Comparison of the expression and activity of the lipogenic pathway in human and rat adipose tissue

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Abstract Lipogenesis is considered less active in human than in rat adipose tissue. This could be explained by different nutritional conditions, namely high-carbohydrate (HCHO) diet in rats and high-fat (HF) diet in humans. Adipose tissue was sampled (postabsorptive state) in rats and humans receiving HCHO or HF diets, ad libitum fed humans, and obese subjects. We measured *1*) mRNA concentrations of fatty acid synthase (FAS), acetyl-CoA carboxylase 1 (ACC1), sterol regulatory element binding protein 1c (SREBP-1c), and carbohydrate response element binding protein (ChREBP), *2*) SREBP-1c protein, and *3*) FAS activity. FAS, ACC1, ChREBP, and SREBP1-c mRNA concentrations were unaffected by diet in humans or in rats. FAS and ACC1 mRNA levels were lower in humans than in rats ($P < 0.05$). FAS activity was unaffected by diet and was lower in humans ($P < 0.05$). SREBP-1c mRNA concentrations were similar in rats and humans, but the precursor and mature forms of SREBP-1c protein were less abundant in humans ($P < 0.05$). ChREBP mRNA concentrations were lower in humans than in rats. In conclusion, the lipogenic capacity of adipose tissue is lower in humans than in rats. This is not related to differences in diet and is probably explained by lower abundance of SREBP-1c protein. A decreased expression of ChREBP could also play a role.—Letexier, D., C. Pinteur, V. Large, V. Fréring, and M. Beylot. **Comparison of the expression and activity of the lipogenic pathway in human and rat adipose tissue. *J. Lipid Res.* 2003.** 44: **2127–2134.**

Supplementary key words sterol regulatory element binding protein 1c • carbohydrate response element binding protein • fatty acid synthase • messenger ribonucleic acid • acetyl-CoA carboxylase 1 • stable isotopes

Adipose tissue and liver are the two main sites of de novo lipogenesis (DNL), which is the synthesis of fatty acid molecules from nonlipid substrates, mainly carbohydrates. DNL is considered a minor metabolic pathway in humans. Indeed, in healthy humans, hepatic DNL is a minor contributor to the fatty acids used for liver triglyceride (TG) synthesis and secretion (usually less than 1 g/day) (1–4). Although hepatic lipogenesis is largely (2- to 4-fold) increased by high-carbohydrate (HCHO) diets (2, 5–8) and increased in ad libitum-fed obese subjects (3, 9) and in hypertriglyceridemic type 2 diabetic patients (10), its contribution (2–5 g/day) to the total amount of TG available daily remains minor compared with the oral intake (usually >80 g/day in Western countries). The contribution of adipose tissue to fat synthesis in humans is less well defined. The key enzymes for fatty acid synthesis are present in human adipose tissue (11–13), but its actual contribution to whole-body lipogenesis is considered very low and less than that of liver (14–17). This situation appears different from that in rats, in which DNL is considered to occur to a similar extent in the liver and in adipose tissue and to be more active than in humans (12). However, this view of a large difference in the lipogenic activity of adipose tissue between humans and rats has been challenged. First, the usual diet of rats in animal facilities is an HCHO diet, contrary to the usual diet of humans, which is much more rich in fat, at least in Western countries. This could play a role in the difference between the two species, given the well-known stimulatory effect of carbohydrate (CHO) and the inhibitory action of fat on lipogenesis (18, 19). Actually, Swierczynski et al. (20) concluded that the difference between the lipogenic potential of human and rat adipose tissue was only moderate when humans and rats were studied while receiving comparable diets. Second, measurements of whole-body and liver lipogenesis in subjects massively overfed with CHO showed that liver lipogenesis explained only a part of the increase in whole-body lipogenesis (6, 21). This suggested that lipogenesis was stimulated in another tissue, probably adipose tissue. On the other hand, we found no evidence for stimulation of the expression and activity of the lipogenic pathway in adipose tissue of control subjects by either acute glucose load or moderate CHO overfeeding, while hepatic lipogenesis was clearly stimulated (2). Thus, adipose tissue DNL appeared less responsive than liver to physiological variations of CHO intake.

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In the present report, we compared directly the expression (mRNA concentrations) and activity of the lipogenic pathway in human and rat adipose tissues. To determine whether the differences between the two species previously reported were or were not related to differences in diet, rats were studied while receiving an HCHO or high-fat (HF) diet, and humans while receiving either a moderate HCHO or a moderate HF diet. Obese human subjects were also included. Because sterol regulatory element binding protein 1c (SREBP-1c) is a key transcription factor controlling the expression of the lipogenic pathway (18, 22, 23), we measured also its mRNA concentration and the amounts of the precursor and mature forms of its protein. The transcriptional activity of SREBP-1c can be stimulated or inhibited by various cofactors such as Sp1, Nfy, Coup-TF1, Ids, and YY1 (24–28). We measured, therefore, the expression level of these cofactors. Finally, a new transcription factor mediating the stimulatory effect of glucose on the expression of lipogenic genes was recently purified from rat liver and called carbohydrate response element binding protein (ChREBP) (29, 30). To date, the role of ChREBP has been firmly established only in rat and mouse liver and for the L-pyruvate kinase gene. To our knowledge, no data on the presence and possible role of ChREBP in adipose tissue are available. Therefore, we checked for and measured the mRNA concentrations of ChREBP in human and rat adipose tissue.

MATERIALS AND METHODS

Studies in animals

Male Sprague Dawley rats (4 weeks old) were obtained from Iffacredo (l'Arbresle, France). After acclimating to the animal facility, they were separated into two groups. One group (n = 5) received a standard diet (rHCHO) (20% proteins, 10% lipids, 70% starch) and the other group (n = 5) an HF diet (rHF) {20% proteins, 60% lipids [74% PUFA; 20% monounsaturated fatty acid (MUFA); 6% saturated fatty acid (SFA)], 20% starch} for 2 weeks. All rats had free access to food. Rats on the HF diet did not gain more weight than those in the HCHO group during the 2 weeks. For each diet, rats were studied in the postabsorptive state (6 h after food withdrawal). All rats were anaesthetized with pentobarbital (6 μg/100 g body weight). Perirenal adipose tissue was quickly removed and snap frozen in liquid nitrogen. Samples were stored at −80°C until analysis.

Studies in human subjects

Subjects. Written informed consent was obtained from 12 healthy subjects and seven obese patients after explanation of the nature, purpose, and possible risks of the study. The control group consisted of seven women and five men [aged 24 ± 2 years, body mass index (BMI) 21 ± 1]. No control subject had a personal or familial history of diabetes or obesity or was taking any medication; all had normal physical examinations and normal plasma glucose and lipid concentrations (**Table 1**). Subjects with unusual dietary habits were excluded. The obese group consisted of seven women (aged 29 ± 4 years, BMI 42 ± 2) with normal physical examinations except for excessive body weight. All had plasma glucose, lipid, and cholesterol levels within normal values but slightly above values of the control group ($P < 0.05$) (Table 1).

TABLE 1. BMI, hormonal, and metabolic parameters of human subjects measured in the postabsorptive state

	Control Subjects	Obese Subjects	hHCHO Subjects	hHF Subjects
Sex (M/F)	3/4	0/7	2/3	2/3
Age (years)	24 ± 1.99	29.33 ± 4.26	26.8 ± 2.86	23.8 ± 1.47
BMI (Kg/m^2)	21.36 ± 0.76	42.56 ± 2.49*	20.12 ± 0.92	21.3 ± 1.01
Glucose (mM)	4.71 ± 0.14	5.60 ± 0.33*	4.45 ± 0.11	4.47 ± 0.08
Insulin (pM)	54 ± 9	ND	43 ± 7	43 ± 8
Cholesterol (mM)	4.37 ± 0.23	5.51 ± 0.42*	3.96 ± 0.46	4.06 ± 0.45
TG (mM)	0.83 ± 0.08	1.76 ± 0.18*	0.75 ± 0.10	0.66 ± 0.16

TG, triglyceride; BMI, body mass index; ND, not determined; HCHO, high-carbohydrate; HF, high-fat.
* $P < 0.05$ versus all groups.

Protocols. The protocols of the study were approved by the Ethical Committee of Lyon and the Institut National de la Santé et de la Recherche Médicale, and the study was conducted according to the Hurriet law. A first group of seven control subjects was studied once while consuming its usual diet (i.e., fed ad libitum). The other five control subjects were studied twice after 3 weeks of a controlled diet, either an HCHO diet (hHCHO) or an HF diet (hHF), with 4 months between the two studies. The order of the diets was randomized. The hHCHO and hHF diets were isoenergetic. The hHCHO diet provided 55% of total energy as carbohydrate (20–25% simple and 30–35% complex carbohydrate) and 30% as fat (10% SFAs, 10% MUFAs, and 10% PUFAs). The hHF diet provided 45% of total energy as lipids (15% SFAs, 15% MUFAs, and 15% PUFAs) and 40% as carbohydrate (same proportions of simple and complex carbohydrates as in the hHCHO diet). These diets were provided by a dietitian who met with each subject before each diet period to obtain a report of the subject's usual diet and to establish the subject's diet during the HF and HCHO controlled diet period. The dietitian met again with each subject at the end of the controlled diet periods. 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. For women, the test was performed during the first 10 days of the menstrual cycle in order to take into account the known variations of lipogenesis during the menstrual cycle (there are no menstrual variations for cholesterol synthesis) (3). All subjects abstained from alcohol or heavy physical activity the week before the study. The evening before the test, in order to measure hepatic lipogenesis, the subjects drank a loading dose of deuterated water (3 g/kg body water; one-half after the evening meal and one-half at 10 PM). Until the end of the study, they drank only water enriched with ^2H_2O (4.5 g ^2H_2O/l of drinking water). All tests were initiated in the postabsorptive state after an overnight fast. At 7:30 AM, an indwelling catheter was placed in a forearm vein and blood samples were drawn for the various concentration and enrichment measurements. Thereafter, a sample of abdominal subcutaneous (SC) adipose tissue (150–250 mg) was obtained by needle biopsy under local anesthesia and immediately stored in liquid nitrogen.

For obese patients, samples of omental (Om) and abdominal SC adipose tissues were obtained during the surgical placement of an adjustable gastric ring by laparoscopy under general anesthesia and immediately stored in liquid nitrogen until analysis.

Analytical procedures

Metabolites and enrichments. Metabolites were assayed using enzymatic methods on neutralized perchloric extracts of plasma (glucose) or on plasma (TG, cholesterol) (9). Plasma insulin and

glucagon concentrations were determined by radioimmunoassay. Measurements of deuterium enrichment in the palmitate of plasma triglycerides were performed as described in detail previously (1, 9, 31). Deuterium enrichment in plasma water was measured by the method of Yang et al. (32). The contribution of hepatic lipogenesis to the plasma pool of TG was calculated as previously described (1, 9).

Measurements of mRNA concentration. Total RNA was extracted from adipose tissue samples using the RNeasy Mini Kit (Qiagen, Coutaboeuf, France). Concentration and purity were verified by measuring optimal density at 260 and 280 nm. Their integrity was checked by 1% agarose gel electrophoresis (Tebu, Le Peray en Yvelines, France). Id1, Id2, Id3, Nfy γ, Sp1, TF1, and YY1 mRNA concentrations were measured by semiquantitative reverse transcription polymerase chain reaction (RT-PCR) using β-actin as a reference. Primer sequences are shown in **Table 2**. For each target mRNA, RT was performed from 0.04 μg of total RNA with 2.5 U of the thermostable reverse transcriptase (*Tth* DNA polymerase, Promega Corp., Charbonnières France) in 10 mM Tris-HCl (pH 8.3), 90 mM KCl, and 10 mM $MnCl_2$ buffer with 4 pmol deoxynucleoside triphosphate and 15 pmol of the specific antisense primer, in a final volume of 20 μl. The reaction consisted of 10 min at 32°C, then 3 min at 60°C, followed by 15 min at 72°C and 5 min at 99°C. After chilling, 5 μl were used for the PCR reaction. The 5 μl of RT medium was added to 45 μl of PCR mix [10 mM Tris-HCl (pH 8.3), 10 mM KCl, 0.75 mM EGTA, 0.05% Tween®, 5% glycerol, and 25 mM $MgCl_2$] containing 8 pmol of deoxynucleoside triphosphate, 2.5 U of *Taq* polymerase (Invitrogen, Cergy Pontoise, France), 15 pmol of corresponding antisense primers, and 22.5 pmol of sense primers. The PCR conditions were 2 min at 94°C followed by 35 cycles (1 min at 94°C, 1 min at 60°C, 1 min at 72°C) and 10 min at 72°C. The β-actin sense primer was added after five complete cycles of PCR. For the determination of ChREBP mRNA concentration, the number of cycles were 33 and 23 for ChREBP and β actin, respectively. Products were analyzed on agarose gel, post-stained with Gelstar® (Tebu). For quantitation of relative band intensities, scanning was performed with a fluorimager (Molecular Dynamics Instruments, CA), and the ratio of each target to β actin was determined for each sample with Gel Grab software (Clara Vision, Paris, France).

Fatty acid synthase (FAS), acetyl-CoA carboxylase 1 (ACC1), and SREBP-1c mRNA concentrations in human adipose tissue were measured by RT followed by competitive PCR as published previously (9, 33). For measurements of FAS and ACC1 mRNA concentrations in rat adipose tissue, we utilized the same method used with the human adipose tissue measurement, as the primer was designed for use in both species. We verified that the expected decrease in rat liver mRNA concentrations during fasting was observed for FAS (postabsorptive state: 103 ± 25 attomoles/μg total RNA, 24 h fasted: 1.1 ± 0.2) and ACC1 (postabsorptive state: 46 ± 11 attomoles/μg total RNA, 24 h fasted: 5 ± 2). For SREBP-1c mRNA, the competitor and the antisense primer used are the same for rats and humans, but the sense primer is different (see Table 2). We verified also that the expected decrease of SREBP-1c mRNA in the liver of fasted rats was found using this method of measurement (postabsorptive state: 6.9 ± 2.4 attomoles/μg total RNA, 24 h fasted: 0.9 ± 0.2)

Western blot analysis. Amounts of Sp1 and SREBP-1c proteins were quantified by immunoblotting. Frozen tissue samples (~200 mg) were crushed in liquid nitrogen and then homogenized in 0.6 ml of 0.25 M sucrose with 1 mM dithioerythritol, 1 mM EDTA, 20 μg/ml leupeptin, 20 μg/ml antipain, and 1 μg/ml pepstatin A, at pH 7 and 4°C. Fat-depleted infranatants were obtained after centrifugation at 12,000 *g* and 4°C for 3 h. Total protein infranatant was measured using BCA protein assay (Pierce, Rockford, IL). Aliquots of the infranatants (200 μg of total proteins) were adjusted to final concentrations of 0.008% bromophenol blue (w/v), 1% sodium dodecyl sulfate (w/v), and 7% glycerol (v/v) and applied to 8% polyacrylamide gels according to the Laemmli method under reducing conditions (3% β-mercaptoethanol). Prestained precision protein standards (Bio-Rad, Marne-la-coquette, France) were used as references. Afterward, electrophoresis proteins were transferred to nitrocellulose membrane. The blot was incubated in blocking buffer (2 h) and then with a primary antibody (1 h). Finally, the blot was incubated with a phosphatase alkaline-conjugated secondary antibody for 1 h and then briefly incubated with ECF (Amersham Pharmacia Biotechnology, UK). The relative amounts of immunodetectable proteins contained in each lane were determined by scanning with a fluorimager and Gel Grab software. The following primary antibodies were used: a rabbit polyclonal anti-Sp1 antibody (1:500) (Santa Cruz Biotechnology, CA) and a mouse monoclonal anti-SREBP-1c antibody (1:200) (IgG-2A4) produced by cell line (American Type Culture Collection, Manassas, VA) and purified from conditioned medium by protein G-Sepharose affinity chromatography as described by the manufacturer (Amersham Pharmacia Biotech). The primary antibodies were visualized with

TABLE 2. Sequence of primers used for RT-PCR

	Sense	Antisense	Size
	(5′ 3′)		*bp*
β-actin	GACGAGGCCCAGAGCAAGAGA	GGGTGTTGAAGGTCTCAAACA	225
h Id1	TGTCTGTCTGAGCAGAGCGT	CTGATCTCGCCGTTCAGGGT	310
r Id1	ATGAAGGTCGCCAGTAGCAGT	CTGATCTCGCCGTTCAGGGT	391
Id2	AACAGCCTGTCGGACCACAG	TGCAAGGACAGGATGCTGAT	314
Id3	TGCCTGTCGGAACGTAGCCT	CTCCTCTTGTCCTTGGAGAT	304
Nfy γ	AGCAGCAGTGATGCCCAGCAAAG	CTGCTGACCTTCTCCAACCTGCAT	572
Sp1	TAGGAACAGCAACAACTCCCA	TGCACCTGGTATGATCTGTA	408
TF1	GCAAGCACTACGGCCAATTC	AGCTCGCAGATGTTCTCGAT	384
YY1	CAGAAGCAGGTGCAGATCAAGA	TGTGCACAGACGTGGACTCTG	370
h SREBP-1c	GCGGAGCCATGGATTGCAC	CTCTTCCTTGATACCAGGCCC	311
r SREBP-1c	ACGACGGAGCCATGGATTG	TTTGATTGGAGGCCCAGGGG	308
ChREBP	CTGGTGTCTCCCAAGTGGAA	CACCGCTGAAGAGGGAGTCAACCA	702
FAS	GGCCTGGACTCGCTCATGGG	TGGGCCTGCAGCTGGGAGCA	514
ACC1	GTTGCACAAAAGGATTTCAG	CGCATTACCATGCTCCGCAC	504

r, rat; h, human; SREBP-1c, sterol regulatory element binding protein 1c; ChREBP, carbohydrate response element binding protein; FAS, fatty acid synthase; ACC1, acetyl-CoA carboxylase 1.

anti-rabbit (1:10,000) for Sp1 or anti-mouse IgG (1:20,000) for SREBP-1c.

FAS enzymatic activity. The activity of the FAS was determined with the method of Linn (34). Briefly, frozen tissue samples (~100 mg) were crushed in liquid nitrogen and then homogenized in 0.3 ml of 10 mM Tris-HCl (pH 7.4), 1 mM EDTA, 250 mM sucrose, and protease inhibitor cocktail. Fat-depleted infranatants were obtained after centrifugation at 800 *g* and 4°C for 10 min. A second centrifugation was performed on the infranatant at 100,000 *g* and 4°C for 1 h. Sixty microliters of the supernatant was added to 220 μl of 100 mM phosphate buffer (pH 6.5) containing 85 μM acetyl-CoA and 126 μM NADPH in the final concentration. The reaction was started by adding malonyl CoA at a final concentration of 115 μM. The oxidation of NADPH was followed at 340 nm and 37°C for 20 min. The results are expressed as nanomoles of NADPH oxidized/min/mg of tissue (35).

Statistical analysis. The results are shown as means ± SEM. Comparisons were performed with Mann-Whitney nonparametric test, except for the comparisons between the hHCHO and hHF diets (Student *t*-test for paired data).

RESULTS

Metabolite concentrations and hepatic lipogenesis in human subjects

Obese subjects had slightly higher values of glucose, cholesterol, and TG than the other groups of human subjects (Table 1). In agreement with a previous report (36), subjects studied when consuming the hHCHO diet had slightly lower cholesterol concentrations ($P < 0.05$) than when consuming the hHF diet. The contribution of hepatic lipogenesis to the circulating TG pool was, as expected, higher ($P < 0.05$) during the hHCHO diet (12.9 ± 1.6%) than during the hHF diet (6.8 ± 0.5%). The value for subjects studied while consuming their usual diet (8.7 ± 1.3%) was intermediate and not significantly different from the values observed during the hHCHO or hHF diets. These subjects also had contributions of CHO (47 ± 4%) and fat (37 ± 4%) to total energy intake intermediate to those actually obtained in the other two groups.

FAS mRNA concentration and activity in adipose tissue

Compared with the other group (rHCHO diet), rats consuming the rHF diet had a moderate decrease of FAS mRNA concentration, which was of borderline significance ($P = 0.11$) (**Figs. 1**, **2**). FAS activity was unaffected by diet. In humans, FAS mRNA concentrations were not different in the hHCHO and the hHF diet groups, and the values were comparable to that found in the control groups; these concentrations were significantly lower in obese patients, for both the SC and visceral adipose tissues ($P < 0.05$ vs. all other human groups). These mRNA concentrations were lower in normal subjects than in rats consuming the rHCHO or rHF diet ($P < 0.05$). Obese patients had values lower than those in both groups of rats ($P < 0.05$). FAS activity could not be determined in human subjects consuming the HCHO and HF diets due to limitations in the amount of tissue. Activities measured in control subjects and in obese patients were comparable; they were all largely lower ($P < 0.05$) than activities measured in rats (Fig. 2).

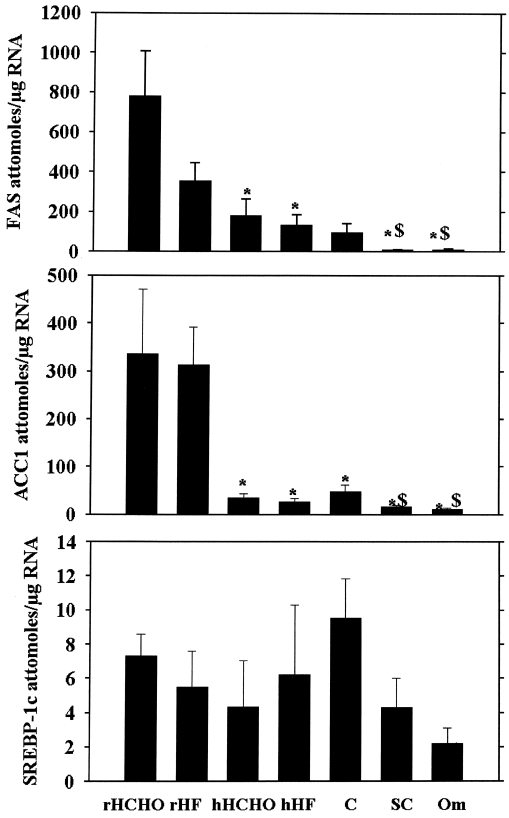

Fig. 1. mRNA concentrations (attomoles/μg of total RNA) of fatty acid synthase (FAS) (upper panel), acetyl-CoA carboxylase 1 (middle panel), and sterol regulatory element binding protein 1c (SREBP1c) (lower panel) in the adipose tissue of rats consuming a high-carbohydrate (HCHO) diet (rHCHO, n = 5) or a high-fat (HF) diet (rHF, n = 5), in subcutaneous (SC) adipose tissue of human subjects fed ad libitum (C, n = 7) or consuming either an HCHO diet (hHCHO, n = 5) or an HF diet (hHF, n = 5), and in SC and omental (Om) adipose tissue of obese human subjects (n = 7). Results are shown as mean and SEM. * $P < 0.05$ versus rHCHO; $ $P < 0.05$ versus control human subject group (C).

ACC1 mRNA concentrations

There were no significant differences between the HCHO and HF diet groups either in rat or in human groups (Fig. 1). The values in the three groups of normal subjects were dramatically lower than in rats ($P < 0.05$). These values were still lower in obese patients ($P < 0.05$ vs. control group for both SC and visceral adipose tissue).

SREBP-1c mRNA concentrations and protein amounts

No difference in mRNA levels was observed according to diet, either in rat groups (rHCHO vs. rHF) or in human groups (hHCHO vs. hHF, and both groups vs. the control group) (Fig. 1). Contrary to what was observed for FAS and ACC1 mRNA levels, there was no difference of SREBP-1c mRNA levels between the adipose tissue of rats and that of normal human subjects. In obese subjects, SREBP-1c mRNA concentrations in SC adipose tissue were comparable to those in rats and normal human subjects but were slightly lower than in rats ($P < 0.05$) for visceral adipose tissue.

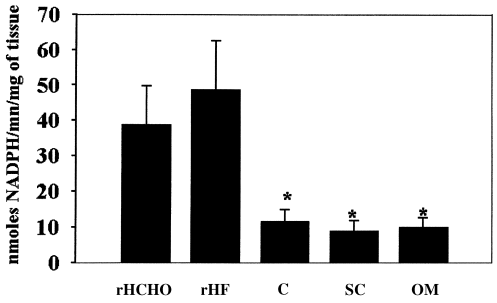

Fig. 2. FAS activity (nmol NADPH oxidized/min/mg of tissue) in adipose tissue of rats consuming an HCHO diet (rHCHO, n = 5) or an HF diet (rHF, n = 5), of human control subjects (C, n = 7), and of obese human subjects (SC and Om adipose tissue, n = 7). Results are shown as mean and SEM. * $P < 0.05$ versus rat rHCHO.

SREBP-1c protein amounts could not be measured in human subjects consuming the HCHO and HF diet due to the small amounts of tissue available. **Figure 3** shows a representative blot obtained in control and obese human samples and in rat adipose tissue samples. In rats, there was an insignificant trend for lower amounts of both the precursor and mature forms of SREBP-1c in the rHF group (**Fig. 4**). Contrary to SREBP-1c mRNA concentrations, both the precursor and mature forms of SREBP-1c protein were clearly decreased ($P < 0.05$) in human adipose tissue compared with values of rats consuming either the HCHO or the HF diet. In control human subjects, the precursor form was barely detectable and could not be quantified. There was no difference between control human and obese adipose tissue (SC and visceral) for the mature form of SREBP1c.

Cofactors of SREBP-1c

We measured the mRNA concentrations of cofactors of SREBP-1c that acted either to stimulate (Sp1, Nfy γ, coup-TF1) or to inhibit (Id1, Id2, Id3, YY1) its transcriptional activity. For all of these cofactors, there was no difference according to diet (rHCHO vs. rHF) in rat groups (data not shown). Coup-TF1 mRNA concentrations were similar in rat and human adipose tissue (data not shown). For the two coactivators, Sp1 and Nfy γ, the mRNA levels were significantly decreased in human groups (control and obese) and compared with rat groups ($P < 0.01$) (**Fig. 5**), with no difference between control and obese human subjects. However, with respect to Sp1, we found no difference in protein amount between rat and human adipose

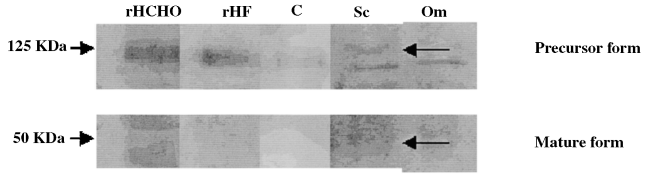

Fig. 3. Representative Western blot of SREBP-1c showing the precursor and mature form in adipose tissue samples from rats fed an HCHO (rHCHO) or an HF (rHF) diet, in control humans (C), and in obese subjects (SC, Om tissue).

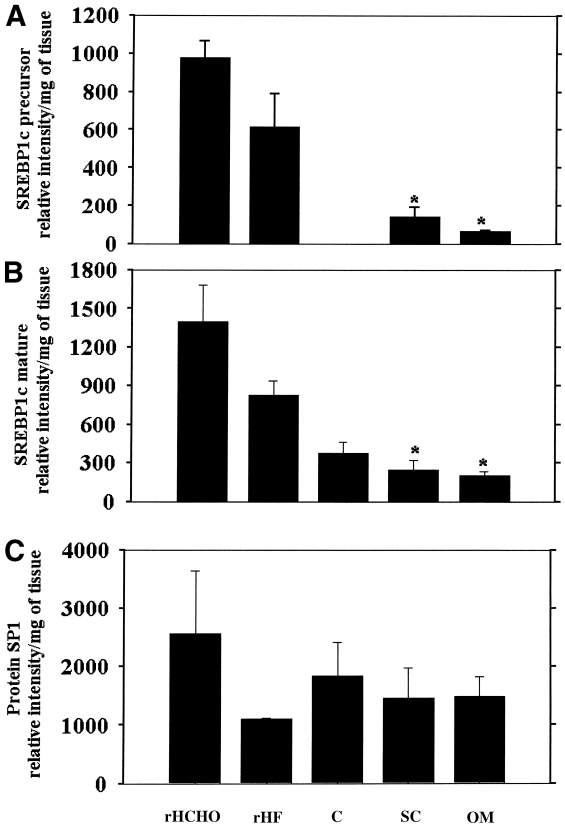

Fig. 4. Results from a Western blot analysis of precursor SREBP-1c (A), mature SREBP-1c (B), and Sp1 (C) proteins (relative intensity/mg of tissue). Measurements were performed in adipose tissue of rats consuming either an HCHO (rHCHO, n = 5) or an HF diet (rHF, n = 5), of human control subjects (C, n = 7), and of obese human subjects (SC and Om adipose tissue, n = 7). Results are shown as mean and SEM. * $P < 0.05$ versus rHCHO.

tissue (Fig. 4). Nfy protein levels were barely detectable by Western blot in either rat or human adipose tissue. Among the possible inhibiting cofactors of SREBP-1c, we found large decreases of the mRNA concentrations of YY1, Id1, and Id3 (Fig. 5) in human adipose tissue (control and obese subjects), whereas the mRNA concentration of Id2 was increased.

ChREBP mRNA

ChREBP mRNA was found in the adipose tissue of rats and humans. In rats, there was a trend for lower values in the HF group (**Fig. 6**). In humans, comparable values were found in the groups of healthy subjects, despite a trend for lower values in the HF group compared with the HCHO group; these values in lean humans were lower than in rats ($P < 0.05$ for each group). Obese patients had lower values than lean subjects, but the difference was significant only in Om adipose tissue ($P < 0.05$).

DISCUSSION

An accurate comparison between human and rat adipose tissue lipogenesis must have similar nutritional con-

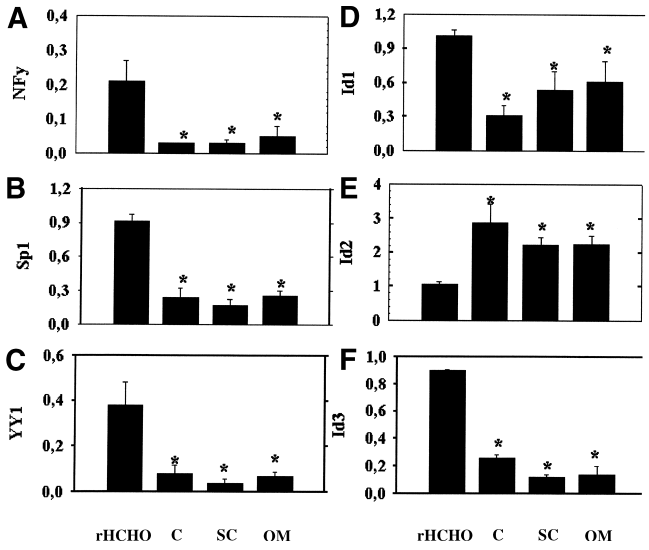

Fig. 5. mRNA (mRNA target/mRNA β actin) concentrations of Nfy (A), Sp1 (B), YY1 (C), Id1 (D), Id2 (E), and Id3 (F) in adipose tissue of rats consuming either an HCHO (rHCHO, n = 5) or an HF diet (rHF, n = 5), of human control subjects (C, n = 7), and of obese human subjects (SC and Om adipose tissue, n = 7). Results are shown as mean and SEM. * $P < 0.05$ versus rHCHO.

ditions for the two species. Most metabolic studies in humans are conducted with subjects in the postabsorptive state (in the morning, 12 h after the last meal). Therefore, we also chose to study rats in the postabsorptive state (i.e., 6 h after food withdrawal) and not, as in most studies, in the fed state and/or after a 24 h or 48 h fast. Moreover, both humans and rats were studied while receiving either an HCHO or an HF diet in order to determine whether the previously reported difference between human and rat adipose tissue lipogenic capacity could be linked, as suggested (20), to differences in diets (HF diet in humans vs. HCHO diet in rats).

We found first that in humans, neither the mRNA concentrations of FAS, ACC1, and SREBP-1c nor the amounts of the precursor and mature forms of SREBP-1c protein were modified by diet. We found that ChREBP mRNA was

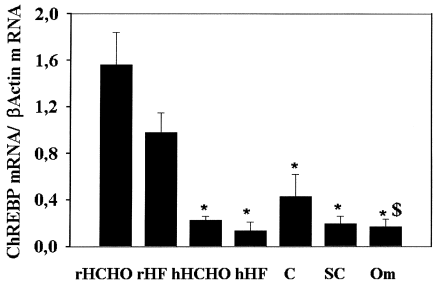

Fig. 6. mRNA (mRNA target/mRNA β-actin) concentrations of carbohydrate response element binding protein in adipose tissue of rats consuming either an HCHO (rHCHO, n = 5) or an HF diet (rHF, n = 5), of lean human subjects fed ad libitum (C, n = 7) consuming an HCHO (hHCHO, n = 5) or an HF (hHF, n = 5), and in obese human subjects (SC and Om adipose tissue, n = 7). Results are shown as mean and SEM. * $P < 0.05$ versus rHCHO and rHF, $ $P < 0.05$ versus C.

present in adipose tissue. This is, to our knowledge, the first evidence that this transcription factor is expressed as well, at least at the mRNA level, in adipose tissue. We observed no significant modification of these mRNA levels by diet. The control of ChREBP activity by glucose and fatty acids in the liver has been clearly demonstrated (30, 37, 38), but little is known on the possible regulation of ChREBP expression by nutrients and/or hormones, except for the finding of a stimulation of ChREBP transcription by glucose in INS-1 insulin-secreting cells (39). Our data do not provide clear evidence for such a regulation in adipose tissue. Hepatic lipogenesis, as measured by the deuterated water method, on the contrary, was clearly responsive to variations in the fat-to-CHO ratio in diet. These results agree with our previous finding that neither an acute oral glucose load nor the feeding of a high-energy HCHO diet stimulated the expression and activity of adipose tissue lipogenesis in normal subjects, while hepatic lipogenesis was stimulated (2). Altogether, these results point to differences between liver and adipose tissue in the regulation of lipogenesis in humans. We also found no significant difference in the expression of lipogenic genes, including ChREBP, in adipose tissue of rats receiving an HCHO or HF diet, despite a more marked variation in the dietary fat-to-CHO ratio than in humans. This contrasts again with the well-demonstrated stimulation and inhibition of hepatic lipogenesis by carbohydrates and PUFAs, respectively (18, 40–43). This lack of variation of adipose tissue lipogenesis in the present report could be linked to our choice of studying rats in the postabsorptive rather than in the fed state. However, some previous studies showed that the response of lipogenic genes to stimulation by insulin/glucose (44), sucrose (45), and HCHO diet (42) and inhibition by PUFA (43, 44) is less important in adipose tissue than in rat liver. Therefore, there also appears to be some difference in the regulation of lipogenesis in these two tissues in rats.

Our main aim was to compare the lipogenic capacity of human and rat adipose tissue. This capacity is clearly reduced in normal humans: FAS and ACC1 mRNA levels were lower, whatever the nutritional conditions, and FAS activity was largely reduced. This adipose tissue lipogenic capacity was also decreased in subjects with long-standing obesity. In agreement with a previous report (9), FAS and ACC1 mRNA concentrations in SC adipose tissue were still lower in obese than in control subjects; these mRNA concentrations were also low in Om adipose tissue, whereas FAS activity was comparable in the adipose tissue of control and obese subjects. SREBP-1c is a key transcription factor controlling the expression of lipogenic genes in liver and adipose tissue (18, 22, 41). SREBP-1c protein is present in two forms: a precursor, membrane-bound form, and a mature form resulting from the proteolytic cleavage of the precursor and the release of the active, N-terminal part of the protein. This mature form is translocated into the nucleus, where it binds to specific sequences in the promoters of lipogenic genes (18). Contrary to what was observed for FAS and ACC1, we found no decrease in SREBP-1c mRNA concentration in control human sub-

jects compared with rats. In agreement with previous studies (9, 46, 47), this SREBP-1c mRNA level was decreased in obese subjects, the decrease being more marked in Om tissue. However, the amounts of both the precursor and mature forms of SREBP-1c protein were largely decreased in the adipose tissue of control and obese subjects compared with rat adipose tissue. This low abundance of SREBP-1c protein probably plays a major role in the decreased lipogenic capacity of human adipose tissue. It remains to be determined why the amount of SREBP-1c protein is decreased despite mRNA levels comparable to those observed in rats.

The transcriptional activity of SREBP-1c is also dependent upon the presence of activating or inhibiting cofactors. The mRNA concentrations of two main activating cofactors, Sp1 and Nfy (25, 27), were decreased in human adipose tissue. However, we found no difference in the protein amount of Sp1, and in both species, the amount of Nfy protein was too low to allow for correct quantification. Moreover, the mRNA level of YY1, a potential inhibitor of SREBP-1c, was also decreased, while those of the various Ids (26) were either decreased (Id1 and Id3) or increased (Id2). Therefore, it is difficult to determine whether these various cofactors play a role in the difference in the expression of the lipogenic pathway between human and rat adipose tissue. Finally, we found that ChREBP mRNA concentrations were clearly lower in human than in rat adipose tissue. Although the role of the transcription factor in the regulation of lipogenic gene expression in adipose tissue remains to be established, the low mRNA level of ChREBP could also be involved in the difference in lipogenic activity between rat and human adipose tissue.

In conclusion, we found that that the lipogenic capacity of adipose tissue is reduced in humans compared with rats. This is not explained by differences in the CHO-to-fat ratio in the diet and appears to be related to a reduced amount of SREBP-1c protein. Differences in the expression of ChREBP could also play a role. Finally, this finding of low lipogenic activity in adipose tissue in humans confirms that most of the TGs stored in adipose tissue are provided by diet and delivered to adipocytes by circulating lipoproteins.

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