Effect of Carbohydrate Overfeeding on Whole Body and Adipose Tissue Metabolism in Humans

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Abstract

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Objective: To evaluate the effect of a 4-day carbohydrate overfeeding on whole body net de novo lipogenesis and on markers of de novo lipogenesis in subcutaneous adipose tissue of healthy lean humans.

Research Methods and Procedures: Nine healthy lean volunteers (five men and four women) were studied after 4 days of either isocaloric feeding or carbohydrate overfeeding. On each occasion, they underwent a metabolic study during which their energy expenditure and net substrate oxidation rates (indirect calorimetry), and the fractional activity of the pentose-phosphate pathway in subcutaneous adipose tissue (subcutaneous microdialysis with $1,6^{13}C_2,6,6^2H_2$ glucose) were assessed before and after administration of glucose. Adipose tissue biopsies were obtained at the end of the experiments to monitor mRNAs of key lipogenic enzymes.

Results: Carbohydrate overfeeding increased basal and postglucose energy expenditure and net carbohydrate oxidation. Whole body net de novo lipogenesis after glucose loading was markedly increased at the expense of glycogen synthesis. Carbohydrate overfeeding also increased mRNA levels for the key lipogenic enzymes sterol regulatory element-binding protein-1c, acetyl-CoA carboxylase, and fatty acid synthase. The fractional activity of adipose tissue pentose-phosphate pathway was 17% to 22% and was not altered by carbohydrate overfeeding.

Discussion: Carbohydrate overfeeding markedly increased net de novo lipogenesis at the expense of glycogen synthesis. An increase in mRNAs coding for key lipogenic enzymes suggests that de novo lipogenesis occurred, at least in part, in adipose tissue. The pentose-phosphate pathway is active in adipose tissue of healthy humans, consistent with an active role of this tissue in de novo lipogenesis.

Key words: de novo lipogenesis, pentose-phosphate pathway, insulin, carbohydrate overfeeding

Introduction

In contrast with hepatic de novo lipogenesis, the quantification of adipose tissue de novo lipogenesis has remained elusive in humans. Although in vitro experiments and rodents studies have been able to document the presence of lipogenic enzymes and the conversion of glucose into fat in insulin-stimulated adipocytes (1–3), the measurement of tracer incorporation into triglycerides (TGs)[1] in adipose tissue in humans in vivo has remained inconclusive, most likely because of the huge dilution of newly synthesized labeled fatty acids in the large pool of adipose lipids (4). However, recent studies provide indirect evidence that extrahepatic de novo lipogenesis occurs in humans, at least under strenuous overfeeding conditions (5,6).

In this study, we studied healthy human volunteers after 4-day periods of either isocaloric feeding or carbohydrate

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[1] Nonstandard abbreviations: TG, triglyceride; SREBP-1c, sterol regulatory element-binding protein-1c; FAS, fatty acid synthase; ACC, acetyl-CoA carboxylase; NADPH, nicotinamide adenine dinucleotide phosphate; FFA, free fatty acid; RT-cPCR, reverse transcription-competitive polymerase chain reaction; NOGD, nonoxidative glucose disposal; EE, energy expenditure; RQ, respiratory quotient.

Table 1. Subjects characteristics*

	At inclusion	Isocaloric feeding	Overfeeding
Body weight (kg)	65.4 ± 1.9	65.5 ± 1.9	67.0 ± 2.1†
Fat mass (kg)	13.8 ± 0.9	13.7 ± 1.0	13.9 ± 0.9†
Fat free mass (kg)	51.5 ± 2.7	51.8 ± 2.7	53.1 ± 2.8

* Mean ± SEM.
† Significantly different from isocaloric feeding.

Table 2. Dietary macronutrient contents*

	Isocaloric feeding	Overfeeding
Energy (kJ/d)	10.2 ± 0.5	17.8 ± 0.8†
Carbohydrate (g)	304 ± 14 (50%)‡	759 ± 35 (71%)†
Protein (g)	91 ± 4 (15%)	91 ± 4 (9%)
Fat (g)	94 ± 4 (35%)	95 ± 4 (20%)

* Mean ± SEM.
† Significantly different from isocaloric feeding (Student's paired *t* test): $p < 0.05$.
‡ Percent energy of macronutrient.

overfeeding. Under each of these two nutritional conditions, we monitored whole body net de novo lipogenesis as well as the expression level of the key lipogenic enzymes [sterol regulatory element-binding protein-1c (SREBP-1c), fatty acid synthase (FAS), and acetyl-CoA carboxylase (ACC)] (7) in subcutaneous adipose tissue biopsies obtained after administration of a large oral glucose load. Because de novo lipogenesis requires nicotinamide adenine dinucleotide phosphate (NADPH) for FAS activity, we also monitored the fractional activity of the NADPH-generating pentose-phosphate pathway in subcutaneous adipose tissue by a technique combining adipose microdialysis and double-labeled glucose (8).

Research Methods and Procedures

Subjects

Nine healthy subjects (five men and four women; age, 26 ± 1 years; BMI, 22 ± 1 kg/m^2) participated in this study (Table 1). Their body composition was assessed by skinfold thickness measurements before and after each diet intervention. The subjects were taking no medication and had no familial history of diabetes and obesity. The study protocol was approved by the Ethical Committee of Lausanne University School of Medicine, and informed written consent from the subjects was obtained for all procedures.

Dietary Conditions

Daily energy requirement was estimated to be equal to 1.6 times the resting metabolic rate measured by indirect calorimetry in fasting conditions. Two controlled dietary interventions were then applied, each lasting 4 days: isocaloric feeding (100% of energy requirement, 50% of total energy as carbohydrate, 35% as lipid, and 15% as protein), or carbohydrate overfeeding (175% of energy requirement, 71% as carbohydrate, 20% as lipid, and 9% as protein). The absolute amount of daily protein and lipids remained identical in these two dietary interventions, which differed only in the amount of daily carbohydrates (Table 2). The diets were prepared at the Institute of Physiology by a registered dietitian (Véronique di Vetta) and were given as normal meals (rice, pasta, crackers, biscuits, cheese, yogurt, creams, etc.). All foods and drinks were provided as prepackaged portions, and subjects were instructed not to consume any other food or drink. The last two meals of each 4-day period were consumed at the Institute of Physiology, where the subjects stayed and slept the day preceding the metabolic studies. The order of the two dietary interventions was randomized, and each intervention was separated by at least 2-week intervals. In female subjects, metabolic studies were performed within 7 days of the beginning of their last menstrual period.

Experimental Protocol

The experimental protocol is described in Figure 1. Controlled diets were consumed from days 1 to 4. In the morning of the fifth day, an indwelling venous cannula was inserted into a wrist vein for hourly withdrawal of arterialized blood samples. A microdialysis catheter (CMA 60; CMA, Stockholm, Sweden) was placed into the subcutaneous abdominal adipose tissue and was infused with saline solution containing 50 mM 1,6 $^{13}C_2$ 6,6 $^{2}H_2$ glucose (MassTrace, Woburn, MA) at a rate of 0.3 μL/min. This infusion started at 8:00 AM (−240 minutes). Indirect calorimetry was performed from 10:00 AM (−120 minutes) until the end of the metabolic study. A lemon-flavored glucose solution (2.5 g glucose/kg body weight) was administered in three divided doses: 50% of the total glucose load at 12:00 AM (0 minutes), 25% at 2:00 PM (120 minutes), and 25% at 3:00 PM (180 minutes). At the end of the metabolic study (5:00 PM; 300 minutes), a biopsy of gluteal subcutaneous adipose tissue was obtained under local anesthesia (1% lidocain) and immediately frozen with liquid nitrogen and kept at −80 °C until analysis.

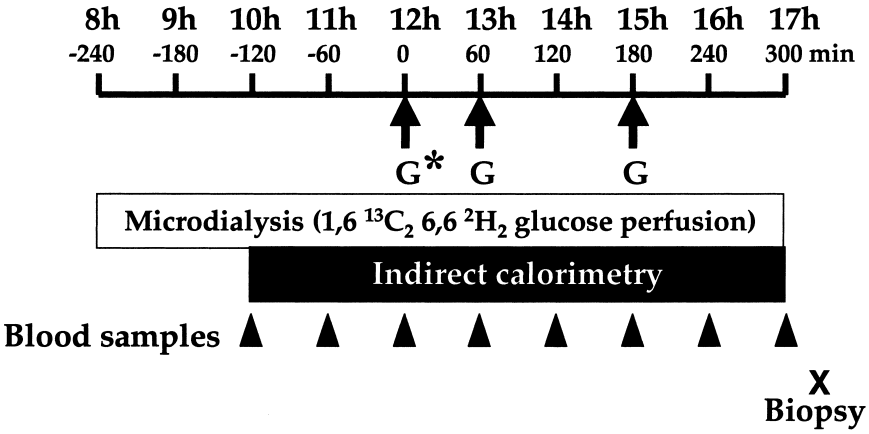

Figure 1: Experimental protocol. After each 4-day dietary intervention, a 9-hour metabolic study was started at 8:00 AM (−240 minutes). 1,6 $^{13}C_2$ 6,6 $^{2}H_2$ glucose was perfused through a microdialysis probe inserted into the subcutaneous adipose tissue. Indirect calorimetry was performed from 10:00 AM to 5:00 PM to monitor energy expenditure and net substrate oxidation. *G, glucose (total dose = 2.5 g/kg body weight) was administered in three divided doses (50% of the dose at time 0, 25% at time 120, and 25% at time 180 minutes). An adipose biopsy was collected at the end of each test.

Analytical Procedure

Plasma glucose concentrations were measured with a Beckman analyzer II (Beckman Instruments, Fullerton, CA). Plasma lactate concentrations were determined enzymatically using a lactate analyzer (Yellow Springs, OH). Plasma insulin (kit from Biochem Immunosystems GmbH, Freiburg, Germany), leptin, and glucagon (kits from Linco, St. Charles, MO) were measured by radioimmunoassay. Plasma free fatty acid (FFA) and TG concentrations were determined by colorimetric methods using a kit from Wako (Freiburg, Germany) for FFAs and from bioMerieux (bioMerieux Suisse S.A., Geneva, Switzerland) for TGs.

Lactate isotopomers in collected dialysates were analyzed by gas chromatography-mass spectrometry in chemical ionization mode with selective monitoring from m/z 219 to m/z 222 after being converted into trimethylsilyl (Me_3Si) derivatives (9).

Competitive Reverse Transcriptase-Polymerase Chain Reaction on Adipose Tissue mRNAs

Biopsy samples (~150 mg) were pulverized in liquid nitrogen, and total RNA was prepared using the RNeasy total RNA kit from Qiagen (Courtaboeuf, France). Average yield of total RNA was 1.5 ± 0.2 μg/100 mg of adipose tissue. The mRNA levels of the target genes (SREBP-1c, FAS, and ACC) were quantified by reverse transcription-competitive polymerase chain reaction (RT-cPCR) (10). The construction of the competitors, the sequences of the primers, the validation of the assays, and the conditions of the RT-cPCR assays have been described in detail previously (11,12). The specific first strand cDNA was synthesized from 0.2 μg of total RNA, and PCR products were analyzed by automated laser fluorescence DNA sequencer (ALFexpress; Pharmacia, Uppsala, Sweden). The concentrations of the target mRNAs were determined at the competition equivalence point as previously described (13).

Calculations

Substrate oxidation, net de novo lipogenesis, and energy expenditure were calculated from VO_2, VCO_2, and urinary nitrogen using the equations of Livesey and Elia (14). Net nonoxidative glucose disposal (net NOGD) and net glycogen synthesis were calculated using the following formula: net NOGD = ingested carbohydrate − carbohydrate oxidation; net glycogen synthesis = net NOGD − glucose used for net de novo lipogenesis (1 g of glucose is used to synthesize 0.35 g of lipids).

Calculation of the activity of the pentose-phosphate pathway was done according to Ben-Yoseph et al. (8). The principles by which fractional pentose-phosphate pathway activity was monitored have been discussed extensively by Ben-Yoseph et al. Briefly, glucose labeled with one heavy atom attached to the first carbon and three heavy atoms attached to the sixth carbon are infused through the microdialysis probe. Label attached to the first carbon is lost when glucose is metabolized in the pentose-phosphate pathway, whereas labels attached to the sixth carbon are retained. Consequently, the ratio of lactate isotopomers labeled with one heavy atom to those labeled with three heavy atoms decreases when fractional pentose-phosphate pathway activity increases.

Statistics

All results were expressed as mean ± SEM. Comparisons of substrate use between groups were made by a Student's paired *t* test. Periodic changes of blood parameters were

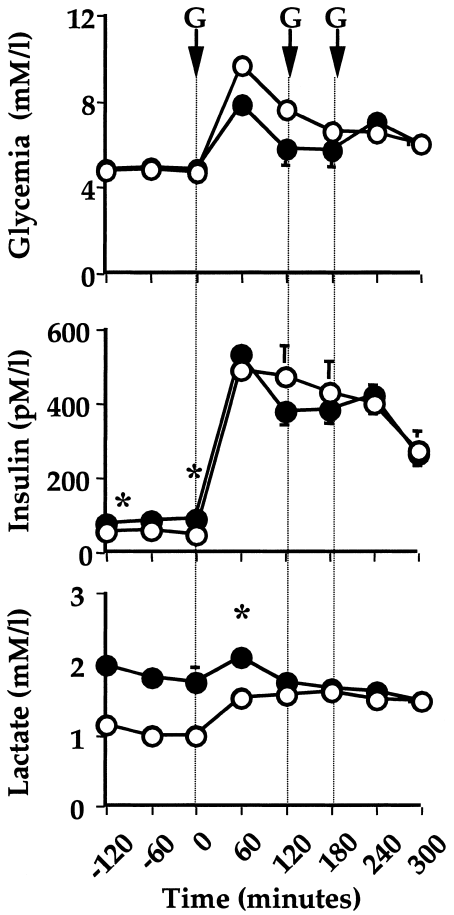

Figure 2: Plasma glucose, insulin, and lactate concentrations after isocaloric feeding (○) or after carbohydrate overfeeding (●) were shown. G+ arrows indicated the glucose administrations. *Significantly different from isocaloric feeding (Student's paired *t* test): $p < 0.05$.

compared by ANOVA with repeated measurements. The mRNA content was analyzed by Wilcoxon signed rank test. All analyses were performed using STATVIEW 4.0 (SAS Institute Inc., Cary, NC).

Results

Plasma Glucose, Lactate, and Hormone Concentrations

The time course of blood substrate and hormone concentrations are shown in Figure 2. Four-day carbohydrate overfeeding resulted in significant increases in basal plasma lactate (from 0.99 ± 0.15 to 1.74 ± 0.20 mM, $p < 0.05$) and insulin (from 47 ± 10 to 90 ± 23 pM, $p < 0.05$). However, glycemia remained the same under each dietary condition. Plasma leptin and glucagon concentrations were not significantly different under the two dietary conditions (glucagon: 62 ± 4 vs. 63 ± 4 ng/L; leptin: 4.7 ± 1.4 vs. 5.3 ± 1.1 μg/L, isocaloric feeding vs. overfeeding). After the oral glucose load, there was no significant difference in plasma glucose, insulin, or glucagon.

Plasma Lipids

After isocaloric conditions, plasma FFA concentrations were moderately increased in basal conditions and were suppressed after glucose ingestion. After carbohydrate overfeeding, basal FFA concentrations were already suppressed and remained low after glucose ingestion. Plasma TG concentration was markedly increased after overfeeding, and this increase persisted until the end of the test (Figure 3).

Substrate Use and De Novo Lipogenesis

Basal energy expenditure (EE), respiratory quotients (RQs), and carbohydrate oxidation were significantly higher after 4 days of carbohydrate overfeeding (EE: 4.8 ± 0.2 vs. 4.5 ± 0.2 kJ/min, $p = 0.019$; carbohydrate oxidation: 22.7 ± 1.3 vs. 10.2 ± 0.7 μmol/kg/min, $p < 0.001$; Figures

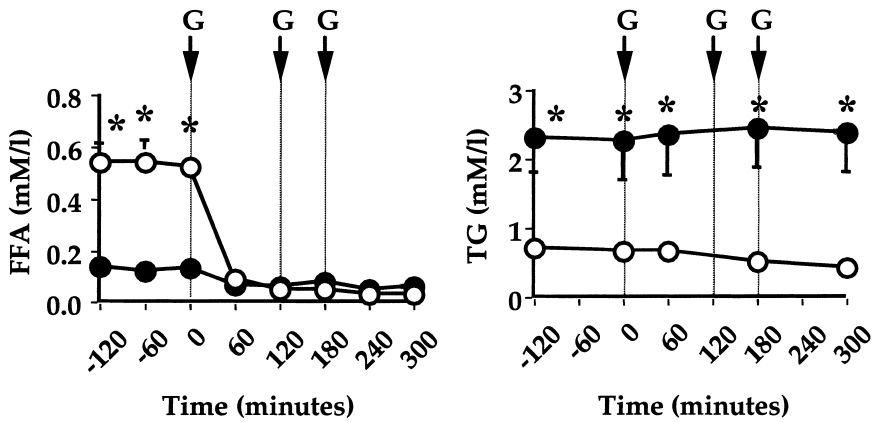

Figure 3: Plasma FFA and TG concentrations after isocaloric feeding (○) or after carbohydrate overfeeding (●) were shown. G+ arrows indicated the glucose administrations. *Significantly different from isocaloric feeding (Student's paired *t* test): $p < 0.05$.

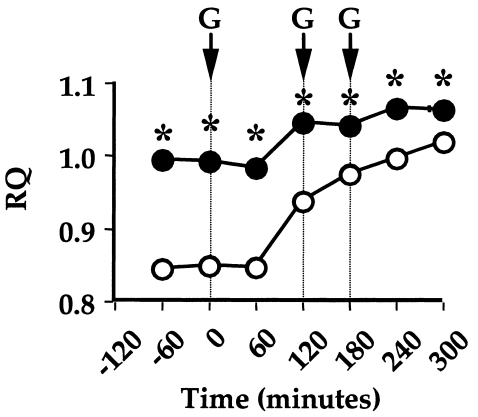

Figure 4: RQs measured by indirect calorimetry after isocaloric feeding (○) or after carbohydrate overfeeding (●) indicate glucose administration. *Significantly different from isocaloric feeding (Student's paired *t* test): $p < 0.05$.

Table 3. Glucose disposal during oral glucose*

g/kg/5 hours	Isocaloric feeding	Overfeeding
Carbohydrate intake	2.49 ± 0.01	2.48 ± 0.02
Carbohydrate oxidation (g glucose)	1.06 ± 0.03	1.44 ± 0.04†
Net NOGD (g glucose)	1.44 ± 0.03	1.07 ± 0.04†
Net glycogen synthesis (g glucose)	1.36 ± 0.04	0.73 ± 0.07†
Net de novo lipogenesis (g lipids)	0.03 ± 0.01	0.12 ± 0.02†

* Values are expressed as mean ± SEM.
† Significantly different from isocaloric feeding (Student's paired *t* test): $p < 0.05$.

4 and 5). Over the 5 hours after ingestion of the glucose load, the differences in EE and RQ were further accentuated (5.3 ± 0.3 vs. 4.8 ± 0.2 kJ/min, $p = 0.003$). Net carbohydrate oxidation remained higher ($p = 0.001$) and net glycogen synthesis was significantly decreased ($p < 0.001$) after carbohydrate overfeeding (Table 3; Figure 5). Net de novo lipogenesis was increased by 296% after overfeeding ($p = 0.001$; Table 3; Figures 4 and 5).

Gene Expression of Lipogenic Enzymes in Adipose Tissue Biopsies

mRNA coding for key lipogenic enzymes was measured in adipose tissue biopsies obtained at the end of the metabolic studies, i.e., after glucose ingestion. Values obtained after isocaloric feeding were compared with those obtained after a 4-day carbohydrate overfeeding. The expression of SREBP-1c mRNA increased by 72% after carbohydrate overfeeding condition ($p = 0.012$, Figure 6). The expression of FAS and ACC mRNAs was also significantly increased by 363% and 72%, respectively, under overfeeding conditions ($p = 0.018$ and $p = 0.018$, respectively; Figure 6).

Pentose-phosphate pathway Activity in Adipose Tissue

In the basal state, fractional pentose-phosphate pathway activity was 17% after isocaloric feeding and 18% after carbohydrate overfeeding at the basal state (p = not significant). After the glucose load, the fractional activity of this pathway was 22% after isocaloric condition and 20% after carbohydrate overfeeding conditions.

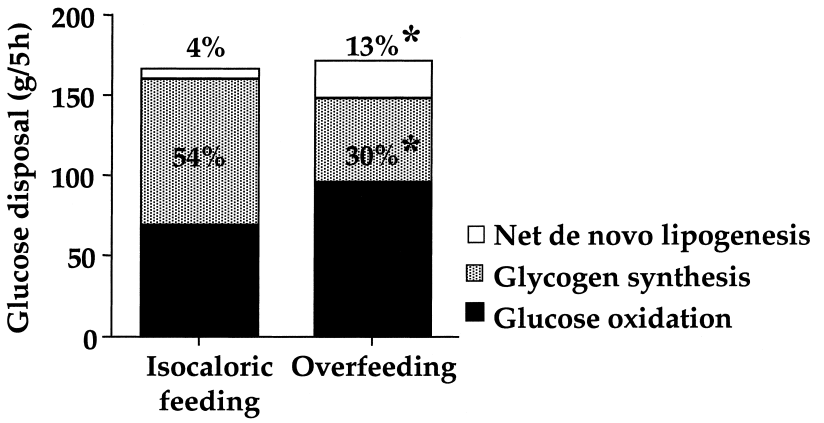

Figure 5: Major pathways of glucose disposal after isocaloric feeding and after carbohydrate overfeeding. *Significantly different from isocaloric feeding (Student's *t* test): $p < 0.05$.

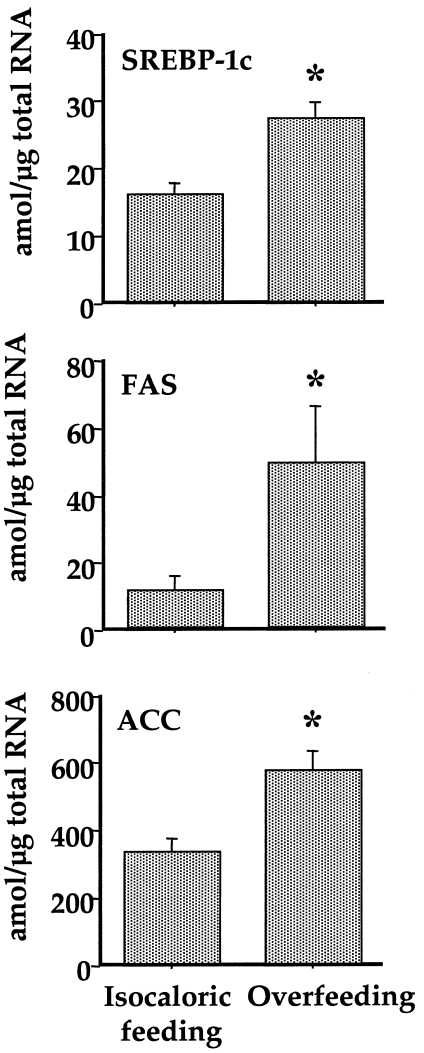

Figure 6: mRNA concentrations were measured by competitive RT-PCR. SREBP-1c, sterol regulatory element-binding protein 1c; FAS, fatty acid synthase; ACC, acetyl-CoA carboxylase. *Significantly different from isocaloric feeding (Student's paired *t* test): $p < 0.05$.

Discussion

This study was designed to evaluate the effect of a 4-day carbohydrate overfeeding on the major pathways of glucose disposal. The overfeeding protocol was specifically designed to provide a total carbohydrate load in excess of total energy expenditure (71% carbohydrate content of a total energy intake equal to 175% energy requirements yield a carbohydrate load corresponding to 125% total energy expenditure). The total excess carbohydrate administered over the 4-day overfeeding period can be estimated to be ~200 g/d, assuming nitrogen balance and net lipid oxidation equal to zero. Cumulated over 4 days, the excess carbohydrate to be disposed of nonoxidatively was therefore roughly 800 g, which is within the glycogen storage capacity of humans, as previously demonstrated by Acheson et al. (15).

The data collected provide a clear picture of the effects of carbohydrate overfeeding on the disposal of a large oral glucose load. Under standard isocaloric conditions, 42% of the ingested glucose was oxidized over the 5-hour postingestive period. The remaining 58% was essentially stored as glycogen (54%), with very little net de novo lipogenesis (4% of the glucose load). There is no indication as to where such de novo lipogenesis occurred. It is likely that part of it took place in the liver because it has been reported that fractional hepatic de novo lipogenesis represents 1–5% of very-low-density lipoprotein-fatty acids turnover under such conditions (16). Because indirect calorimetry measures only *net* de novo lipogenesis (i.e., fat synthesis in excess of fat oxidation), it is quite possible that this figure somewhat underestimates absolute de novo lipogenesis. Isotopic measurements of hepatic de novo lipogenesis in humans after carbohydrate administration, however, confirm that the absolute amount of fat synthesized is small under such conditions (17).

Carbohydrate overfeeding had marked effects on energy and substrate metabolism in these healthy individuals. Compared with what was observed after isocaloric feeding, net glucose oxidation was increased in basal conditions and further rose after glucose ingestion. Net nonoxidative glucose disposal was consequently reduced and represented only 43% of the glucose load. Simultaneously, net glycogen synthesis was reduced and represented only 30% of ingested glucose, whereas net de novo lipogenesis increased by 296% and corresponded to 13% of ingested glucose. There was also a marked increase in fasting and postprandial plasma TG concentrations after carbohydrate overfeeding together with suppressed plasma FFA concentrations. This increase in plasma TGs may be caused by both stimulation of hepatic de novo lipogenesis (18) and a decreased clearance of very-low-density lipoprotein-TGs (19). There is clear evidence in the literature that carbohydrate overfeeding stimulates hepatic de novo lipogenesis. However, a recent study during which healthy humans were overfed with carbohydrates reported that total hepatic de novo lipogenesis amounted to 43 g/d (5). Because the absolute carbohydrate load in this study was higher than in our present study and because this value of 43 g/d hepatic de novo lipogenesis is far lower than the whole body net de novo lipogenesis observed in our study, we can safely conclude that the bulk of de novo lipogenesis occurred in extrahepatic tissues. Basal and postprandial energy expenditure were increased by 7% and 10%, respectively, consistent with the high energetic cost of de novo lipogenesis. Other factors, such as sympathetic nervous system activation, which was not documented in these experiments, may be also involved in this stimulation of energy expenditure.

We next turned our attention to markers of de novo lipogenesis in adipose tissue biopsies obtained 5 hours after the beginning of the oral glucose administration. SREBP-1c

is a transcription factor that regulates the expression of lipogenic enzymes such as FAS and ACC (20,21). Carbohydrate overfeeding increased the expression of mRNAs coding for SREBP-1c, FAS, and ACC. Although de novo lipogenesis may be stimulated through an increase in lipogenic enzyme activity and substrate concentrations even in the absence of changes in lipogenic enzymes on RNA levels, these observations indicate that carbohydrate overfeeding also increased lipogenic enzyme transcription. This observation is entirely consistent with a stimulation of adipose tissue de novo lipogenesis.

Of interest, carbohydrate overfeeding increased glucose oxidation and de novo lipogenesis after glucose ingestion without altering plasma glucose and insulin concentrations compared with isocaloric conditions. This indicates that insulin sensitivity was not altered by overfeeding. This also suggests that changes in insulin concentrations were not responsible for the alteration of intracellular glucose metabolism observed after overfeeding. However, it is likely that plasma insulin and perhaps glucose concentrations were higher during the 4 days of overfeeding because of the 2.5-fold larger carbohydrate load. A continuous stimulation of SREBP-1c gene expression has been likely to occur in this condition as a consequence of hyperinsulinemia and/or increased glucose metabolism within the adipocyte (22–25). SREBP-1c may in turn have upregulated FAS and ACC, leading to increased partitioning of intracellular glucose into the de novo lipogenic pathway (26). Similar upregulation of other genes involved in carbohydrate oxidation may have occurred as well, but this was not documented in this study.

We further focused our attention on the fractional activity of the pentose-phosphate pathway as an indirect indicator of adipose tissue de novo lipogenesis, because this pathway supplies NADPH for de novo lipogenesis. The basal fractional activity of the pentose-phosphate pathway was ~20% under isocaloric conditions. This figure is consistent with that reported in the literature in various conditions in rats adipose cell cultures (27). This approach assumes that the carbon label of 1,6 $^{13}C_2$ 6,6 $^{2}H_2$ glucose in position one is lost both in the pentaphosphate pathway and during oxidation in the Krebs cycle, whereas the carbon in position six is lost exclusively during oxidation. It also assumes that the bulk of deuterium tracer in position six of the glucose molecule is retained when lactate is formed. This latter assumption may not be entirely correct in case of lactate-pyruvate and pyruvate-phosphoenol pyruvate cycling (28), and the present results should therefore be held as semiquantitative. In preliminary experiments (data not shown), we have observed in healthy humans that an oxidative stress applied locally by infusing 3% H_2O_2 in subcutaneous abdominal adipose increases this activity by 30% to 150%. We therefore feel confident that this approach gives a useful estimate of pentose-phosphate pathway activity in human adipose tissue in vivo. In the present study, the fractional activity of the pathway remained constant after glucose ingestion. Data from the literature, however, indicate that glucose use in adipocytes, which represents $<$1.0 mmol/100 g adipose per minute in basal conditions (29), increased by 50% after carbohydrate feeding (30). That such an increase in adipose tissue metabolism occurred after glucose ingestion is indeed supported by our observation that interstitial lactate concentrations, a marker of local glucose uptake, were increased. As a consequence, the absolute amount of glucose metabolized through the pentose-phosphate pathway was likely increased after glucose ingestion.

Contrary to our expectation, the fractional activity of the pentose-phosphate pathway was not altered by carbohydrate overfeeding. This may possibly be caused by the semiquantitative nature of our measurements. Furthermore, the absolute activity of the pentose-phosphate pathway may have increased if adipocytes' glucose use was increased. No data are available in the literature regarding the effect of carbohydrate overfeeding on subcutaneous adipocyte glucose use. The observation of a highly increased basal interstitial lactate concentration may suggest that basal glucose use was stimulated under such condition. No firm conclusion can be made in the absence of an accurate documentation of adipocytes glucose use.

In conclusion, the present data indicate that whole body de novo lipogenesis after glucose ingestion was markedly enhanced by a 4-day carbohydrate overfeeding and that the amount of lipid newly synthesized markedly exceeded the maximal reported rates for hepatic de novo lipogenesis. Increased mRNA expression of major lipogenic enzymes after carbohydrate overfeeding strongly suggests that de novo lipogenesis occurs in adipose tissue under such condition. Adipose tissue de novo lipogenesis was, however, not directly quantified in these experiments, and whether de novo lipogenesis occurs in other tissues as well remains an open question. Approximately 20% of the glucose taken up by adipocytes were metabolized in the pentose-phosphate pathway. Because this pathway is involved in NADPH synthesis, which is required for de novo lipogenesis, this observation is entirely consistent with active de novo lipogenesis in adipocytes.

Acknowledgments

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