A Role for Adipose Tissue De Novo Lipogenesis in Glucose Homeostasis During Catch-up Growth

A Randle Cycle Favoring Fat Storage

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Catch-up growth, a risk factor for type 2 diabetes, is characterized by hyperinsulinemia and accelerated body fat recovery. Using a rat model of semistarvation-refeeding that exhibits catch-up fat, we previously reported that during refeeding on a low-fat diet, glucose tolerance is normal but insulin-dependent glucose utilization is decreased in skeletal muscle and increased in adipose tissue, where de novo lipogenic capacity is concomitantly enhanced. Here we report that isocaloric refeeding on a high-fat (HF) diet blunts the enhanced in vivo insulin-dependent glucose utilization for de novo lipogenesis (DNL) in adipose tissue. These are shown to be early events of catch-up growth that are independent of hyperphagia and precede the development of overt adipocyte hypertrophy, adipose tissue inflammation, or defective insulin signaling. These results suggest a role for enhanced DNL as a glucose sink in regulating glycemia during catch-up growth, which is blunted by exposure to an HF diet, thereby contributing, together with skeletal muscle insulin resistance, to the development of glucose intolerance. Our findings are presented as an extension of the Randle cycle hypothesis, whereby the suppression of DNL constitutes a mechanism by which dietary lipids antagonize glucose utilization for storage as triglycerides in adipose tissue, thereby impairing glucose homeostasis during catch-up growth.

Catch-up growth during infancy and childhood is now recognized as an important risk factor for the development of type 2 diabetes and cardiovascular diseases later in life (1–4). Although the mechanisms by which catch-up growth leads to these chronic diseases remain obscure, there is compelling evidence both in humans and other mammals that catch-up growth is characterized by a disproportionately higher rate of body fat recovery than lean tissue recovery, and that an early feature of such preferential catch-up fat is hyperinsulinemia (5).

Using a rat model showing catch-up fat in response to semistarvation-refeeding (6), we previously showed that the insulin-resistant state of catch-up fat persists in the absence of hyperphagia (7) and that it is associated with diminished in vivo glucose utilization in skeletal muscle but enhanced glucose utilization in white adipose tissue (WAT) (8). These data have led to the proposal that the preferential catch-up fat during catch-up growth is characterized by glucose redistribution from skeletal muscle to WAT (8). Consistent with this hypothesis are subsequent demonstrations, in this same rat model of catch-up fat, of diminished mitochondrial mass and lower insulin receptor substrate-1 (IRS1)–associated phosphatidylinositol-3-kinase activity in skeletal muscle (9,10). Importantly, the increased glucose utilization in WAT during catch-up fat is associated with enhanced glucose flux toward lipogenesis as well as enhanced adipogenesis, which limit and delay adipocyte hypertrophy during catch-up fat (11). It is therefore possible that the enhanced glucose flux toward lipogenesis in WAT could significantly contribute to blood glucose homeostasis by compensating for the diminished glucose utilization in skeletal muscle.

Despite the adaptive nature of accelerated fat deposition during catch-up growth in restoring the body's main energy stores, this catch-up fat phenomenon may have deleterious consequences in the context of the modern lifestyle where energy-dense diets rich in fat are often consumed. Indeed, we have reported that rats showing catch-up fat on a high-fat (HF) diet display excess adiposity and glucose intolerance compared with rats showing catch-up fat on an isocaloric low-fat (LF) (high-carbohydrate) diet or compared with rats growing spontaneously on isocaloric amounts of the same HF diet (7). Understanding the mechanisms by which refeeding on the HF diet leads to these metabolic disturbances is clearly of importance for elucidating the pathophysiological consequences of catch-up growth pertaining to impaired glucose homeostasis. In the study reported here, we provide evidence suggesting a major role for de novo lipogenesis (DNL) in glucose homeostasis during catch-up growth. We show that exposure to an HF diet leads to rapid suppression of the increased WAT glucose utilization observed during catch-up fat on an LF diet. Moreover, we demonstrate that these effects of dietary lipids in suppressing glucose utilization in WAT are not due to excess caloric intake, an overt defect in proximal insulin signaling, or adipose tissue hypertrophy and inflammation, but can be explained by Randle-like lipid/glucose substrate competition for fat storage in adipocytes.

RESEARCH DESIGN AND METHODS

Animals and diets. Male Sprague-Dawley rats (Elevage Janvier, Le Genest Saint Isle, France), caged singly in a temperature-controlled room (22 ± 1°C) with a 12-h light-dark cycle, were maintained on a commercial chow diet

(Kliba, Cossonay, Switzerland) consisting, by energy, of 24% protein, 66% carbohydrate, and 10% fat and had free access to tap water. During the experiments, they were fed or refed on isocaloric amounts of either an LF or HF semisynthetic diet. The composition of these diets has been presented in details previously (7); the LF and HF diets provided approximately 6 and 53% energy as fat, respectively, and lard was the main source of fat in the HF diet. Animals were maintained in accordance with the regulations and guidelines of the Department of Medicine (University of Fribourg) for the care and use of laboratory animals. **Design of study.** The experiments were performed according to our previously reported design of semistarvation-refeeding that established this rat model of catch-up fat when consuming either an LF or HF diet (7,11). In brief, groups of 7-week-old rats were food restricted at 50% of their spontaneous food intake for 2 weeks, after which they were refed isocaloric amounts of LF or HF diets for variable refeeding periods of 3–14 days and compared with fed controls of similar body weight at the onset of refeeding. Under these conditions, the refed animals show similar gain in lean mass but greater gain in body fat than controls (6,7); the higher efficiency of fat deposition during refeeding on the LF diet was exacerbated by isocaloric refeeding on the HF diet (7).

In vivo glucose utilization during hyperinsulinemic-euglycemic clamps. The animals were fasted for 4–7 h and anesthetized with Nembutal (50 mg/kg i.p.; Abbott Laboratories, Chicago, IL), and the glucose infusion rate (GIR) to maintain euglycemia was determined under basal and insulin-stimulated conditions (200 mU/mL; Actrapid HM; Novo Nordisk, Bagsvaerd, Denmark), as previously described (12,13). At the end of the hyperinsulinemic-euglycemic clamps, the in vivo insulin-stimulated GUI of individual tissues, namely various skeletal muscles and WAT fat pads, was determined with the labeled 2-deoxy-D-glucose technique (12,13). Plasma glucose and insulin levels were determined under basal and clamp conditions by the glucose oxidase method (Roche Diagnostics GmbH, Rotkreuz, Switzerland) and ELISA (SPIbio, Montigny Le Brotenneux, France), respectively.

In vivo bolus administration of insulin. The rats were fasted from 7:00 A.M., and 4–7 h later, subgroups were anesthetized by injection of ketamine/xylazine (39/5 mg/kg body weight [bw]) and surgically prepared for a bolus injection (through the jugular vein) of insulin (10 units/kg bw) (Actrapid) or an equal volume of saline vehicle, as previously reported (11). Insulin or saline was injected 3 min before the animal was killed, and adipose tissue was harvested, frozen in liquid nitrogen, and stored at −80°C until analysis.

Adipocyte number and size. Fixation with osmium tetroxide and isolation of adipocytes for cell counting/sizing were performed according to the method of Hirsch and Gallian (14), as previously reported (11); suspensions of adipocytes were analyzed using the Multisizer 3 Coulter Counter. For determination of cell size (adipocyte diameter distribution), similar amounts of cells (~8,000) were aspirated by the machine and classified according to their diameter and frequency.

In vivo measurement of DNL. De novo lipogenesis was determined as previously described (15), with minor modifications. In brief, after 3–4 days of refeeding, freely moving rats were injected intraperitoneally with 2 mCi of ^3H_2O (Anawa, Zürich, Switzerland) and 10 μCi of [U-^{14}C]-glucose (Anawa). Plasma samples obtained at 0, 10, 30, and 60 min after injections were used for the determination of plasma glucose levels, as well as plasma water and glucose-specific activities. Rats were killed at 60 min by an overdose of pentobarbital sodium salt (Esconarkon; Streuli Pharma AG, Uznach, Switzerland). When the animal was killed, inguinal and epididymal adipose tissue depots and the liver were rapidly dissected and stored at −80°C until use. Lipids were extracted with chloroform/methanol (2:1 vol/vol), using the Floch method (16). Fatty acids were obtained after saponification and extraction using petroleum ether (17). Incorporation of ^{3}H and ^{14}C into lipids and saponified fatty acids was then measured using a full-spectrum disintegrations per minute (DPM) method on a Tricarb2900 TR (Perkin Elmer, Inc.). It was considered that 13.3 moles of H_2O are incorporated into each newly synthetized C16 fatty acid, and incorporation of [U-^{14}C]glucose into fatty acids was normalized to the time-average specific activity of plasma glucose over the whole experiment (15).

Enzymatic and molecular assays. Measurements of fatty acid synthase (FAS) and glucose-6-phosphate dehydrogenase (G6PDH) activities were performed as previously described (18). Akt (Ser 473) and extracellular signal–related kinase (ERK) (p44/42 MAPK) were evaluated by immunoblot analysis, as detailed previously (11). Total RNA from 50–150 mg of crushed WAT was isolated using the method of Chomczynski and Sacchi (19). After phase separation, RNA was precipitated with isopropanol, cDNA was synthesized from 250 ng of total RNA, and RT-PCR was performed as detailed in Supplementary Table 1.

Proteins and fatty acid analysis. Plasma cytokines and other markers of inflammation were measured using commercial ELISA kits for tumor necrosis factor-α, interleukin-6 (IL-6), IL-10, and interferon-γ (eBioscience, Inc., San Diego, CA), for IL-1-α, IL-1-β, IL-1 RA, and C-reactive protein (R&D Systems Europe, Ltd., Abingdon, U.K.), and also for adiponectin (AssayPro, St. Charles, MO) and leptin (Crystal Chem, Downers Grove, IL). Fatty acid analysis of tissues and diet were determined on extracted lipids by automated gas liquid chromatography as previously detailed (20).

Data analysis and statistics. All data are presented as means ± SEM. For studies comparing data across fed and refed animals consuming the LF or HF diet, two-factor ANOVA was used for the main effects of group (refed vs. fed controls) and diet (HF vs. LF) and for the group × diet interaction. For the study comparing data from animals consuming the HF or LF diets in response to insulin or saline, the data were analyzed by two-factor ANOVA for the main effects of diet (HF vs. LF), treatment (insulin vs. saline), and diet × treatment interaction; these latter analyses being conducted separately under conditions of feeding or refeeding. Pairwise comparisons between diet (HF vs. LF) or treatment (insulin vs. saline) were performed using unpaired Student t test. Between-group differences in distribution curves for adipocyte diameter were analyzed by the Kolmogorov-Smirnov test. The statistical treatment of data was performed using the computer software STATISTIX, version 8.0 (Analytical Software, St. Paul, MN).

RESULTS

HF diet impairs glucose tolerance during catch-up fat. In a previous study (7), we observed during a glucose tolerance test on day 12–13 of refeeding that the refed animals showed hyperinsulinemia both on LF and HF diets, but glucose tolerance was normal in animals refed the LF diet, whereas it was impaired in those refed the HF diet. These data are confirmed here in a new set of experiments with GTT conducted on day 11–12 of refeeding (Supplementary Fig. 1). **HF diet blunts the enhanced adipose tissue glucose utilization during catch-up fat.** Because we also showed in a subsequent study (8) using hyperinsulinemic-euglycemic clamps that glucose utilization is diminished in skeletal muscle but enhanced in adipose tissue depots, we investigated here whether the observed loss of glycemic control during refeeding on HF diet could arise from the loss of the enhanced glucose utilization in adipose tissue shown during refeeding on the LF diet. To this end, we performed hyperinsulinemic-euglycemic clamps with 2-deoxy-glucose tracer in rats fed or refed on the HF or LF diet for 11–12 days. As shown in Table 1 (day 11–12), the GIR is significantly lower in refed animals on the HF diet than on the LF diet by ~20% ($P < 0.01$); no significant between-diet differences are observed in the fed controls. The data on tissue-specific insulin-stimulated glucose utilization index (GUI) (Fig. 1A) indicate that GUI is higher in adipose tissue depots of refed animals than in controls when on the LF diet, but not when refed the HF diet, such that GUI is significantly lower in WAT from animals refed on the HF diet compared with the LF diet, particularly in epididymal (EWAT) and inguinal WAT (IWAT). No differences between LF and HF diets are observed in adipose tissues of control animals (Fig. 1A) or in skeletal muscles of either refed or control animals (data not shown).

We next investigated whether the effect of HF diet on WAT insulin sensitivity is an early event of catch-up fat rather than a consequence of the excess adiposity observed after 7–10 days of refeeding (10,11). Thus, we performed hyperinsulinemic-euglycemic clamp on day 3–4 of refeeding, an early time point of catch-up growth where the weight of WAT depots of the refed animals is not higher than that of control-fed rats (Supplementary Fig. 2). During this clamp study, the GIR is significantly lower (-25%, $P < 0.05$) in the refed rats on the HF diet (RF-HF group) than in the control animals on the LF diet (C-LF group; day 3–4) (Table 1). The data on tissue-specific GUI (Fig. 1B) indicate that, as for day 11–12, GUI in WAT depots on day 3–4 are higher in refed animals than in controls when on the LF diet but not when refed the HF diet.

Thus, the increased GUI observed in WAT during refeeding on the LF diet is completely blunted by isocaloric refeeding on the HF diet for only a few days, and hence represents an early event during catch-up fat on the HF diet.

TABLE 1
Metabolic parameters during hyperinsulinemic-euglycemic clamps in control (C) and refed (RF) rats on an LF or HF diet for 11–12 days and 3–4 days of refeeding
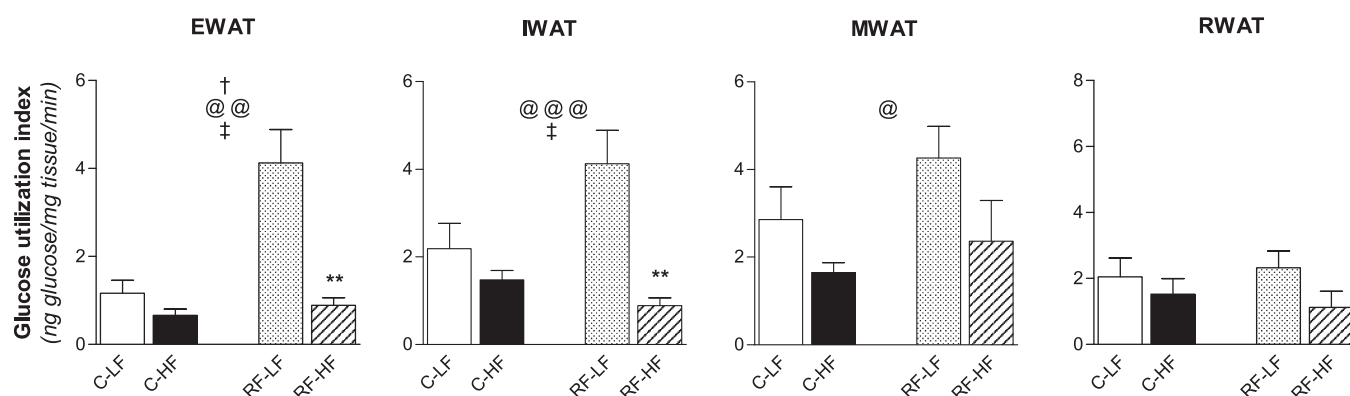
	Day 11–12				Day 3–4			
	C-LF	C-HF	RF-LF	RF-HF	C-LF	C-HF	RF-LF	RF-HF
Plasma glucose (mg/100 mL)								
Basal	94 ± 2	99 ± 5	96 ± 5	99 ± 4	113 ± 4	110 ± 3	111 ± 3	107 ± 3
Insulin stimulated	100 ± 3	104 ± 4	103 ± 3	103 ± 2	91 ± 2	92 ± 1	94 ± 2	93 ± 2
Plasma insulin (ng/mL)								
Basal	4.11 ± 0.52	4.50 ± 1.01	4.63 ± 0.28	4.25 ± 0.47	3.90 ± 0.59	4.05 ± 0.74	4.07 ± 0.59	3.74 ± 0.38
Insulin stimulated	9.09 ± 0.54	8.32 ± 0.71	8.59 ± 0.47	8.98 ± 0.86	9.09 ± 1.18	9.28 ± 0.88	9.11 ± 1.27	9.36 ± 0.69
Glucose infusion rate (mg · min^{-1} · kg^{-1})	20.4 ± 1.2	18.6 ± 0.9	21.4 ± 0.9	17.1 ± 0.9*	22.3 ± 1.2	19.6 ± 0.9	19.8 ± 1.4	16.7 ± 1.0¶

Data are means ± SE (n = 6–8). For data on plasma glucose and plasma insulin, whether for day 11–12 or day 3–4, two-factor ANOVA analysis indicates no significant effect of group (RF vs. C), diet (HF vs. LF), or group × diet interaction. For data on glucose infusion rate under day 11–12, two-factor ANOVA analysis indicates significant effect of diet ($P < 0.01$). For data on GIR under day 3–4, two-factor ANOVA analysis indicates significant effect of group ($P < 0.05$) and diet ($P < 0.05$). *Significant difference ($P < 0.01$) between RF-HF and RF-LF by unpaired Student t test. ¶Significant difference ($P < 0.05$) of RF-HF vs. C-LF group by Dunnett test.

By contrast, no significant differences are found between the HF and LF diets in skeletal muscle GUI (data not shown), such that the previously reported lower muscle GUI in refed than in control animals (8) is independent of diet type.

Impaired insulin signaling is not required for reduced glucose utilization in WAT. To investigate whether WAT insulin signaling is impaired by exposure to the HF diet over a few days, we assessed Akt/protein kinase B and
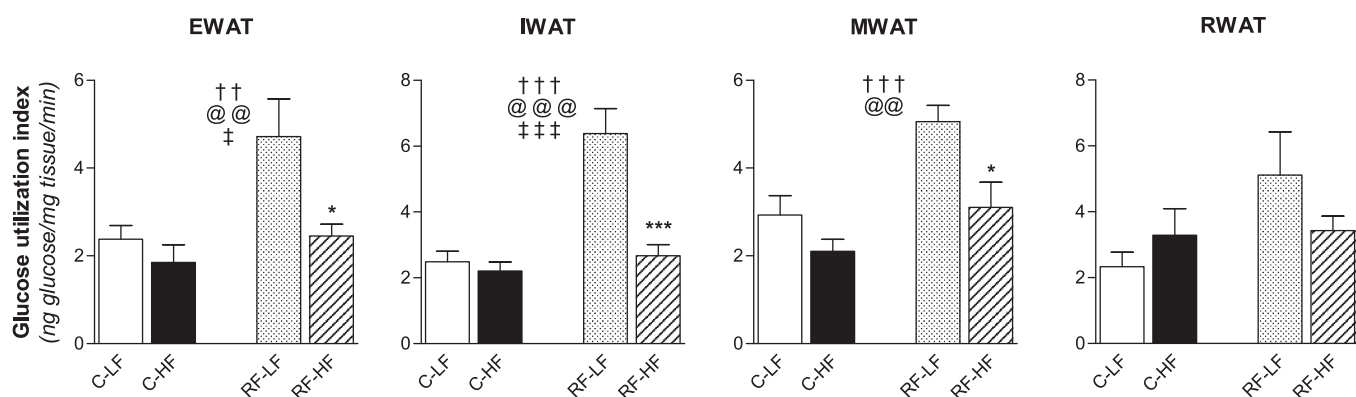

FIG. 1. In vivo insulin-stimulated glucose utilization. Tissue GUI was assessed by hyperinsulinemic-euglycemic clamp on day 11–12 (*A*) or day 3–4 (*B*) of refeeding in control (C) and refed (RF) rats on an LF or HF diet. EWAT, epididymal WAT; IWAT, inguinal WAT; MWAT, mesenteric WAT; RWAT, retroperitoneal WAT. All values are means ± SE (n = 6–8). Statistical significance of differences, assessed by two-factor ANOVA, is indicated as follows: †Group effect (RF vs. C); @Diet effect (LF vs. HF); ‡Group × diet interaction. *Significant difference by post hoc pairwise comparison between diets within either refed animals (RF-LF vs. RF-HF) or within control animals (C-LF vs. C-HF). Single, double, and triple symbols imply $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively.

ERK signaling in IWAT and EWAT after in vivo administration of saline or a low dose of insulin (10 units/kg bw) on day 3–4 of refeeding. In EWAT, insulin-stimulated Akt and ERK phosphorylations are unaltered by the HF diet in both control and refed groups (Fig. 2A). In IWAT, insulin-stimulated Akt and ERK phosphorylation are also not altered by the HF diet in the control group (Fig. 2B), but they are significantly lower in the refed group on the HF diet relative to the LF diet, although this difference for insulin-stimulated Akt phosphorylation is modest (<25%). Overall, whereas proximal insulin signaling is significantly reduced in IWAT by refeeding on an HF diet, we could not detect any defect in insulin-induced Akt and ERK phosphorylation in EWAT of rats exhibiting catch-up fat on the HF diet. As glucose utilization during the clamp studies is similarly reduced in EWAT and IWAT, these results suggest that mechanisms other than defective proximal insulin signaling are implicated in the effects of dietary lipids in impairing adipose tissue glucose utilization during catch-up growth.

Early catch-up fat on the HF diet is not associated with overt adipocyte hypertrophy or inflammation. Adipocyte hypertrophy and consequent adipose tissue inflammation have been proposed to impair normal insulin signaling cascades, causing insulin resistance (21). To assess whether the loss of adipose tissue insulin hyperresponsiveness could be attributed to hypertrophic adipocytes, morphometric studies were conducted in EWAT

and IWAT harvested at similar time points compared with the glucose-clamp studies, i.e., on day 3–4 of refeeding. The results for EWAT (Fig. 3A) show that tissue weight is higher with the HF than the LF diet in both refed and control rats, and that the refed groups display higher tissue weight but also higher adipocyte number than the control rats. They also have smaller adipocytes than their respective diet controls, as suggested by a shift to the left in their frequency distribution of adipocyte diameter. In IWAT (Fig. 3B), tissue weight is also higher with the HF than the LF diet in both refed and control groups. Although there are no significant between-diet differences in adipocyte number within refed or control groups, the adipocyte number in the refed group on the HF diet is higher than in controls on the HF diet (RF-HF > C-HF by 28%, $P < 0.05$), and the refed group has smaller adipocytes (RF-HF < C-LF, $P < 0.05$). Furthermore, between-diet comparisons in the refed animals indicate that although those on the HF diet display heavier IWAT than those on the LF diet, which is partly due to a modest increase in adipocyte size, the population of hypertrophic adipocytes with a diameter >100 μm is only marginally increased.

To assess whether the insulin-resistant state of catch-up fat and the loss of WAT insulin hyperresponsiveness during HF refeeding could be linked to metabolic inflammation, we assessed the gene expression of key markers of inflammation in both EWAT and IWAT harvested on day 3–4
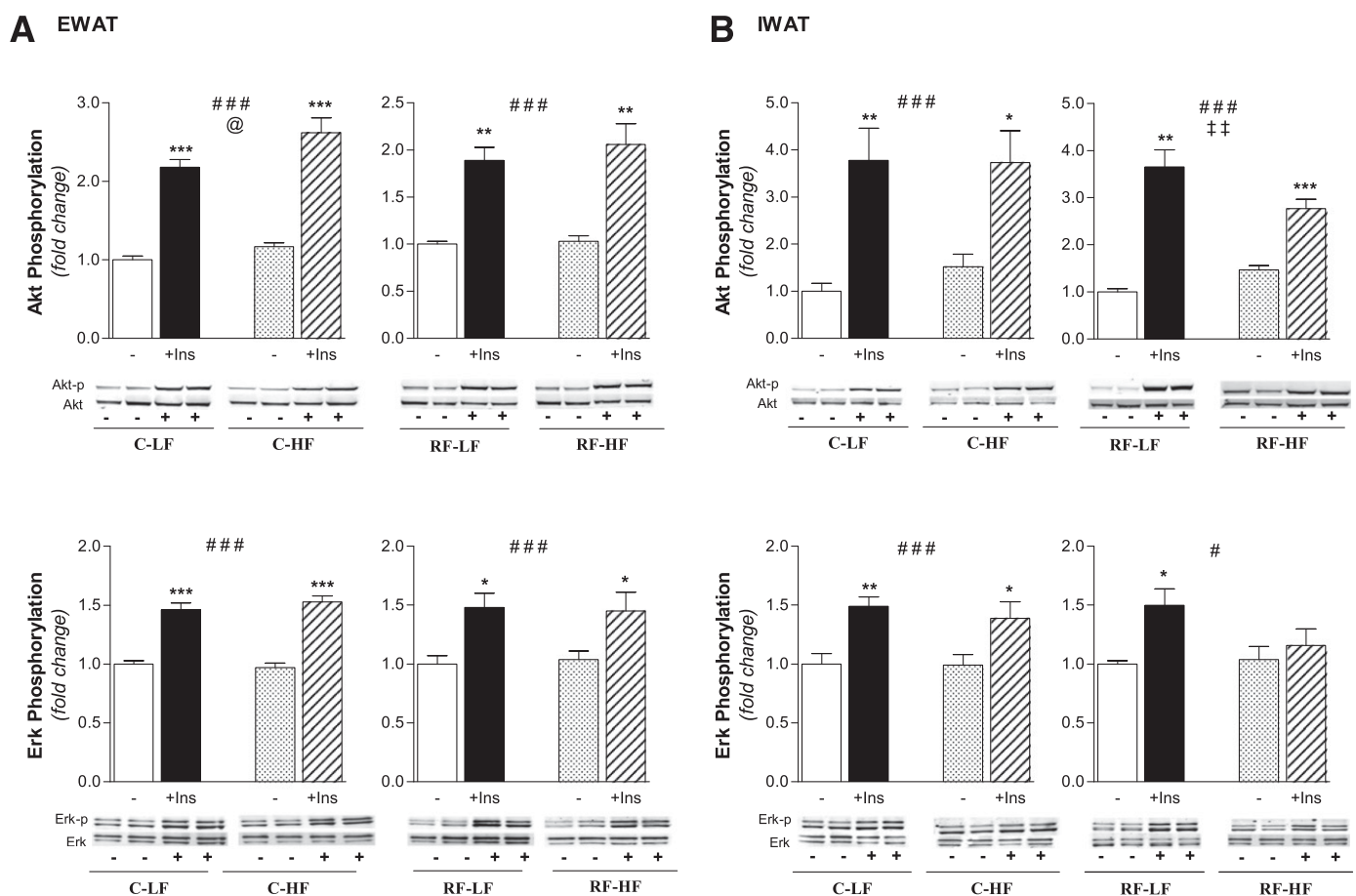

FIG. 2. Adipose tissue insulin signaling. Akt and ERK phosphorylation in EWAT (A) and IWAT (B) in response to intravenous injection of 10 units/kg insulin (+Ins) or saline (−) in controls (C) or refed (RF) groups on day 3–4 of refeeding on LF or HF diet. Values are means ± SE ($n = 6$). Statistical significance of differences, assessed by two-factor ANOVA, is indicated as follows: #Effect of treatment (insulin vs. saline); @Effect of diet (HF vs. LF); ‡Group × diet interaction. *Significant difference by post hoc pairwise comparison between insulin and saline within each group. Single, double, and triple symbols imply $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively.

of catch-up fat. The results (Supplementary Table 1) show no increase in the mRNA levels of the macrophage marker CD-68. Importantly, rats showing catch-up fat on the HF diet do not show increased levels of inflammatory markers (tumor necrosis factor-α and IL-6) compared with their respective controls or with refed rats on the LF diet. Similarly, plasma concentrations of various cytokines and C-reactive peptide, generally implicated in inflammation, as well as adiponectin and leptin, are not significantly different across the various groups at this time point (day 3–4)

FIG. 3. Adipose tissue morphometry. WAT weight, adipocyte number (from total EWAT or total IWAT), as well as adipocyte diameter frequency distribution on day 3–4 of refeeding in control (C) and refed (RF) animals on LF or HF diet. Data for EWAT and IWAT are shown under *A* and *B*, respectively. Values are means ± SE ($n = 6$). For data on WAT weight and adipocyte number, the statistical significance of differences is as indicated in the legend of Fig. 1. ¶Significant differences ($P < 0.05$) between RF-HF and C-HF. For data on adipocyte diameter frequency distribution, pairwise group comparisons, performed by Kolmogorov-Smirnov test for analysis of curve shifts, can be summarized as follows: EWAT, RF-LF < C-LF = RF-HF < C-HF, i.e., significant pairwise differences ($P < 0.05$) across all four groups with the exception of C-LF vs. RF-HF; IWAT, RF-LF < RF-HF < C-LF < C-HF, i.e., significant pairwise differences ($P < 0.05$) across all four groups.

(Supplementary Table 2). Furthermore, no significant differences are observed in markers of oxidative stress in WAT (or in liver), as judged by data showing no change in the activities of aconitase and superoxide dismutase (Supplementary Table 3).

Adipose tissue lipid composition and gene expression analysis of dietary lipid storage. Since the fatty acid composition of adipose tissue triglycerides generally reflects the fatty acid composition of the diet (22), the possibility arises that the loss of enhanced glucose flux into adipose tissue during catch-up fat on the HF diet might reside in the enhanced flux of dietary fatty acid in adipose tissue during

HF diet consumption. To substantiate this hypothesis, we show here in Fig. 4 that oleic acid (C18:1), by far the most abundant fatty acid in the HF diet used here (Fig. 4A), is increased in the adipose tissue of animals on the HF diet (Fig. 4B). Conversely, palmitoleic acid (16:1), which is found in negligible amounts in the HF diet, is reduced. This specific increase in oleic acid in adipose tissue occurs despite indications of diminished capacity for desaturation of saturated fatty acids in lipogenic tissues (Fig. 4C), as suggested by the marked reduction in mRNA levels of stearoyl-CoA desaturase 1 (SCD1), the rate-limiting enzyme catalyzing the synthesis of monounsaturated fatty acids (C16:1 and C18:1)
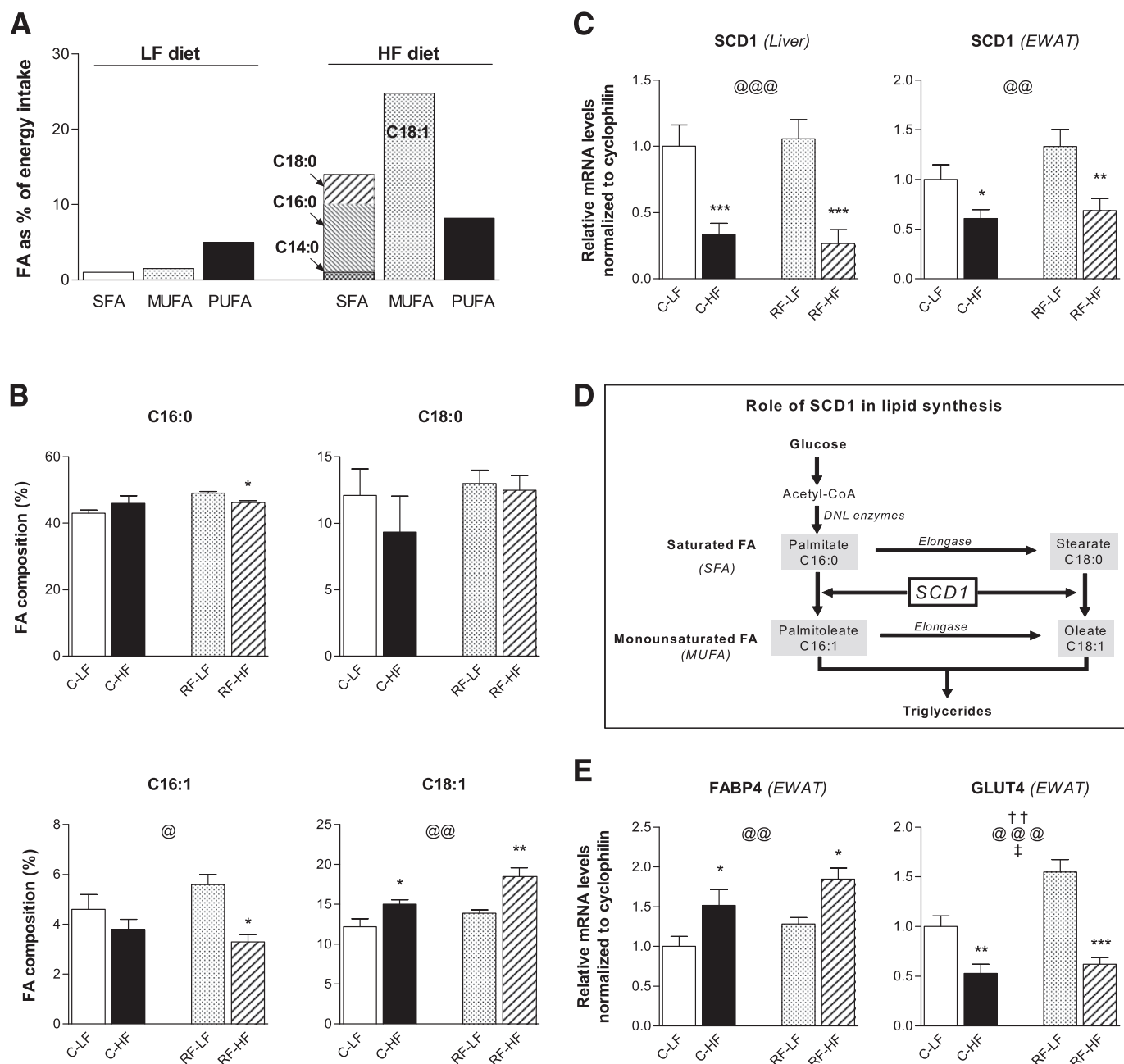

FIG. 4. Impact of dietary fat on markers of glucose and fatty acid flux into adipose tissue. *A*: Fatty acid (FA) composition of LF and HF diets, indicated as the percentage of energy intake in the form of saturated (SFA), monounsaturated (MUFA), and polyunsaturated (PUFA) fatty acids. *B*: Proportion of palmitate (C16:0), palmitoleate (C16:1), stearate (C18:0), and oleate (C18:1) in the triglyceride fraction of EWAT. *C*: mRNA levels of SCD1 in EWAT and liver. *D*: Metabolic pathways of lipid synthesis influenced by SCD1. *E*: mRNA levels of transporters of glucose (GLUT4) and fatty acids (FABP4) in EWAT; tissues were obtained on day 3–4 of refeeding. Statistical significance of differences is as indicated in the legend of Fig. 1.

from saturated fatty acids (C16:0 and C18:0) (Fig. 4*D*). Furthermore, the data on the gene expression of glucose and fatty acid transporters, indicating lower GLUT4 but increased FABP4 mRNA levels in EWAT during HF consumption (Fig. 4*E*), are in line with an enhanced fatty acid flux in adipose tissue during catch-up fat at the expense of the glucose flux.

Dietary fat rapidly inhibits DNL during catch-up fat. The idea that dietary lipids compete with glucose utilization for adipose tissue lipid storage implies that the glucose flux into DNL would be elevated during refeeding on an LF but not an HF diet. To test this hypothesis, we assessed DNL in vivo by measuring the rate of incorporation of ^{3}H and ^{14}C into newly synthesized fatty acids and total lipids. The results show that glucose incorporation into fatty acids, via DNL, is specifically increased during refeeding on an LF diet, and that it is blunted by the HF diet in both EWAT and IWAT, as well as in the liver (Fig. 5*A* and *B*). The data for the incorporation of ^{3}H and ^{14}C into total lipids indicate similar changes in DNL (Supplementary Fig. 3). Consistent with these in vivo DNL data, it is shown that exposure of refed animals to the HF diet results in a blunting of the activities of two key enzymes implicated in DNL: FAS and G6PDH (Fig. 5*C* and *D*). Furthermore, both FAS and G6PDH mRNA levels are lower in refed animals on an HF diet relative to those on an LF diet (Fig. 6). The measurement of various transcription factors (SREBP1c, ChREBP, PPARγ, and CEBPα) implicated in the modulation of genes controlling glucose and lipid metabolism show, however, that they are not altered by the HF diet, with the exception of SREBP1c, which is lower in IWAT from the group refed the HF diet. Overall, dietary lipids rapidly cause a defect in DNL during catch-up growth.

DISCUSSION

We previously reported that catch-up fat on an LF (high-carbohydrate) diet is characterized by tissue-specific changes in insulin-dependent glucose utilization; it is reduced in skeletal muscle but enhanced in WAT (8), leading to the notion that catch-up fat is a state of glucose redistribution between skeletal muscle and adipose tissue. It is shown here that refeeding on an isocaloric HF diet has no additional impact on insulin-stimulated glucose utilization in skeletal muscle, whereas it blunts the enhanced insulin-stimulated glucose utilization in WAT. This effect of dietary lipids is specific for catch-up growth as spontaneously growing (fed control) rats on the HF diet show no significant differences in insulin-stimulated glucose utilization in WAT compared with controls fed the LF diet. These findings are in line with previous reports showing that, unlike in skeletal muscle, WAT insulin-stimulated glucose uptake is not significantly reduced after exposure of spontaneously growing animals to HF diets even for several weeks (23,24).

Mechanisms by which dietary fat impairs adipose tissue insulin-dependent glucose utilization. The findings here that the blunting effect of the HF diet on insulin-stimulated glucose utilization in WAT of refed animals is as pronounced during the early phase of catch-up fat (day 3–4) as during the late phase (day 11–12) (Fig. 1) indicate that the loss of WAT insulin hyperresponsiveness during catch-up fat is an early and sustained response to dietary lipids, which is not initiated by excess caloric intake or adiposity or by adipocyte hypertrophy per se. This loss is also not linked to overt adipose tissue inflammation, as judged by unchanged gene expression levels of CD68 and adipokines implicated in

metabolic inflammation (21). Although insulin-stimulated Akt and ERK phosphorylation was decreased in the IWAT of refed animals on the HF diet, proximal insulin signaling was not impaired in their EWAT. Still, dietary lipids in rats showing catch-up growth decreased glucose utilization to a similar extent in EWAT and IWAT, thereby underscoring a poor overall association between changes in insulin signaling and reduced adipose tissue glucose utilization in this model. In this respect, it is important to consider that insulin signaling was evaluated using a relatively low insulin dose and that adipose tissue can achieve maximal glucose uptake when as little as 2% of insulin receptors are activated (25,26), such that a modest defect in insulin signaling is likely to be compensated by the large number of spare receptors on adipocytes during hyperinsulinemic clamp.

By contrast, DNL was clearly associated with alterations in insulin-dependent glucose utilization both in EWAT and IWAT, being increased during refeeding on the LF diet and unchanged or decreased by refeeding on the HF diet. These changes in DNL measured by in vivo glucose incorporation into fatty acids in adipose tissues and liver correlate with changes in the activity and gene expression of FAS and G6PDH, two key lipogenic enzymes. However, the differences in mRNA levels of FAS and G6PDH could only partially explain the differences in FAS and G6PDH activity, suggesting that additional posttranscriptional mechanisms are likely implicated in the effects of dietary lipids on DNL.

A role for DNL in adipose tissue glucose uptake. It should be pointed out that during catch-up fat on an LF diet, a hyperinsulinemic state, consequent to skeletal muscle insulin resistance, occurs as early as on day 1 of refeeding (11) and persists after 1 week (7), on day 11–12 (Supplementary Fig. 1) and on day 12–13 (7), such that hyperinsulinemia is most likely a major factor in initiating and sustaining the enhanced glucose uptake and DNL in WAT. DNL not only contributes to the rapid recovery of fat in adipose tissue but also acts as a glucose sink that allows glycemia to be maintained within the physiological range. Consistent with this view is the past demonstration by Fried et al. (27) that pharmacological inhibitors of DNL decrease the ability of insulin to stimulate both the pentose shunt pathway and overall glucose utilization. Thus, the increased demand in acetyl-CoA and NADPH for lipogenesis would lead to enhanced glucose metabolism through glycolysis and the pentose phosphate pathway, thereby driving glucose influx. These studies in rats have led these authors (27) to propose that the enzymatic capacity for fatty acid synthesis is an important factor in determining insulin-stimulated glucose uptake and utilization in WAT. Consistently, Guerre-Millo et al. (28) showed a strong correlation between FAS activity and the insulin effect on glucose metabolism in rat adipocytes.

In humans, there is increasing evidence that adipocytes are able to synthesize fatty acids and triglycerides from nonlipid precursors (29–32) and that adipose tissue DNL can be induced by high-carbohydrate overfeeding (30,34). Like past studies in rats (27,28), the recent demonstrations from human studies of strong associations between low adipocyte DNL and insulin resistance in adipocytes and/or systemic insulin resistance (32,33,35–37) are hence in line with a role for adipose tissue DNL in human glucose homeostasis. Furthermore, the findings that both basal and insulin-stimulated DNL in adipocytes of human infants and children are two to four times higher than in adults (38) underscore the fact that DNL may play an important role in fat storage coupled
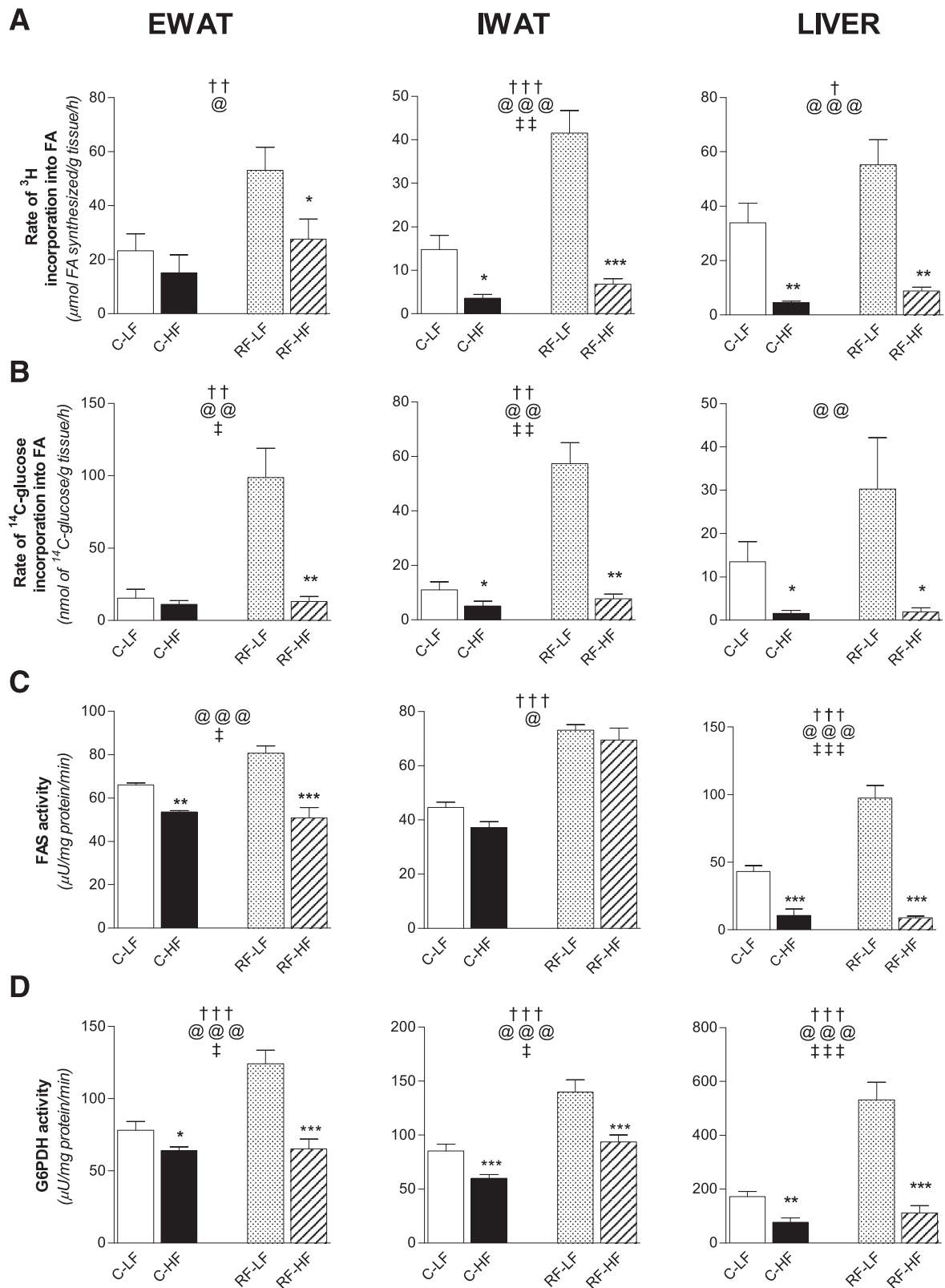
A

EWAT

IWAT

LIVER

FIG. 5. DNL in adipose tissue depots and liver. *A* and *B*: DNL measured in vivo by incorporation of tritium (^{3}H) or ^{14}C-labeled glucose into newly synthesized fatty acids. *C* and *D*: Activities of key DNL enzymes, FAS and G6PDH. All tissues were obtained on day 3–4 of refeeding. Statistical significance of differences is as indicated in legend of Fig. 1.
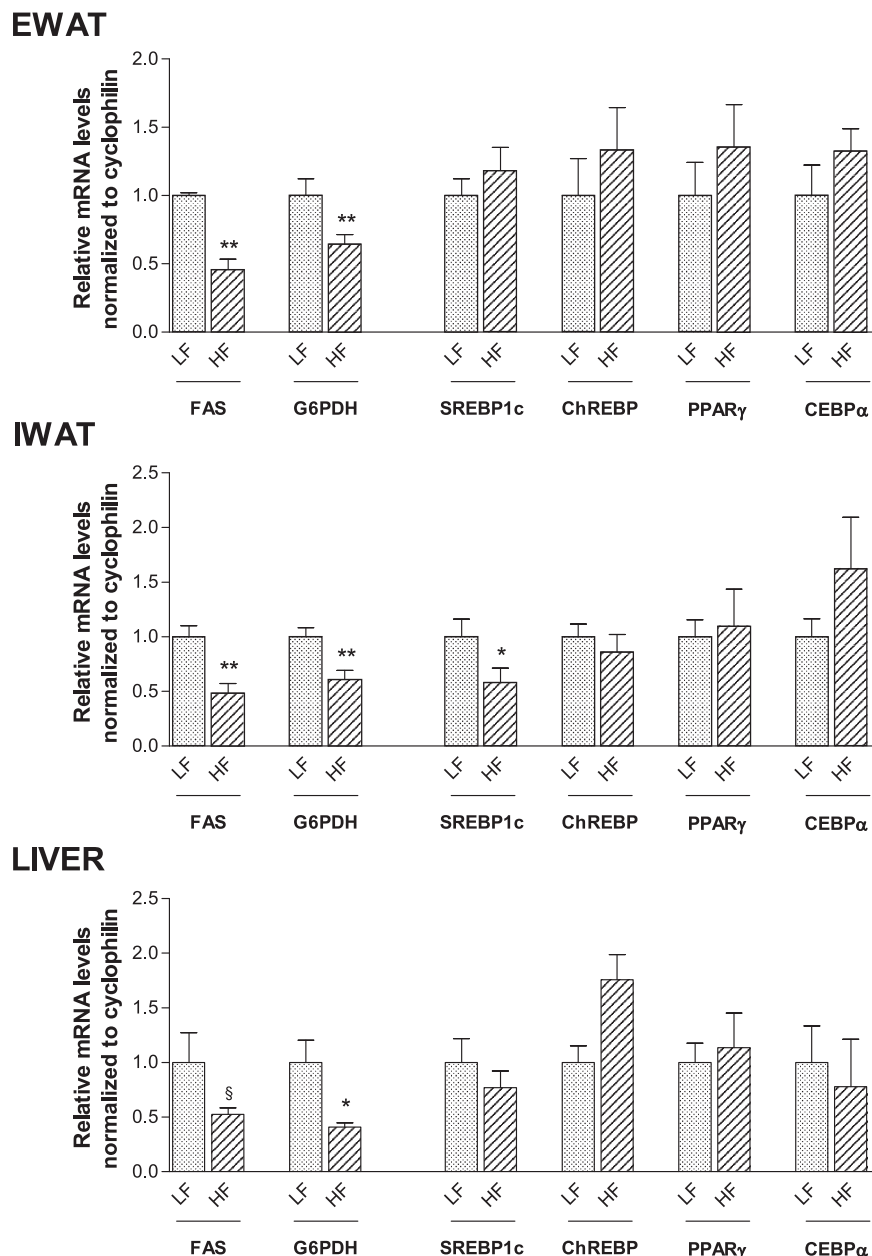
8

EWAT

IWAT

LIVER

FIG. 6. Gene expression of DNL enzymes and transcription factors. All tissues were obtained on day 3–4 of refeeding. Statistical differences (by unpaired Student *t* test) are denoted as follows: *$P < 0.05$, **$P < 0.01$, and §$P = 0.1$.

to glucose homeostasis during early growth, with potentially greater relevance during catch-up growth (4)

Randle cycle for fat storage in adipose tissue. We find our results to be reminiscent of the Randle cycle hypothesis proposed ~50 years ago (39), which stipulates that fatty acids and glucose directly compete as the energy source for liver and skeletal muscles. Thus, our findings here can be viewed as an extension of the Randle cycle, whereby suppression of de novo lipogenic machinery constitutes a mechanism by which dietary fat competes with carbohydrates for storage as triglycerides in WAT (Fig. 7). Support for this contention can be derived from our data indicating that oleic acid (C18:1), by far the most abundant fatty acid in the HF diet used here, is increased in adipose tissue despite downregulation of SCD1, the rate-limiting enzyme catalyzing the synthesis of monounsaturated fatty acids

from saturated fatty acids. The findings of increased expression of the adipose tissue fatty acid transporter FABP4 and decreased expression of the glucose transporter GLUT4 with HF consumption also contribute to our view of an extended Randle cycle in adipose tissue. Thus, by blunting the enhanced capacity for glucose flux into WAT during catch-up fat, dietary fat offsets the ability of WAT to buffer against glucose spared from insulin resistance in skeletal muscle. It is postulated here that upon exposure to HF foods, this Randle cycle for fat storage in WAT may be an early event initiating the deleterious effects of dietary lipids on glucose homeostasis during catch-up growth. It could also be of importance in the excessive fat storage encountered in obesity and the accompanying metabolic defects involving impaired glucose tolerance as a first step toward the development of type 2 diabetes.
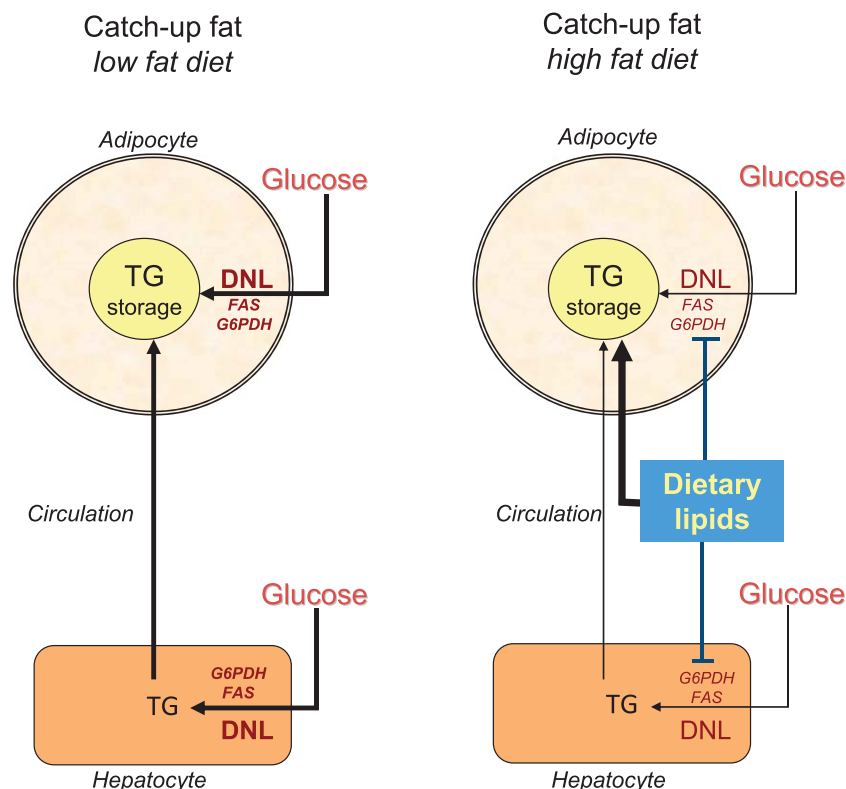
FIG. 7. Schematic diagram depicting direct competition between glucose and lipids for fat storage as triglycerides in adipocytes during catch-up fat. During catch-up fat on an LF (high-carbohydrate) diet, the increased DNL pathway leads to enhanced insulin-dependent glucose utilization in adipose tissue and liver, which contributes to maintaining normal blood glucose homeostasis. By contrast, during catch-up fat on an HF diet, the increased availability of dietary lipids markedly inhibits the activity of key lipogenic enzymes (G6PDH and FAS) and provides substrates for triglyceride (TG) synthesis and storage in adipose tissue. Thus, substrate competition between dietary fat and carbohydrates for TG storage in adipose tissue, via the inhibition of DNL, will lead to diminished glucose utilization in adipocytes and hepatocytes, thereby promoting glucose intolerance during catch-up growth.

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