

SUBJECTS AND METHODS

Subjects

Written informed consent was obtained from 15 normal subjects after explanation of the nature, purpose, and possible risks of the study. This group consisted of seven women and eight men (aged 19 to 51 years, BMI 19 to 25). No subject had a personal or familial history of diabetes or obesity or was taking any medication; all had a normal physical examination and normal plasma glucose and lipids concentrations (**Table 1**). Subjects with unusual dietary habits were excluded.

Protocols

The protocol was approved by the INSERM and the local ethical committee, and the study was conducted according to the French Hurriet law. Three studies were performed. Studies 1 and 2 were conducted in subjects consuming their usual diets during the previous weeks, while Study 3 was carried out in subjects who consumed during the previous 2 weeks a high-energy (140–150% of energy needs), HC (60–65% of total energy intake), mainly complex carbohydrates (60% of carbohydrates) diet. This diet was provided by a dietitian who met with each subject before the high-energy, HC diet period to obtain a report of the subject's usual diet and to establish the subject's requirements during the controlled diet period. The dietitian met with each subject during and at the end of the controlled diet period. A detailed report of each subject's dietary intake during the last week of the controlled diet period was obtained, and the actual intakes were calculated using the Cuqual tables. A detailed report of the dietary intake during the week preceding the metabolic study was also obtained for each subject studied while consuming his/her usual diet (Studies 1 and 2).

TABLE 1. Dietary intakes during the week preceding the metabolic studies

	Study 1	Study 2	Study 3
Energy intake cal. kg^{-1} day^{-1}	31.8 ± 1.0	28.6 ± 1.1	54.4 ± 4.6[b]
Proteins (% of energy)	15.8 ± 0.4	16.8 ± 0.6	15.7 ± 0.4
CH (% of energy)	49.3 ± 1.1	47.1 ± 0.6	60.7 ± 1.2[b]
Fat (% of energy)	35.5 ± 1.0	36.1 ± 0.7	23.3 ± 0.7[b]
Fibers (g/day)	16.1 ± 1.6	13.1 ± 0.6	26.8 ± 1.5[a]
Fructose (g/day)	10.8 ± 3.4	8.1 ± 2.0	14.0 ± 2.2[a]
SFA (g/day)	32.2 ± 2.1	27.7 ± 2.0	44.2 ± 4.4[a]
MUFA (g/day)	25.3 ± 1.1	24.3 ± 1.0	32.6.± 3.0[a]
PUFA (g/day)	10.6 ± 0.9	9.3 ± 0.4	15.7 ± 1.0[a]
Simple CH/total CH	0.49 ± 0.03	0.49 ± 0.02	0.38 ± 0.02[a]

CH, cholesterol; SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid. Values are mean ± SEM.
[a] $P < 0.05$ versus the other two studies.
[b] $P < 0.01$ versus the other two studies.

The general protocol was the same for the three studies. Tests were initiated at 07:00 h in the postabsorptive state. An indwelling catheter was inserted in a forearm vein for blood sampling. After initial blood sampling and collection of expired gas, a sample of abdominal subcutaneous adipose tissue (150–250 mg) was obtained by needle biopsy under local anesthesia and immediately stored in liquid nitrogen. The subjects then drank a loading dose (LD) of deuterated water (99% MPE) (3 g/l of body water) and [1,2-$^{13}C_2$]acetate (99% MPE) (5 mg/kg of body weight), both from Cambridge Isotopes. Blood samples and expired gas were collected every hour for 12 h. Abdominal subcutaneous adipose tissue samples were collected again at 6 h and 12 h, each time at a different site separated from the previous site(s) by at least 8–10 cm. Respiratory gas exchange was measured from 0 h to 1 h and then from 5 h to 6 h and 11 h to 12 h. During the first study, the subjects (n = 5) remained in the fasting state and drank 2.5 mg/kg of [1,2-$^{13}C_2$]acetate diluted in water enriched (6 g/l) with deuterated water each hour. During the second study, the subjects (n = 5) also ingested glucose (1 g/kg) at 1 h, followed by the ingestion of 20 g glucose every hour until the end of the test. This represented 1,100–1,150 calories/12 h, thus above the resting energy expenditure of the subjects (700 ± 25 calories/12 h). The third study (n = 5) was comparable to the first one except that the subjects were studied after 2 weeks of the HC diet.

Analytical procedures

Metabolites were assayed using enzymatic methods on neutralized perchloric extracts of plasma (glucose) or on plasma (NEFA, TAG). Plasma insulin and glucagon concentrations were determined by radioimmunoassay. Methods for preparing samples for the measurement of deuterium and ^{13}C enrichment in the palmitate of plasma TAG [previous studies showed that comparable results are obtained using either VLDL-TAG or plasma TAG (19)] have been published in detail previously (19, 20). Determinations of deuterium and ^{13}C enrichments were performed on a gas chromatograph coupled with a mass spectrometer (HP5890, Hewlett-Packard, Palo Alto, CA) operating in the electronic impact ionization mode (70 eV). Ions 270 to 273 were selectively monitored. The ratio 271:270 was used to measure deuterium enrichment since, at the deuterium enrichment level obtained in body water, deuterium incorporation in the molecules synthesized produces only singly labeled palmitate (19). The ratio 272:270 was used to calculate ^{13}C enrichment since incorporation of [1,2-$^{13}C_2$]acetate gives palmitate labeled with two ^{13}C. Since any increase in the 271:270 ratio results in a rise in the 272:270 ratio, this contribution was subtracted from the measured 272:270 in order to obtain the true enrichment in ^{13}C (21). These measurements of deuterium and ^{13}C enrichments in the palmitate of plasma TG assume that there was no significant recycling in liver of [1,2-$^{13}C_2$]acetate resulting in the appearance of singly labeled acetate molecules. Deuterium enrichment in

plasma water (IEw) was measured by the method of Yang et al. (22). For all the enrichments measured by gas chromatography-mass spectrometry, the lowest enrichment measurable was 0.1%. ^{13}C enrichment in the CO_2 of expired gas was measured by isotope ratio mass spectrometry (IRMS) (23).

Since labeled TAG incorporated in adipose tissue was diluted by a large pool of unlabeled TAG, deuterium and ^{13}C enrichment were measured by IRMS. Briefly, adipose tissue lipids were extracted using the Folch procedure (24), and TAG was purified by thin-layer chromatography, dried under nitrogen, and transferred into 18 cm sealed combustion tubes (Vycor, Corning Glass Works, NY). Cupric oxide (0.5 g) and a 2 cm length of silver wire were added and tubes sealed at less than 20 mtorr pressure. TAG samples were then combusted to ^{13}C-enriched CO_2 and deuterium-enriched water for 4 h at 520°C. The generated CO_2 was transferred under vacuum into Vycor tubes for measurement of ^{13}C enrichment. ^{13}C enrichment in CO_2 was then measured by IRMS (SIRA 12, Isomass, Cheshire, UK). Deuterium-enriched water was vacuum distilled into sealed tubes containing 60 mg zinc reagent and reduced to deuterium-enriched hydrogen gas at 520°C for 30 min. Deuterium enrichment was measured by IRMS using a manually operated dual inlet system (VG Isomass 903D, Cheshire, UK). The lowest enrichment measurable by IRMS for deuterium and ^{13}C was 0.5 δ per mille above reference value.

mRNA measurements

Procedures for the extraction of total RNA and the measurements of mRNA concentrations of FAS, ACC1, and SREBP-1c have been described previously (11, 25, 26).

Calculations

Carbohydrate and lipid oxidation rates were calculated from respiratory gaseous exchange and urinary nitrogen excretion data (27). The fractional contribution (FSR) of lipogenesis to the plasma TAG pool was calculated from the deuterium enrichments in TAG palmitate and in plasma water as previously described (19, 20). An important assumption (28), and possible limitation, in these calculations of lipids synthesis is that the number of incorporation sites of deuterium in the molecules synthesized is not significantly modified by the metabolic state. Hepatic lipogenesis was not calculated from ^{13}C incorporation since, with the dose of labeled acetate used, there was no measurable apparition of molecules of palmitate labeled with two labeled acetates, and therefore mass isotopomer distribution analysis could not be used to calculate ^{13}C enrichment in the lipogenic pool of hepatic acetyl-CoA.

Fat-free mass (FFM) was calculated from the LD of deuterated water ingested, and the IEw as FFM = (LD-IEw)−0.732 (29). The ratios of deuterium over ^{13}C enrichment in TAG of adipose tissue and in palmitate of plasma TAG were calculated. These ratios were used to differentiate the direct (through in situ DNL) labeling of adipocytes TAG from indirect labeling (through uptake of FAs synthesized and released by the liver in VLDL TAG). This ratio approach used the following modeling assumptions. Deuterated water equilibrates in the whole-body pool of water. Acetate, on the contrary, is efficiently taken up and produced by the liver, and during oral administration of ^{13}C acetate, the enrichment in peripheral blood is considerably less than in portal blood (30). Therefore, hepatic lipogenesis incorporates in the synthesized palmitate both deuterium and ^{13}C, and produces VLDL-TAG labeled by both tracers. On the contrary, adipose tissue lipogenesis, if active, will give FA labeled only with deuterium. Comparison of the deuterium and ^{13}C enrichments measured in circulating TAG and in the TAG of abdominal subcutaneous adipose tissue obtained by needle biopsy thus permits determination of whether adipose tissue lipogenesis is active (higher deuterium-^{13}C enrichment ratio in adipose tissue than in circulating TAG) or not (comparable ratio).

All results are shown as means ± SEM. Comparisons were performed with one-way (between-test comparison) or two-way (within-test comparisons) ANOVA followed by Student's *t*-test to locate the differences.

RESULTS

Dietary intake

There was no difference between the dietary intakes preceding Studies 1 and 2. As expected, total energy intake and the contribution of carbohydrate to this intake were largely increased during the 2 weeks preceding Study 3, while the contribution of fat was decreased ($P < 0.01$ for all). The increase in carbohydrate intake was associated with a parallel increase in fibers and fructose intakes ($P < 0.05$). Absolute fat intake was also moderately increased during the HC, this increase being significant ($P < 0.05$) for saturated, monounsaturated, and polyunsaturated FAs.

Hormones and metabolites values

In the postabsorptive state, there were no significant differences between the three groups of subjects despite the expected trend for lower NEFA and increased insulin and TAG concentrations in the subjects of Study 3 (**Table 2**). In addition, these subjects gained body weight ($P < 0.05$) during the 2 weeks of controlled HC (mean body weight gain: 1.5 kg).

Figures 1 and **2** show the evolutions of glucose, insulin, NEFA, and TAG during the three tests. As expected, both glucose and insulin were markedly higher and NEFA lower ($P < 0.01$) during Study 2 (with oral glucose ingestion) than during the two other studies. The evolution of these parameters, with a progressive decline in insulin and rise in NEFA, was comparable for Studies 1 and 3, although insulin was slightly higher ($P < 0.05$) at time 660 min and 720 min of Study 3 (after HC). The evolution of TAG was comparable during the three studies, with a trend for higher values throughout the whole test in Study 3. As expected, respiratory quotient decreased throughout the tests during Studies 1 and 3 and increased during the tests of Study 2. The initial values during Study 3 were higher ($P < 0.05$) than during the two other series of tests. Carbohydrate oxidation rates decreased while lipid oxidation increased throughout the tests during Studies 1

TABLE 2. Hormone and metabolite concentrations measured in the initial, postabsorptive state during the three studies

	Study 1	Study 2	Study 3
Insulin pmol/l	40 ± 4	53 ± 5	60 ± 8
Glucagon ng/l	176 ± 14	143 ± 9	157 ± 4
Glucose mM	3.84 ± 0.15	4.05 ± 0.17	4.00 ± 0.12
NEFA μM	395 ± 23	565 ± 68	317 ± 47
TAG mM	0.57 ± 0.03	0.53 ± 0.05	0.79 ± 0.14

TAG, triacylglycerol. Results are shown as mean ± SEM.

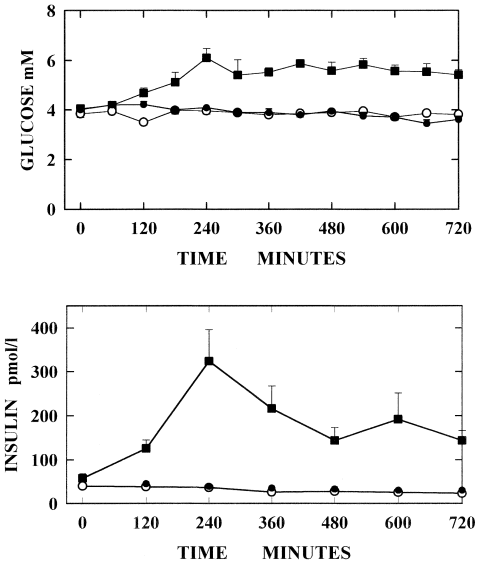

Fig. 1. Evolution of the concentrations of glucose (upper panel) and insulin (lower panel) throughout the 12 h of the three studies [normocaloric diet (NC), no oral glucose: open circles; NC, with oral glucose: closed squares; high-energy, high-carbohydrate diet (HC): closed circles].

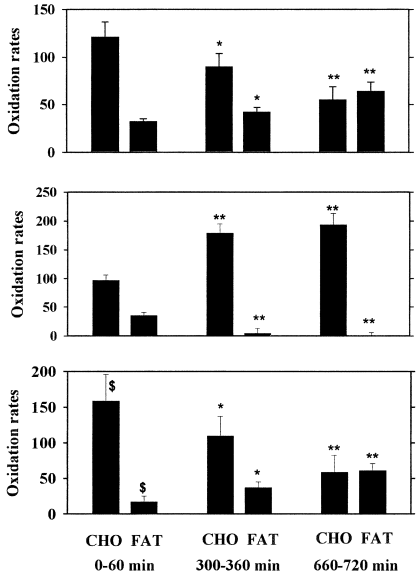

Fig. 3. Lipid and carbohydrate oxidation rates during the three studies (upper panel: NC, no glucose; middle panel: NC with oral glucose; lower panel: HC). Values are in mg/kg/h. *$P < 0.05$, ** $P < 0.01$ versus the initial (0–60 min) values; $ $P < 0.05$ versus the corresponding values of the other two studies.

and 3 (**Fig. 3**). Oral glucose ingestion (Study 2) induced a rise in carbohydrate oxidation and a fall in lipid oxidation rates.

Deuterium and ^{13}C IE and in plasma and adipose tissue TAG

Figure 4 shows deuterium enrichment of plasma water and ^{13}C enrichment in the CO_2 of expired gas during the three studies. Plateau values of deuterium enrichment were obtained for all studies. The kinetics of ^{13}C enrichment in expired gas were identical, showing that ^{13}C acetate absorption and oxidation during the three tests were comparable. The values for deuterium and ^{13}C enrichment in plasma and adipose tissue TAG are shown in **Table 3**. During Study 1, these values in plasma TAG decreased between 6 h and 12 h, and hepatic lipogenesis, calculated from the deuterium enrichments, decreased ($P < 0.05$) (**Table 4**). During Study 2 (with oral ingestion of glucose),

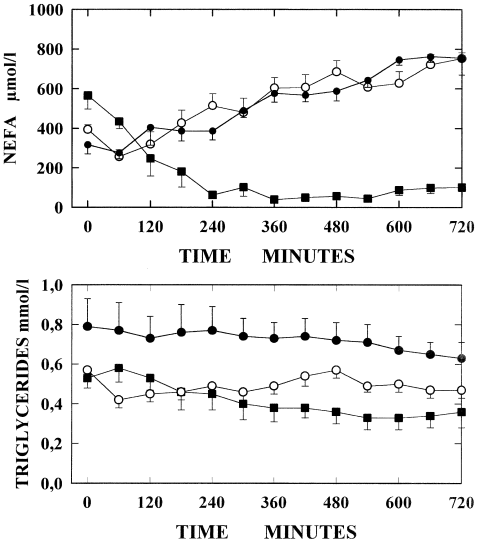

Fig. 2. Evolution of the concentrations of plasma NEFA (upper panel) and triacylglycerol (TAG) (lower panel) throughout the 12 h of the three studies (NC, no oral glucose: open circles; NC, with oral glucose: closed squares; HC: closed circles).

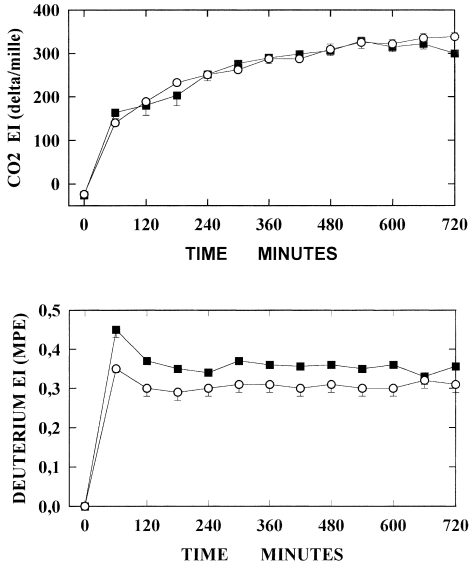

Fig. 4. Evolution of the enrichments of ^{13}C in CO_2 of expired gas (upper panel) and of deuterium in plasma water (lower panel) throughout the 12 h of the three studies (NC, no oral glucose: open circles; NC with oral glucose: closed squares; HC diet: closed circles).

TABLE 3. ^{13}C and deuterium enrichments in plasma TAG palmitate and in adipose tissue TAG

	Study 1		Study 2		Study 3	
	6 h	12 h	6 h	12 h	6 h	12 h
Plasma						
^{13}C (MPE)	0.26 ± 0.04	0.18 ± 0.02	0.96 ± 0.34	1.46 ± 0.53	1.83 ± 0.70	0.80 ± 0.28
Deuterium (MPE)	0.23 ± 0.02	0.18 ± 0.02	0.30 ± 0.09	0.44 ± 0.11	0.77 ± 0.22	0.41 ± 0.12
Adipose tissue						
^{13}C (δ per ml)	0	0	0	0	0	0
Deuterium (δ per ml)	0	11.4 ± 4.6	0.6 ± 0.4	5.6 ± 1.9	3.7 ± 0.8	22.0 ± 1.0

MPE, mol% excess. Enrichments in plasma samples are shown as MPE and in adipose tissue TAG as δ per milliliter.

deuterium enrichment in plasma TAG was slightly higher at 6 h than in Study 1, and increased further at 12 h. Therefore, hepatic lipogenesis increased ($P < 0.05$) during the test instead of decreasing, and was at 12 h higher ($P < 0.05$) than in Study 1 (Table 4). ^{13}C enrichment of plasma TAG was also higher than in Study 1, and increased between 6 h and 12 h, confirming thus the increase in hepatic lipogenic rate. During Study 3 (after the HC), both ^{13}C and deuterium enrichments were higher at 6 h than during the two other studies ($P < 0.05$), but the values declined thereafter, as in Study 1. At 12 h, the values, although always higher than in Study 1, were comparable to those observed at the end of Study 2. Hepatic lipogenesis was at 6 h greater ($P < 0.05$) than during the other two studies (Table 4), and declined after to values comparable to those of the end of Study 2. A quantitative estimate of hepatic lipogenesis can be obtained if we assume a secretion rate of TAG (TAGrt) by the liver of about 0.13–0.15 mg/kg/min (7, 20) as: lipogenesis (g/12 h) = TAGrt.lipogenesis(%).body weight(kg).720(min). The value used in this calculation for lipogenesis(%) is the average of the values obtained at 6 h and 12 h. Hepatic lipogenesis would represent during the 12 h of testing a net production of 0.21 ± 0.04 g, 0.33 ± 0.16 g, and 0.58 ± 0.22 g ($P < 0.05$ vs. Study 1) during Studies 1, 2, and 3, respectively.

During the three studies, there was no detectable appearance of excess ^{13}C in adipose tissue TAG either at 6 h or at 12 h. On the contrary, deuterium abundance increased during the three studies; this enrichment was slightly more important during Study 3 than during the two other studies. Therefore, the ^{13}C-deuterium enrichment ratio in adipose tissue was 0 at the end of the three studies. Since in situ lipogenesis was the only detectable source of TAG labeling by deuterium in adipose tissue, we used this deuterium enrichment to estimate the FSR of adipose tissue lipogenesis to adipose TAG stores during the 12 h of the test. These FSRs were 0.00011 ± 0.00004, 0.00004 ± 0.00001, and 0.00014 ± 0.00004 for Studies 1, 2, and 3, respectively. These FSRs were then converted in estimates of absolute synthetic rates (ASRs) using the total body fat mass (FM) calculated from the dilution of deuterated water, as a measure of TAG stores: ASR = FSR.FM. Adipose tissue lipogenic rates were estimated to be 0.27 ± 0.14 g, 0.16 ± 0.05 g, and 0.41 ± 0.19 g during Studies 1, 2, and 3, respectively (no differences between the three studies despite a trend for higher values in Study 3). These values are comparable to those obtained for hepatic lipogenesis for Study 1, but lower ($P < 0.05$) during Study 2 (acute stimulation by oral glucose) and Study 3 (2 weeks overfeeding). These values for adipose and liver lipogenesis should be considered as estimates rather than truly quantitative, but, considering both the fractional values and these quantitative estimates, adipose tissue lipogenesis clearly appears poorly responsive to both acute and chronic stimulation by carbohydrate ingestion, contrary to liver lipogenesis. It is also clear that the sum during Study 2 of hepatic and adipose lipogenesis (0.5–1 g) was low compared with the total amount of glucose ingested (260–280 g) and of total carbohydrate oxidation rate calculated from indirect calorimetry (around 120 g). This sum also represented only a minor pathway for the disposal of the large amount of carbohydrate ingested by the subjects during the HC diet.

TABLE 4. Hepatic lipogenesis during the three studies (% of the circulating TAG pool)

	6 h	12 h
Study 1	3.57 ± 0.50	2.77 ± 0.32[a]
Study 2	4.01 ± 1.27	5.84 ± 1.33[a,c]
Study 3	10.61 ± 3.29[b]	5.40 ± 1.18[a,c]

[a] $P < 0.05$ versus the value at 6 h of the same study.
[b] $P < 0.05$ versus the 6 h values of the two other studies.
[c] $P < 0.05$ versus the 12 h value of Study 1.

Adipose tissue mRNA concentrations

mRNA concentrations measured at 0 h and 12 h are shown in **Table 5**. FAS mRNA decreased during Study 1 ($P < 0.05$). When oral glucose was given, these concentrations remained at the initial values. After 2 weeks of hypercaloric HC feeding, the initial values of FAS mRNA were not modified, and decreased again ($P < 0.05$) during the 12 h of the test. There were no significant variations of ACC1 mRNA values during Studies 1 and 2. The values observed at 0 h and 12 h during Study 3 were lower than the corresponding values of the two other studies. SREBP-1c mRNA levels were at time 0 h slightly decreased ($P < 0.05$) in Study 3 compared with Studies 1 and 2. These values decreased at 12 h in Study 1 ($P < 0.05$ vs. time 0), and remained at basal levels in Study 2. The decline in Study 3 failed to reach significance ($P = 0.15$).

DISCUSSION

We compared the response of liver and adipose tissue lipogenesis to acute and chronic stimulation by carbohydrate ingestion. Hepatic lipogenesis was, in agreement with previous studies (7, 8, 31), clearly responsive to both acute and chronic stimulation while adipose tissue DNL appeared, on the contrary, poorly responsive. This conclusion is based on several lines of evidence. First, although oral glucose ingestion in Study 2 prevented the decrease in both FAS and SREBP-1c mRNA concentrations observed in Study 1, there was no increase in these concentrations or in ACC1 mRNA value. This strongly suggests that the expression of lipogenic genes, and presumably the activity of the corresponding proteins, were only minimally stimulated in adipose tissue by the 12 h rise in plasma insulin and glucose. Measurements of protein amounts and enzyme activity were precluded by the small amount of adipose tissue obtained by needle biopsy. However, there is no short-term regulation of FAS activity, and changes in activity are linked to changes in mRNA level (32). With respect to SREBP-1c, the present evidence is that its main regulation is at the transcriptional level and that its activation by proteolytic cleavage is probably a constitutive, nonregulated process (33). The present in vivo result contrasts with in vitro studies showing a stimulation of FAS expression and activity by insulin in human adipocytes (12). Second, FAS, ACC1, and SREBP-1c mRNA levels were not increased, but rather decreased, during the HC, suggesting strongly that the lipogenic pathway was not stimulated in adipose tissue by carbohydrate overfeeding. Third, these results are consistent with the tracer-derived estimates of adipose tissue lipogenesis we obtained. The comparison of deuterium and ^{13}C enrichments in plasma and adipose tissue TAG clearly shows that adipose tissue lipogenesis was active under the three situations studied. The lack of detectable enrichment in ^{13}C of adipose TAG does not exclude that some uptake of TAG-FA occurred, but shows that this uptake, if present, is low and does not contribute to the deuterium enrichment measured in adipose TAG. There was, however, no stimulation of lipogenesis in adipose tissue by acute glucose oral load, and only a nonsignificant trend for a higher lipogenic rate after chronic carbohydrate overfeeding. As a result, adipose tissue lipogenesis, which was quantitatively comparable to liver lipogenesis in the absence of stimulation (Study 1), became less important during either acute or chronic stimulation by carbohydrate feeding. Overall, our results agree with those of previous studies that used the incorporation in adipose tissue lipids of ^{14}C from glucose to estimate lipogenesis (4, 5, 15, 34). We therefore think that the stimulation of adipose tissue lipogenesis by massive carbohydrate overfeeding suggested by the study of Aarsland et al. (8) is, if real, present only during such extreme, unphysiological conditions.

The regulation of adipose tissue lipogenesis therefore appears to be different in human beings compared with some other mammalian species. In rats, for example, adipose tissue lipogenesis is more active and is, as is liver lipogenesis, responsive to high insulin/glucose levels and to variations of carbohydrate intake (35, 36). SREBP-1c plays a major role in the regulation of lipogenic genes expression, at least in their response to insulin (18, 37, 38). This transcription factor is a major determinant of the lipogenic capacity of mammalian and avian tissues (39). Therefore, it is possible that SREBP-1c expression is low in human adipose tissue compared with other species, but such a comparison between human beings and other species has not been performed to our knowledge. The lack of response in our study of adipose tissue FAS and ACC1 expression and lipogenesis to acute or chronic carbohydrate overfeeding could be related to the lack of increase of SREBP-1c mRNA. Since SREBP-1c expression is stimulated in rats by dietary carbohydrates and/or insulin (18, 40, 41), it remains to be determined why this stimulation is absent in human adipose tissue.

The comparison of the quantitative estimate of hepatic and adipose lipogenesis during Study 2 with the amount of glucose ingested (less than 1 g vs. 250–270 g) shows that the contribution of this metabolic pathway to the disposal of ingested glucose was minimal. The increase of hepatic and adipose lipogenesis after a hypercaloric HC diet (1–1.5 g/day) was also moderate, and can thus explain only a minimal part of the weight gain of the subjects (1,500 g on average during the 2 weeks of controlled diet). Since liver biopsies for measurement of tracer incorporation in liver TAG were not performed for obvious ethical reasons, we cannot exclude the possibility that some newly synthesized FAs remained within liver TAG stores. However, it seems very unlikely that an increase in liver TAG stores could explain a large part of the disposal of ingested glucose in Study 2 and of the weight gain observed during the HC diet. An increase in lipogenesis in another tissue is unlikely. Therefore, the most probable explana-

TABLE 5. mRNA concentrations (attomol/μg total RNA) in adipose tissue

	Study 1		Study 2		Study 3	
	0 h	12 h	0 h	12 h	0 h	12 h
FAS	47.1 ± 5.6	26.6 ± 6.9[a]	47.9 ± 9.1	55.2 ± 12.8	32.5 ± 10.5	10.1 ± 2.5[a]
ACC1	13.8 ± 4.8	27.5 ± 4.2	32.0 ± 4.8	18.2 ± 1.6	7.1 ± 1.6[b]	2.4 ± 1.0[b]
SREBP-1c	8.9 ± 3.0	1.5 ± 0.4[a]	6.6 ± 1.7	5.6 ± 0.4	3.5 ± 0.8[b]	1.9 ± 0.8

ACC1, acetyl-CoA carboxylase 1; FAS, fatty acid synthase.
[a] $P < 0.05$ versus the value at 0 h of the same study.
[b] $P < 0.05$ versus the values at 0 or 12 h of the two other studies.

tions for the observed body weight increase are merely a repletion of muscles and liver glycogen stores, along with the simultaneous storage of water and the suppression of fat oxidation leading to the deposition of ingested fat. Moreover, although the contribution of fat to total energy intake was decreased during the HC diet, the total amount ingested was increased.

In conclusion, our results show that in normal humans, adipose tissue lipogenesis, although active, is quantitatively a minor pathway and is less responsive than hepatic lipogensis to acute or prolonged carbohydrate overfeeding. The picture could be different in obesity, but the recent finding that lipogenic gene expression is decreased in the adipose tissue of obese subjects (11) makes this possibility improbable. Thus, DNL in adipose tissue is an unlikely contributor to the development of dietary-induced obesity in humans.

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REFERENCES

1. Large, V., L. Hellstrom, S. Reynisdottir, F. Lonnqvist, P. Eriksson, L. Lannfelt, and P. Arner. 1997. Human beta-2 adrenoceptor gene polymorphisms are highly frequent in obesity and associate with altered adipocyte beta-2 adrenoceptor function. *J. Clin. Invest.* **100:** 3005–3013.
2. Large, V., S. Reynisdottir, D. Langin, K. Fredby, M. Klannemark, C. Holm, and P. Arner. 1999. Decreased expression and function of adipocyte hormone-sensitive lipase in sub-cutaneous fat cells of obese subjects. *J. Lipid Res.* **40:** 2059–2066.
3. Binnert, C., C. Pachiaudi, M. Beylot, D. Hans, J. Vandermander, P. Chantre, J. Riou, and M. Laville. 1998. Influence of human obesity on the metabolic fate of dietary long and medium-chain triacylglycerols. *Am. J. Clin. Nutr.* **67:** 595–601.
4. Bjorntorp, P., and L. Sjostrom. 1978. Carbohydrate storage in man: speculations and some quantitative considerations. *Metabolism.* **27:** 1853–1865.
5. Acheson, K., Y. Schutz, T. Bessard, E. Ravussin, E. Jéquier, and J. Flatt. 1984. Nutritional influences on lipogenesis and thermogenesis after a carbohydrate meal. *Am. J. Physiol.* **246:** E62–E70.
6. Parks, E. 2002. Dietary carbohydrate's effect on lipogenesis and the relationship of lipogenesis to blood insulin and glucose concentrations. *Br. J. Nutr.* **87:** S247–S253.
7. Parks, E., R. Krauss, C. M. P. R. Neese, and M. Hellerstein. 1999. Effects of a low-fat, high carbohydrate diet on VLDL-triglyceride assembly, production, and clearance. *J. Clin. Invest.* **104:** 1087–1096.
8. Aarsland, A., D. Chinkes, and R. Wolfe. 1997. Hepatic and whole body fat synthesis in humans during carbohydrate overfeeding. *Am. J. Clin. Nutr.* **65:** 1174–1182.
9. Hudgins, L., M. Hellerstein, C. Seidman, R. Neese, J. Tremaroli, and J. Hirsch. 2000. Relationship between carbohydrate-induced hypertriglyceridemia and fatty acid synthesis in lean and obese subjects. *J. Lipid Res.* **41:** 595–604.
10. Faix, D., R. Neese, C. Kletke, S. Wolden, D. Cesar, M. Countlangus, C. Shackleton, and M. Hellerstein. 1993. Quantification of menstrual and diurnal periodocities in rates of cholesterol and fat synthesis in humans. *J. Lipid Res.* **34:** 2063–2075.
11. Diraison, F., E. Dusserre, H. Vidal, M. Sothier, and M. Beylot. 2002. Increased hepatic lipogenesis but decreased expression of lipogenic gene in adipose tissue in human obesity. *Am. J. Physiol.* **282:** E46–E51.
12. Claycombe, K., B. Jones, M. Standridge, Y. Guo, J. Chun, J. Taylor, and N. Moustaid-Moussa. 1998. Insulin increases fatty acid synthase gene transcription in human adipocytes. *Am. J. Physiol.* **274:** R1253–R1259.
13. Moustaid, N., B. Jones, and J. Taylor. 1996. Insulin increases lipogenic enzyme activity in human adipocytes in primary culture. *J. Nutr.* **126:** 865–870.
14. Parks, R. 2001. Effect of dietary carbohydrate on triglyceride metabolism in humans. *J. Nutr.* **131:** 2772S–2774S.
15. Marin, P., I. Hogh-Christiansen, S. Jansson, M. Kratkiewxky, G. Holm, and P. Bjorntorp. 1992. Uptake of glucose carbon in muscle glycogen and adipose tissue triglycerides in vivo in humans. *Am. ET J.* **263:** E473–E480.
16. Sjöström, L. 1973. Fatty acid synthesis de novo in adipose tissue from obese subjects on a high-carbohydrate diet. *Scand. J. Clin. Lab. Invest.* **32:** 339–349.
17. Acheson, K., Y. Schutz, T. Bessard, K. Anantharaman, J. Flatt, and E. Jequier. 1988. Glycogen storage capacity and de novo lipogenesis during massive carbohydrate overfeeding in man. *Am. J. Clin. Nutr.* **48:** 240–247.
18. Foretz, M., C. Guichard, P. Ferre, and F. Foufelle. 1999. Sterol regulatory element binding protein-1c is a major mediator of insulin action on the hepatic expression of glucokinase and lipogenesis-related genes. *Proc. Natl. Acad. Sci. USA.* **96:** 12737–12742.
19. Diraison, F., C. Pachiaudi, and M. Beylot. 1997. Measuring lipogenesis and cholesterol synthesis in humans with deuterated water: use of simple gas chromatography mass spectrometry techniques. *J. Mass Spectrom.* **32:** 81–86.
20. Diraison, F., and M. Beylot. 1998. Role of human liver lipogenesis and reesterification in triglycerides secretion and in FFA reesterification. *Am. J. Physiol.* **274:** E321–E327.
21. Peroni, O., V. Large, and M. Beylot. 1995. Measuring gluconeogenesis with [2–13C]glycerol and mass isotopomer distribution anlysis of glucose. *Am. J. Physiol.* **269:** E519–E523.
22. Yang, D., F. Diraison, M. Beylot, Z. Brunengraber, M. Samols, and H. Brunengraber. 1998. Assay of low deuterium enrichment of water by isotopic exchange with [U-13C]acetone and gas chromatography mass spectrometry. *Anal. Biochem.* **258:** 315–321.
23. Guilly, R., E. Billion-Rey, C. Pachiaudi, S. Normand, J. P. Riou, E. S. Jumeau, and J. L. Brazier. 1992. On-line purification and carbon 13 isotopic analysis of carbon dioxide in breath: evaluation of on-line gas chromatography isotope ratio mass spectrometry. *Anal. Chim. Acta.* **259:** 193–202.
24. Folch, J., M. Lees, and G. H. Sloane Stanley. 1957. A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.* **226:** 497–509.
25. Cachefo, A., P. Boucher, C. Vidon, E. Dusserre, F. Diraison, and M. Beylot. 2001. Hepatic lipogenesis and cholesterol synthesis in hyperthyroid patients. *J. Clin. Endocrinol. Metab.* **86:** 5353–5357.
26. Letexier, D., F. Diraison, and M. Beylot. 2003. Addition of insulin to a moderately high-carbohydrate diet reduces hepatic lipogenesis and plasma triacylglycerol concentration in humans. *Am. J. Clin. Nutr.* **77:** 559–564.
27. Frayn, K. N. 1983. Calculation of substrate oxidation rates in vivo from gaseous exhange. *J. Appl. Physiol.* **55:** 628–634.
28. Jones, P. J. 1997. Regulation of cholesterol biosynthesis by diet in humans. *Am. J. Clin. Nutr.* **66:** 438–446.
29. Waki, M., J. G. Kral, M. Mazariegos, J. Wang, R. N. Pierson, and S. B. Heymsfield. 1991. Relative expansion of extracellular fluid in obese vs nonobese women. *Am. J. Physiol.* **261:** E199–E203.
30. Puchowicz, M., I. Bederman, B. Comte, D. Yang, F. David, E. Stone, K. Jabbour, D. Wasserman, and H. Brunengraber. 1999. Zonation of acetate labeling across the liver: implications for studies of lipogenesis by MIDA. *Am. J. Physiol.* **277:** E1022–E1027.
31. Hellerstein, M., M. Christiansen, S. Kaempfer, C. Kletke, K. Wu, S. Reid, N. Hellerstein, and C. Schackleton. 1991. Measurement of de novo lipogenesis in humans using stable isotopes. *J. Clin. Invest.* **87:** 1841–1852.
32. Foufelle, F., J. Girard, and P. Ferré. 1996. Regulation of lipogenic enzyme expression by glucose in liver and adipose tissue: a review of the potential cellular and molecular mechanisms. *Adv. Enzyme Regul.* **36:** 199–226.
33. Foufelle, F., and P. Ferré. 2002. New perspectives in the regulation of hepatic glycolytic and lipogenic genes by insulin and glucose: a

role for for the transcription factor sterol regulatory element binding protein-1c. *Biochem. J.* **366:** 377–391.
34. Marin, P., M. Rebuffe-Scrive, U. Smiyh, and P. Bjorntorp. 1987. Glucose uptake in human adipose tissue. *Metabolism.* **36:** 1154–1160.
35. Fukuda, H., N. Iritani, T. Sugimoto, and H. Ikeda. 1999. Transcriptional regulation of fatty acid synthase gene by insulin/glucose, polyunsaturated fatty acids and leptin in hepatocytes and adipocytes in normal and genetically obese rats. *Eur. J. Biochem.* **260:** 505–511.
36. Assimacopoulos-Jeannet, F., S. Brichard, F. Rencurel, I. Cusin, and B. Jeanrenaud. 1995. In vivo effects of hyperinsulinemia on lipogenic enzymes and glucose transporter expression in rat liver and adipose tissue. *Metabolism.* **44:** 228–233.
37. Koo, S., A. Dutcher, and H. Towle. 2001. Glucose and insulin function through two distinct transcription factors to stimulate expression of lipogenic enzyme genes in liver. *J. Biol. Chem.* **276:** 9437–9445.
38. Foretz, M., C. Pacot, I. Dugail, P. Lemarchand, C. Guichard, X. Leliepvre, C. Berthelier-Lubrano, B. Spiegelman, J. Kim, P. Ferre, and F. Foufelle. 1999. ADD1/SREBP-1c is required in the activation of hepatic lipogenic gene expression by glucose. *Mol. Cell. Biol.* **19:** 3760–3768.
39. Gondret, F., P. Ferré, and I. Dugail. 2001. ADD-1/SREBP-1 is a major determinant of tissue differential lipogenic capacity in mammalian and avian species. *J. Lipid Res.* **42:** 106–113.
40. Hasty, A., H. Shimano, N. Yahagi, M. Amenmiya-Kudo, S. Perrey, T. Yoshikawa, J. Osuga, H. Okazaki, Y. Tamura, Y. Iizuka, F. Shionoiri, K. Ohashi, K. Harada, T. Gotoda, R. Nagai, S. Ishibashi, and N. Yamamda. 2000. Sterol responsive element binding protein 1 is regulated by glucose at the transcriptional level. *J. Biol. Chem.* **275:** 31069–31077.
41. Foufelle, F., B. Gouhot, J. Pegorier, D. Perdereau, J. Girard, and P. Ferre. 1992. Glucose stimulation of lipogenic enzyme gene expression in cultured white adipose tissue. *J. Biol. Chem.* **267:** 20543–20546.