Adipose Tissue Plasticity During Catch-Up Fat Driven by Thrifty Metabolism

Relevance for Muscle-Adipose Glucose Redistribution During Catch-Up Growth

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OBJECTIVE—Catch-up growth, a risk factor for later type 2 diabetes, is characterized by hyperinsulinemia, accelerated body-fat recovery (catch-up fat), and enhanced glucose utilization in adipose tissue. Our objective was to characterize the determinants of enhanced glucose utilization in adipose tissue during catch-up fat.

RESEARCH DESIGN AND METHODS—White adipose tissue morphometry, lipogenic capacity, fatty acid composition, insulin signaling, in vivo glucose homeostasis, and insulinemic response to glucose were assessed in a rat model of semistarvation-refeeding. This model is characterized by glucose redistribution from skeletal muscle to adipose tissue during catch-up fat that results solely from suppressed thermogenesis (i.e., without hyperphagia).

RESULTS—Adipose tissue recovery during the dynamic phase of catch-up fat is accompanied by increased adipocyte number with smaller diameter, increased expression of genes for adipogenesis and de novo lipogenesis, increased fatty acid synthase activity, increased proportion of saturated fatty acids in triglyceride (storage) fraction but not in phospholipid (membrane) fraction, and no impairment in insulin signaling. Furthermore, it is shown that hyperinsulinemia and enhanced adipose tissue de novo lipogenesis occur concomitantly and are very early events in catch-up fat.

CONCLUSIONS—These findings suggest that increased adipose tissue insulin stimulation and consequential increase in intracellular glucose flux play an important role in initiating catch-up fat. Once activated, the machinery for lipogenesis and adipogenesis contribute to sustain an increased insulin-stimulated glucose flux toward fat storage. Such adipose tissue plasticity could play an active role in the thrifty metabolism that underlies glucose redistribution from skeletal muscle to adipose tissue. *Diabetes* 58:2228–2237, 2009

The pattern of growth early in life is now recognized to be an important predictor of chronic metabolic diseases. In particular, people who had low birth weight or whose growth faltered during infancy and childhood, but who subsequently showed catch-up growth, had higher propensity for the development of abdominal obesity, type 2 diabetes, and cardiovascular diseases later in life (1–8). The mechanistic basis of the link between catch-up growth and risks for these chronic diseases is poorly understood. There is, however, compelling evidence that mammalian catch-up growth is characterized by a disproportionately higher rate of body fat than lean tissue gain (9) and that an early feature of such "preferential catch-up fat" is concomitant hyperinsulinemia (10).

Using a rat model of semistarvation-refeeding (11), in which catch-up fat is studied in the absence of hyperphagia, we previously showed that the hyperinsulinemic state of catch-up fat preceded the development of excess adiposity and could be linked to suppressed thermogenesis, per se, in the absence of hyperphagia (12). Subsequent studies of hyperinsulinemic-euglycemic clamps during catch-up fat showed that in vivo insulin-mediated glucose utilization was diminished in skeletal muscle but enhanced in white adipose tissue (WAT), suggesting that preferential catch-up fat is characterized by glucose redistribution from skeletal muscle to WAT (13). Consistent with this hypothesis are the demonstrations, in this rat model of catch-up fat, of diminished mitochondrial mass and lower insulin receptor substrate (IRS)-1–associated phosphatidylinositol-3-kinase (PI3K) activity in the skeletal muscle (14,15). Furthermore, ex vivo studies in WAT have previously shown that glucose uptake and utilization are enhanced during refeeding after fasting or caloric restriction (16,17).

Elucidating the mechanisms that underlie such enhancement in glucose uptake and glucose flux toward lipid synthesis in WAT is therefore of central importance in understanding the mechanisms of glucose redistribution during catch-up fat. In addressing this topic, we have characterized our rat model of catch-up fat for changes in adipose tissue morphometry (adipocyte size and number) and fatty acid composition given their importance as determinants of WAT responsiveness to the action of insulin on glucose utilization. Indeed, it is established that small adipocytes have a greater capacity for insulin-

mediated glucose uptake and de novo lipogenesis than larger ones (18–22), while alterations in adipocyte membrane phospholipid composition in favor of a high ratio of polyunsaturated fatty acids (PUFAs) to saturated fatty acids (SFAs) correlates with increased rate of insulin-stimulated glucose transport and glucose flux toward de novo lipogenesis in WAT (23–24). We have therefore investigated here the extent to which differences in adipocyte number and diameter, key gene markers for adipocyte proliferation, as well as the fatty acid composition of phospholipid and triglyceride lipid fractions of WAT, might be involved in the enhanced glucose flux toward lipogenesis. Furthermore, given the importance of insulin signaling in adipocyte growth (25) and in controlling glucose flux toward lipogenesis (26,27), we have also evaluated the in vivo insulinemic response to glucose and investigated proximal insulin signaling in WAT under basal and in vivo insulin-stimulated conditions during catch-up fat.

RESEARCH DESIGN AND METHODS

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 standard diet (Kliba, Cossonay, Switzerland), consisting, by energy, of 24% protein, 66% carbohydrates, and 10% fat, and had free access to tap water. Animals were maintained in accordance with our institute's regulations and guide for the care and use of laboratory animals.

The experiments were performed in rats within an age range characterized by high rate of growth for controls and by catch-up growth in the refed group during ad libitum access to food (online appendix [available at http://diabetes.diabetesjournals.org/cgi/content/full/db08-1793/DC1]). The experimental design is similar to that previously described in establishing a rat model for studying changes in energy expenditure that occur during accelerated fat deposition (i.e., catch-up fat) upon refeeding after growth arrest (11,12) (i.e., an approach that allows suppressed thermogenesis specific for accelerated fat recovery [catch-up fat] to be studied in the absence of confounding variables such food intake and differential rates of protein gain) on energy expenditure. In brief, groups of 7-week-old rats (mean body weight of 225 g) are food restricted at 50% of their spontaneous food intake for 2 weeks. After this period of growth arrest, they are refed the same standard diet at a level equal in metabolizable energy content to the spontaneous food intake of control rats matched for weight at the onset of refeeding. Under these conditions, the refed animals show similar gain in lean mass but about twofold increase in body fat gain than controls for a period of 2 weeks, due to 10–13% lower energy expenditure resulting from suppressed thermogenesis (11,12).

Body composition. Body composition was determined at the end of semi-starvation (corresponding to day 0 of refeeding) and subsequently on days 5, 10, 15, and 20 of refeeding in refed and control groups, as indicated in Fig. 1. After the animals were killed, the whole carcasses were dried to a constant weight in an oven maintained at 70°C and subsequently homogenized for analysis of fat content by the Soxhlet extraction method as previously described (11); the dry lean body mass (a proxy of protein mass) was determined by subtracting total body fat and body water content from body weight.

Glucose tolerance test. Intraperitoneal glucose tolerance test (GTT) was performed as previously described (12). Plasma glucose was determined using a Beckman glucose analyzer (Beckman Instruments, Palo Alto, CA), while plasma insulin was assessed using a rat insulin enzyme-linked immunosorbent assay kit (Crystal Chem, Downers Grove, IL).

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 (28), and suspensions of adipocytes were analyzed using Multisizer 3 Coulter Counter. For determination of cell size (adipocyte diameter distribution), similar amounts of cells (~10,000) were aspirated by the machine and classified according to their diameter and frequency.

Molecular measurements and lipid biochemistry. After harvesting, the adipose tissue was snap frozen in liquid nitrogen and stored at −80°C. Total RNA was isolated from 50 to 150 mg of powdered adipose tissue using the method of Chomczynski and Sacchi (29). After phase separation, RNA was precipitated with isopropanol, cDNA was synthesized from 250 ng of total RNA, and RT-PCR was performed. Cyclophilin was used as an invariant control since we found that its mRNA levels in WAT are not significantly altered in response to semistarvation or to refeeding in our rat model. The relative quantification for a given gene was thus corrected for the cyclophilin mRNA values.

Fatty acid synthase (FAS) activity was measured according to the protocol described by Pénicaud et al. (30). For fatty acid profiling, equal amounts of tissue samples were suspended (1:2:0.8, vol/vol/vol), vortexed, and incubated for 10 min at room temperature. Lipids were extracted using the method of Bligh and Dyer (31), and the fatty acid methyl esters were prepared as described by Morrison and Smith (32) and were analyzed by gas chromatography/mass spectrometry as detailed previously (33).

For PI3K assay, 200–500 mg of protein extract was immunoprecipitated with IRS-1 (Upstate) polyclonal antibody. The kinase reaction, thin-layer chromatography separation, and signal detection were performed as previously described (15,34). Akt (Ser473) and extracellular signal–regulated kinase (ERK) (p44/42 mitogen-activated protein kinase) were evaluated by immunoblot analysis. A total of 40 µg of protein extract were separated by SDS-PAGE and blotted on polyvinylidene fluoride membranes that were analyzed with polyclonal antibodies (Cell Signaling) raised against the indicated phosphorylation sites. Bands within the linear range were quantified densitometrically. Total protein amount of Akt and ERK were measured in the same way by using corresponding antibodies (Cell Signaling).

In vivo bolus administration of insulin. The rats were fasted from 0700 h, and 4–7 h later subgroups were anesthetized by injection of ketamine/xylazine (39/5 mg/kg body wt) and surgically prepared for a bolus injection (through the jugular vein) of insulin (10 units/kg body wt) (Actrapid; Novo Nordisk) or an equal volume of saline vehicle. Insulin or saline was injected 3 min before rats were killed and the tissue was harvested. Immediately after the rats were killed, the epididymal adipose tissue as well as skeletal muscles were rapidly dissected, frozen in liquid nitrogen, and stored at −80°C until analysis. The timing for tissue harvesting after hormonal administration, and the dose of insulin utilized, correspond to maximal activation of PI3K assessed in preliminary studies and are consistent with those obtained by others (35).

Data analysis and statistics. All data are presented as means ± SE. Direct comparisons between the two groups were performed using unpaired t tests. Between-group differences in distribution curves for adipocyte diameter were analyzed by the Kolmogorov-Smirnov test, which takes into account the ordering (i.e., cell diameter) of the categories and is therefore a suitable test for analysis of curve shifts. Between-group differences across time were analyzed by regression analysis incorporating between-group comparison of slopes. For the study comparing refed and control animals in response to hormone or saline, the data were analyzed by two-factor ANOVA for the main effects of groups (refed versus control), treatment (hormone versus saline), and group-times-treatment interactions. All statistical treatment of data were performed using the computer software STATISTIX 8 (Analytical Software, Tallahassee, FL).

RESULTS

Body composition and GTT. In this rat model of catch-up fat, the end of semistarvation (or onset of refeeding) is characterized by the fact that the semistarved and control animals display similar body weight (Fig. 1A) and dry lean body mass (Fig. 1C), but compared with control rats, the semistarved animals have 50% less fat mass (8 vs. 15.7 g, $P < 0.001$) (Fig. 1D) and higher body water content (155 vs. 148 g, $P < 0.01$). Between days 0 and 15 of refeeding on an isocaloric food intake relative to the controls (Fig. 1B), the refed rats gained fat at a rate that is about two times greater than that of controls (Fig. 1D), whereas during days 15–20, both groups display similar rate of fat gain. This enhanced efficiency of catch-up fat during days 0–15 contrasts with no differences in lean body mass gain (Fig. 1C). GTT conducted on days 8 and 9 of refeeding show no between-group differences in glucose homeostasis (Fig. 1E) but reveal plasma insulin concentrations after the glucose load that are clearly higher in refed than in control animals ($P < 0.01$ by ANOVA test) (Fig. 1F).

Adipocyte number and size. The data on adipocyte cell size distribution (Fig. 2A, *left panel*), mean adipocyte diameter (Fig. 2D), and adipocyte cell number (Fig. 2C) from epididymal WAT (EWAT) are presented for refed and control animals at different time points during the phase of
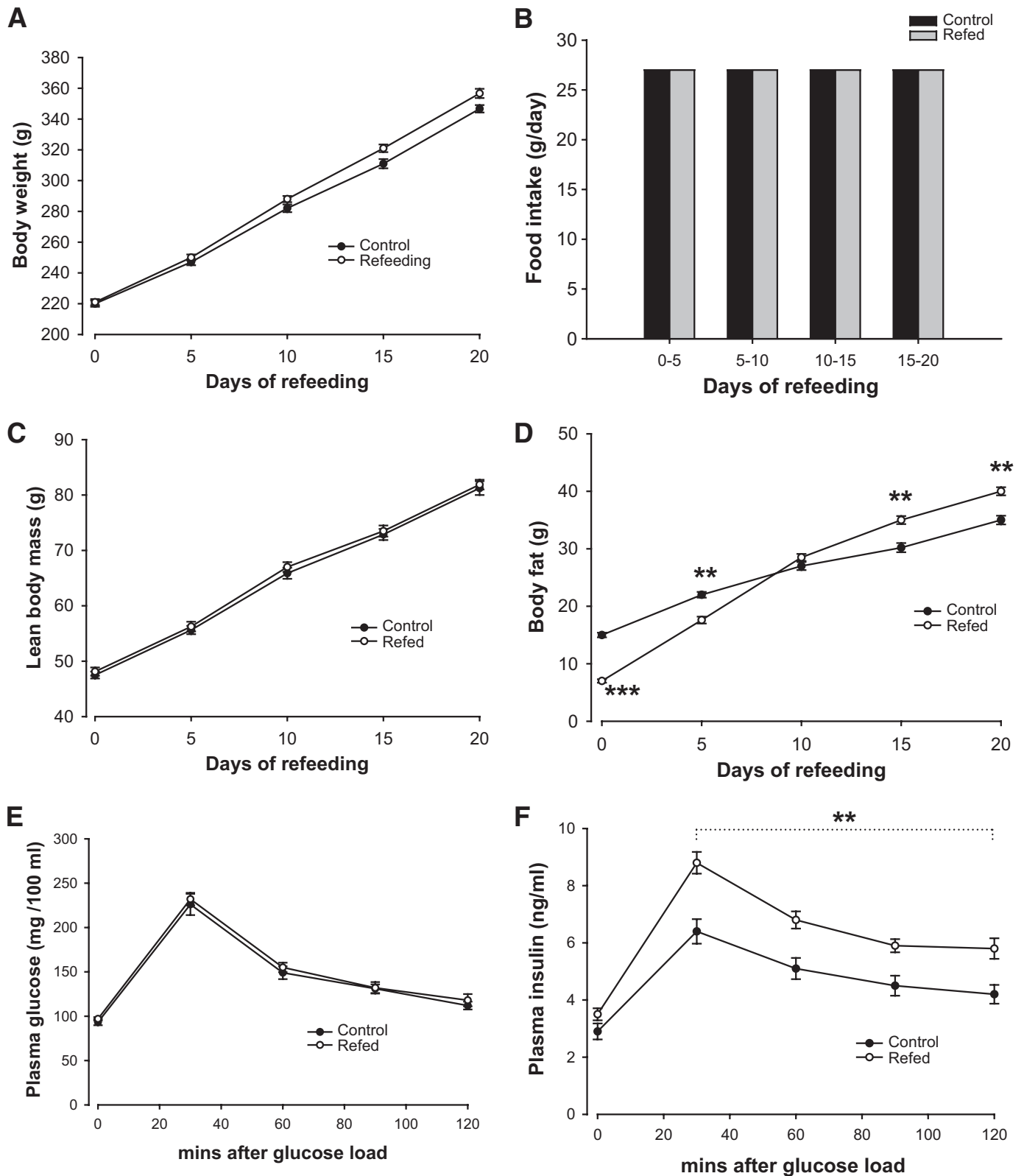
FIG. 1. Hyperinsulinemia in our rat model of semistarvation-refeeding, in which the accelerated fat recovery (catch-up fat) results only from diminished energy expenditure (suppressed thermogenesis) and not from hyperphagia. In this particular investigation here, body weight and body composition (*A*, *C*, and *D*) were determined at day 0, 5, 10, 15, and 20 of refeeding, with both groups consuming isocaloric amount of standard diet (*B*). The acceleration of fat mass but not that of lean mass (i.e., preferential catch-up fat) lasts for about 2 weeks. *E* and *F*: Shows the plasma glucose and insulin concentrations before and for 2 h after an intraperitoneal injection of glucose (2 g/kg body wt); the test of glucose tolerance was performed on days 8 and 9 of refeeding. All values are means ± SE (*n* = 6); **$P < 0.01$; ***$P < 0.001$.
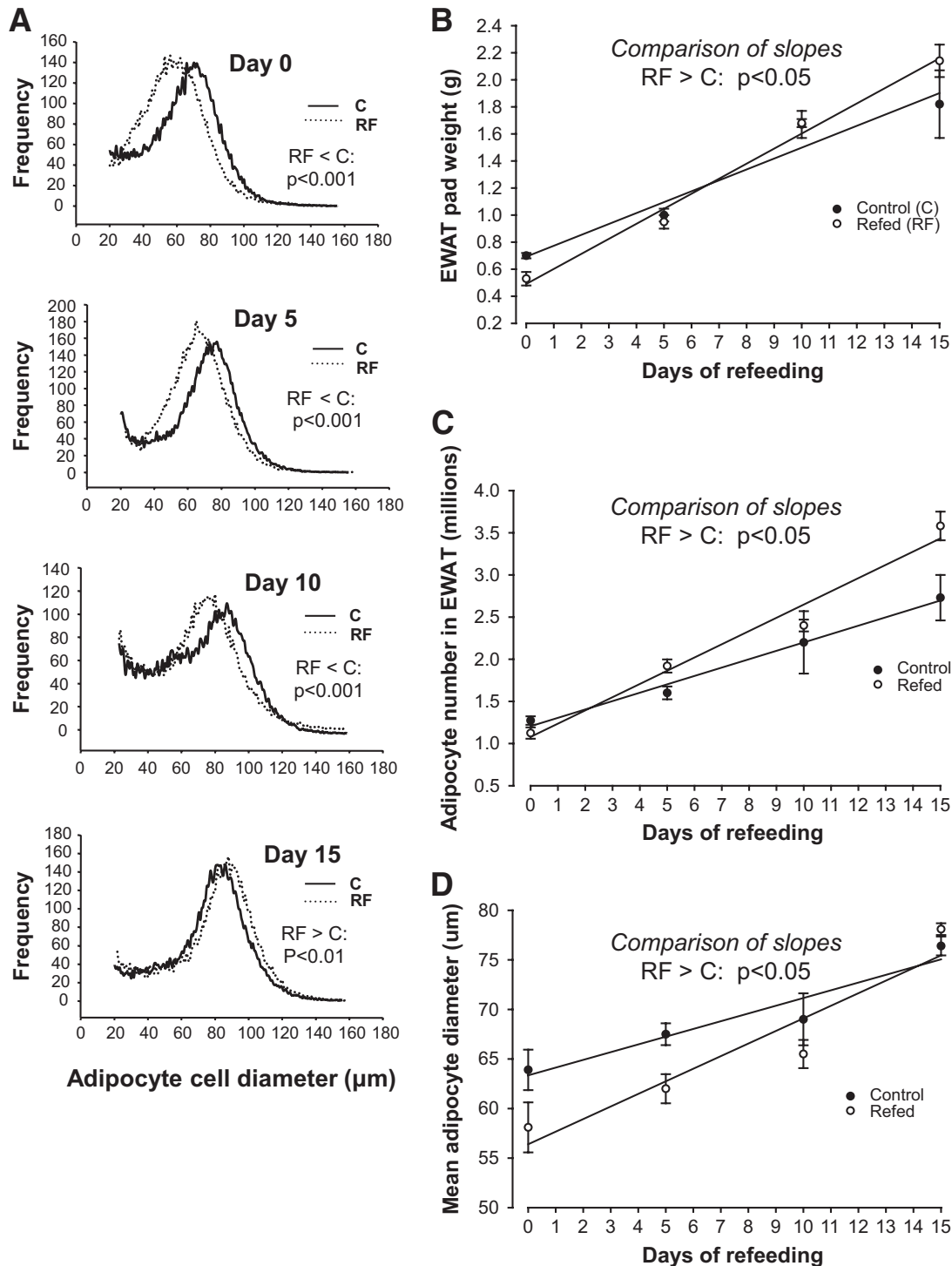
FIG. 2. EWAT morphometry showing the frequency distribution curves for adipocyte cell diameter (*A*), the EWAT pad weight (*B*), the total adipocyte cell number per EWAT pad (*C*), and the mean adipocyte diameter (*D*) in refed (RF) or control (C) animals at the end of semistarvation (day 0) and days 5, 10, and 15 of isocaloric refeeding. Values are means ± SE (*n* = 6). Statistical analysis for differences in refed and controls for adipocyte diameter distribution curves were performed by Kolmogorov-Smirnov tests, while between-group differences across time in EWAT pad weight, adipocyte number, and mean adipocyte diameter were determined by regression analysis and comparison of slopes. The regression coefficients (r^2) of the regression lines in *B–D* are as follows. *B*: r^2 = 0.64 and 0.93 for control and refed, respectively. *C*: r^2 = 0.52 and 0.90 for control and refed, respectively. *D*: r^2 = 0.47 and 0.71 for control and refed, respectively. In *B–D*, the above-mentioned r^2 values are statistically significant (*P* < 0.01).

catch-up fat. After 2 weeks of semistarvation (corresponding to day 0 of refeeding), the weight of EWAT in semi-starved animals is found to be significantly lower than in controls (Fig. 2*B*) and is associated with a significant shift-to-the-left of adipocyte diameter distribution (*P* <

0.001) and hence reduction in average adipocyte diameter, without a significant reduction in adipocyte cell number (Fig. 2*C*). During the first 15 days of isocaloric refeeding, EWAT weight increases faster in refed than in control groups (Fig. 2*B*), and the rate at which adipocyte cell
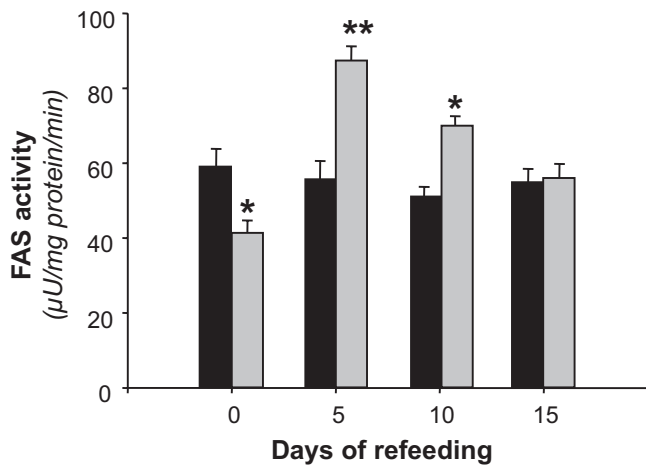
FIG. 3. Time-course analysis of FAS activity in EWAT in refed (▨) or control (■) animals at the end of semistarvation (day 0) and on days 5, 10, and 15 of isocaloric refeeding. Values are means ± SE (n = 6); statistical significance of differences is indicated as follows: * P < 0.05; ** P < 0.01, as assessed by unpaired t test.

number increases is also found to be significantly greater in refed than in control groups, as indicated by statistical comparisons of their slopes of regression (Fig. 2C). Adipocyte diameter remains significantly lower in EWAT from refed animals than in controls on day 5 and on day 10 (Fig. 2A and D) and only exceeded those of controls at day 15. Thus, the recovery of adipose tissue, even in the absence of hyperphagia, is accompanied by an increase in adipocyte number, while the adipocyte diameter remains lower in refed than in control animals during the dynamic phase of catch-up fat, lasting for at least 10 days in this study.

Adipose tissue FAS activity. The data on FAS activity in EWAT on days 0, 5, 10, and 15 of refeeding are shown in Fig. 3. At the end of semistarvation (day 0), the activity of FAS is found to be lower than in controls by ~30% (P < 0.05) but overshoots above control upon refeeding. It is significantly higher in refed animals than in controls on

day 5 (+60%, P < 0.01) and on day 10 (+25%, P < 0.05), whereas on day 15, there are no longer significant differences between refed and controls.

Adipose fatty acid composition. The data on the fatty acid profile of the triglyceride and phospholipid fractions obtained from EWAT of animals on days 8 and 9 of refeeding are presented in Fig. 4. In the triglyceride fraction (Fig. 4A), the proportion of SFAs is significantly higher (P < 0.01) in refed than in control animals, while the proportion of PUFAs, as well as the PUFA-to-SFA ratio are significantly lower (P < 0.001) in refed than in control animals. By contrast, the proportions of SFAs, monounsaturated fatty acids (MUFAs), total PUFAs, and the PUFA-to-SFA ratio in the phospholipid fraction (Fig. 4B) are not different in EWAT from refed and control groups.

Adipose insulin signaling. To assess proximal insulin signaling in adipose tissue, we measured IRS-1–associated PI3K activity in EWAT from refed and control animals after in vivo administration of insulin or saline control on days 8 and 9 of refeeding. The results, presented in Fig. 5A, show that PI3K activity is marginally higher in refed than in control animals (overall group effect by two-factor ANOVA, i.e., refed versus control: P = 0.045), with post hoc pairwise comparisons by unpaired t test indicating that between-group difference in insulin-stimulated PI3K activity (+12%, P = 0.09) or in basal PI3K activity (+31%, P = 0.07) failing to reach statistical significance. Similar lack of statistical significance is also observed with other pairwise comparison tests (e.g., Scheffe's, Tukey). Furthermore, measurement of downstream signaling (Akt phosphorylation) (Fig. 5B) shows no statistically significant group effect by ANOVA. Similarly, there are no differences between refed and controls in ERK phosphorylation (data not shown). Taken together, these data on days 8 and 9 of catch-up fat suggest that at the dose of insulin tested, there is no significant difference in the sensitivity of the insulin receptor IRS-1/PI3K/Akt signaling pathway.

Analysis of early catch-up fat. We also investigated in vivo insulinemic response to glucose, adipose tissue insu-
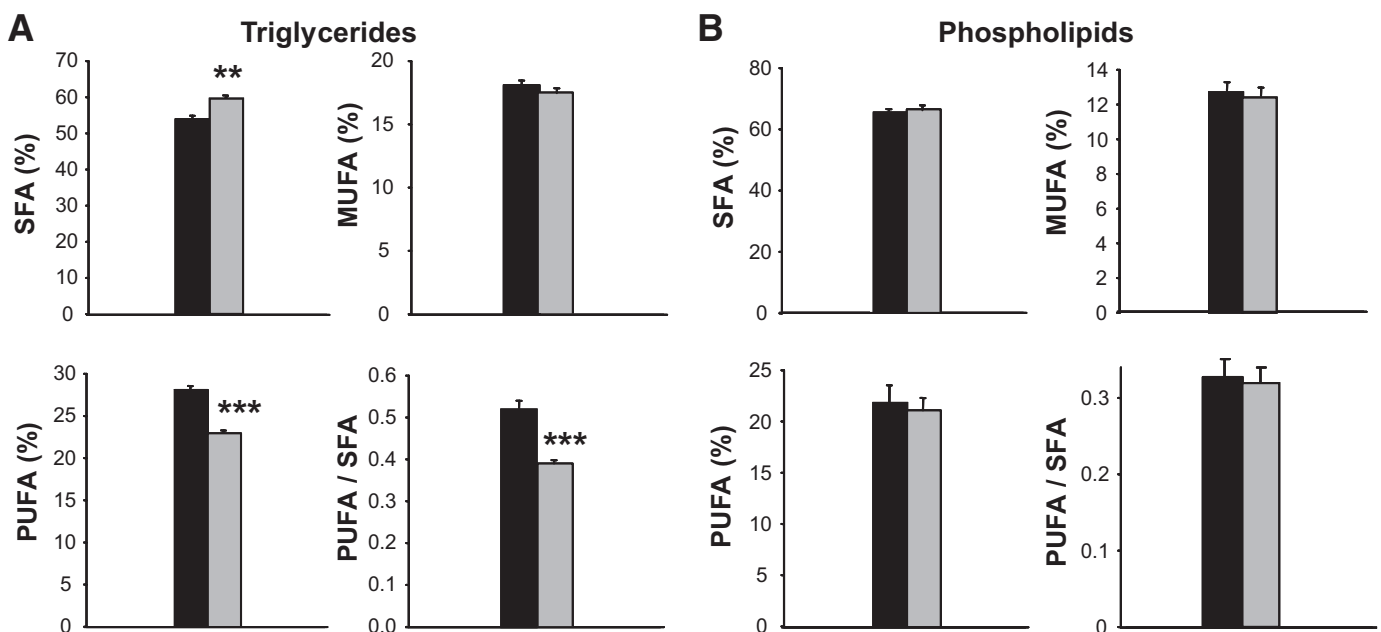
FIG. 4. Fatty acids composition of triglyceride fraction (A) and phospholipid fraction (B) extracted from EWAT pads of control (■) and refed (▨) animals on days 8 and 9 of refeeding. Values are means ± SE (n = 6). **P < 0.01; ***P < 0.001, as assessed by unpaired t test.

A

B
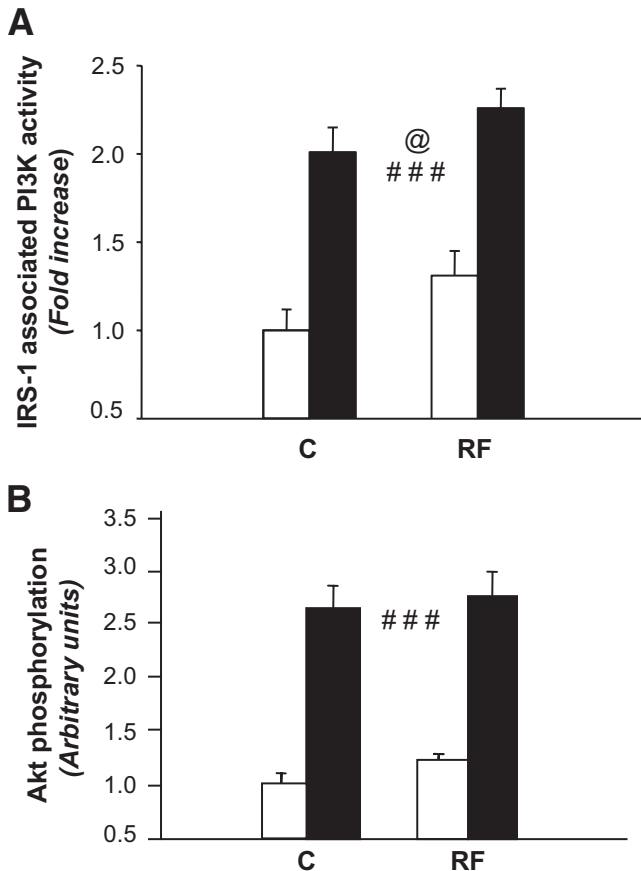
FIG. 5. PI3K activity and Akt phosphorylation in epididymal fat pads in response to either an acute bolus of intravenous administration of 10 units/kg of insulin (■) or a similar volume of saline (□) in refed (RF) and control (C) groups on days 8 and 9 of refeeding. Values are means ± SE ($n = 6$); statistical significance of differences is indicated as follows: @, effect of group (C versus RF); #effect of treatment (hormone versus saline), as assessed by ANOVA. ###$P < 0.001$. Post hoc between-group comparison treated with either saline or insulin was performed by unpaired t test.

lin signaling, and gene expression reprogramming within the first day of catch-up fat (20–23 h after the start of the semistarvation-refeeding transition). This time point was chosen to be as close as possible to the initial process of catch-up fat, while minimizing the gorging effects that typically occur at the onset of refeeding. This was achieved by providing the first day's refeeding diet in three equal parts at 1500, 1900, and 2300 h during this transition, with all food removed at 0700 h the next day and GTT measurements starting 4 h later. As shown in Fig. 6A and B, refed and control rats display similar glucose tolerance, but the hyperinsulinemic response to the glucose load is already present within day 1 of refeeding. Furthermore, we found that on day 1 of refeeding, FAS activity was higher in refed than in control animals 2 h after the glucose load but not in saline controls (Fig. 6C). FAS activity 2 h after the glucose load was also found to correlate positively with both basal and postglucose insulin levels, with the strongest correlations observed with the 30-min (peak) insulin values ($r^2 = 0.5$, $P < 0.02$). To investigate postreceptor events at this early stage of catch-up fat, we measured Akt phosphorylation, ERK phosphorylation, and GLUT4 protein expression in EWAT harvested from refed and control rats after injection of insulin or saline. The results (Fig. 6D) did not reveal any difference in these postreceptor events.

Induction of genes implicated in lipogenesis and adipogenesis. We performed a detailed time course of the expression of genes involved in lipogenesis (*GLUT4, FAS,* and *SREBP1c*) and in adipogenesis (*PPARγ* and *CEBPα*). The data in Fig. 7 show that the expression of these genes are all rapidly induced within day 1 of refeeding relative to semistarvation (day 0), with FAS expression significantly exceeding fed control values on this first day of refeeding. By day 3 of refeeding, all these genes for lipogenesis and adipogenesis were upregulated relative to fed controls. These inductions were still present after 9 days of catch-up fat ($P < 0.01$). By contrast, whereas the gene expression of steoryl CoA desaturase 1 (SCD1) was rapidly induced upon refeeding (relative to semistarvation), SCD1 mRNA levels were similar in refed and controls during catch-up fat.

DISCUSSION

Using a rat model of semistarvation-refeeding in which, from a perspective of energy balance, catch-up fat results solely from lower energy expenditure (and not from hyperphagia), we previously reported that during catch-up fat, the in vivo glucose utilization, under insulin stimulation, is diminished in skeletal muscle but enhanced in WAT (13). The studies reported here suggest that this enhanced capacity for glucose utilization in WAT during catch-up fat is associated with *1*) adipose tissue hyperplasia and controlled hypertrophic growth, with adipocyte size remaining significantly smaller than controls; *2*) an early and sustained enhancement in the capacity for lipogenesis, in parallel to glucose-induced hyperinsulinemia and preserved proximal insulin signaling; and *3*) an increase in the proportion of SFAs specifically in the triglyceride (storage) fraction of WAT but not in the phospholipids (membrane) component. Taken together, these findings during catch-up fat underscore adipose tissue plasticity in favor of an enhanced glucose flux toward fat storage in WAT and suggests that hyperinsulinemia might play a major role in the intitiation and promotion of catch-up fat.

Adipose tissue morphometry. An inverse relationship between adipocyte size and insulin-stimulated glucose utilization is well established (18–22) and is directly supported by in vitro studies (20,21) comparing large and small adipocytes isolated from animals varying in size or by comparing adipocytes varying in size from the same animal (36). While the mechanisms of enhanced insulin sensitivity pertaining to glucose uptake in small adipocytes are poorly understood, they have often been linked to an increase in the number of adipocytes and to enhanced adipogenesis (37). Consequently, the question arose whether the enhanced in vivo insulin-mediated glucose utilization in WAT found in our rat model of catch-up fat (13) could also be related to the progressive recovery of adipocyte size accompanied by an increase in the number of adipocytes. The results presented here show that adipocyte size, which is lower at the end of semistarvation, remains smaller in refed animals than in controls for at least 10 days of refeeding (Fig. 2A). This lower adipocyte size during catch-up fat can in part be explained by enhanced adipogenesis as evidenced from the faster increase in adipocyte number, together with the upregulation of *PPARγ* and *CEBPα* gene expression. While these observations are consistent with several studies (38–41) describing WAT hyperplasia during refeeding
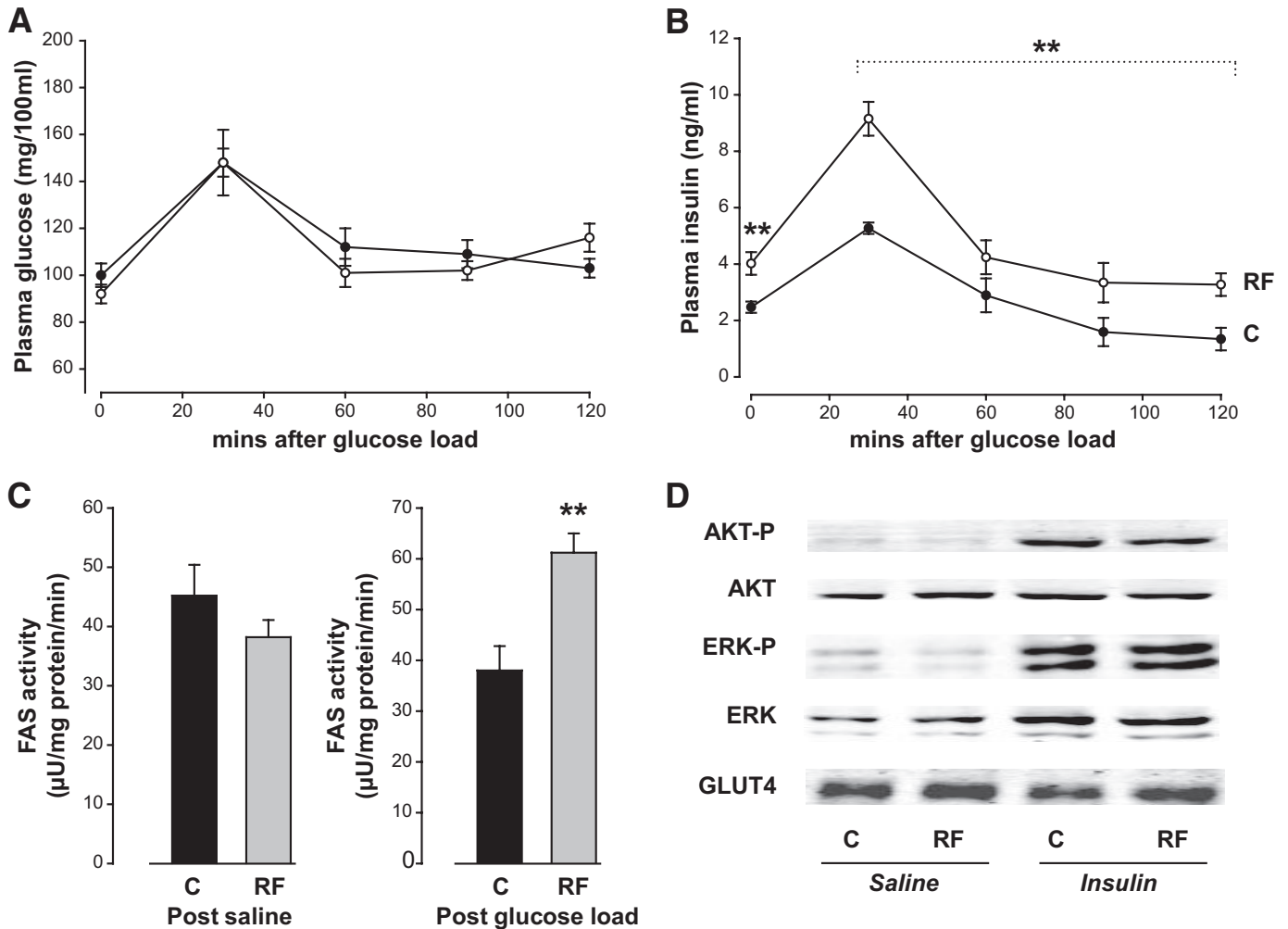
FIG. 6. Analysis of early catch-up fat (day 1 of refeeding). *A* and *B*: The plasma glucose and insulin concentrations before and for 2 h after an intraperitoneal injection of glucose (2 g/kg body wt) during glucose tolerance test. ●, control; ○, refed. *C*: The activity of FAS in EWAT obtained at the end of the GTT test (i.e., 2-h postglucose load) or after saline injection. *D*: The results of a representative Western blot pertaining to proximal insulin signaling in response to bolus administration of insulin or saline; for ERK, the *upper* and *lower bands* correspond to P44 and P42, respectively. C, control; RF, refed. All values are means ± SE ($n = 6$–8); **$P < 0.01$ by unpaired t test.

following caloric restriction, our studies here, however, demonstrate for the first time that hyperphagia is not a requirement for the induction of hyperplasia in response to refeeding after growth arrest.

Adipose tissue de novo lipogenesis and fatty acid profiling. The present studies showing that the adipose tissue of refed animals display marked increases in FAS activity and in the expression of key genes implicated in de novo lipogenesis (*GLUT4*, *SREBP1c*, and *FAS*) are also consistent with our previous demonstration of catch-up fat as a state of increased insulin-mediated glucose utilization in WAT (13). In contrast to the marked upregulation of de novo lipogenic genes in WAT, however, the expression of SCD1, the enzyme that catalyzes desaturation of SFAs to monounsaturates, was not found to differ from that of controls during catch-up fat. The enhancement of de novo lipogenesis (relative to fed controls) without a parallel increase in SCD1-induced desaturation could explain the increase in the proportion of SFAs in the triglyceride lipid fraction of adipose tissue during catch-up fat.

Adipose tissue de novo lipogenesis versus insulin signaling. To test whether the increased lipogenesis and glucose uptake in WAT observed during catch up-fat depend on increased insulin sensitivity, we measured proximal-insulin signaling following in vivo administration of an insulin bolus on days 8 and 9 of refeeding. Measurements of IRS-1–associated PI3K activity, AKT, and ERK phosphorylation revealed a small statistically significant induction of IRS-1–associated PI3K activity during catch-up fat, which, however, was not reflected in an increased AKT phosphorylation or in ERK activation. Although these results show only a marginal increase in insulin signaling, it is important to emphasize that insulin signaling is clearly not impaired, which is in sharp contrast to the diminished PI3K activity in skeletal muscle obtained from the same animals (15). Furthermore, unlike in skeletal muscle, where the insulin-resistant state can also be associated with a decreased ratio of PUFA to SFA in the phospholipid fraction (33), the data presented here on adipose tissue show no such alterations in phospholipids composition. These findings of differential regulation of PI3K signaling and phospholipid composition in skeletal muscle and adipose tissue are in line with the previously reported studies of hyperinsulinemic-euglycemic clamps (13), showing that glucose uptake is reduced in skeletal muscle and enhanced in WAT during catch-up fat.

Whether adipose tissue reprogramming precedes or is consequential to skeletal muscle insulin resistance is not
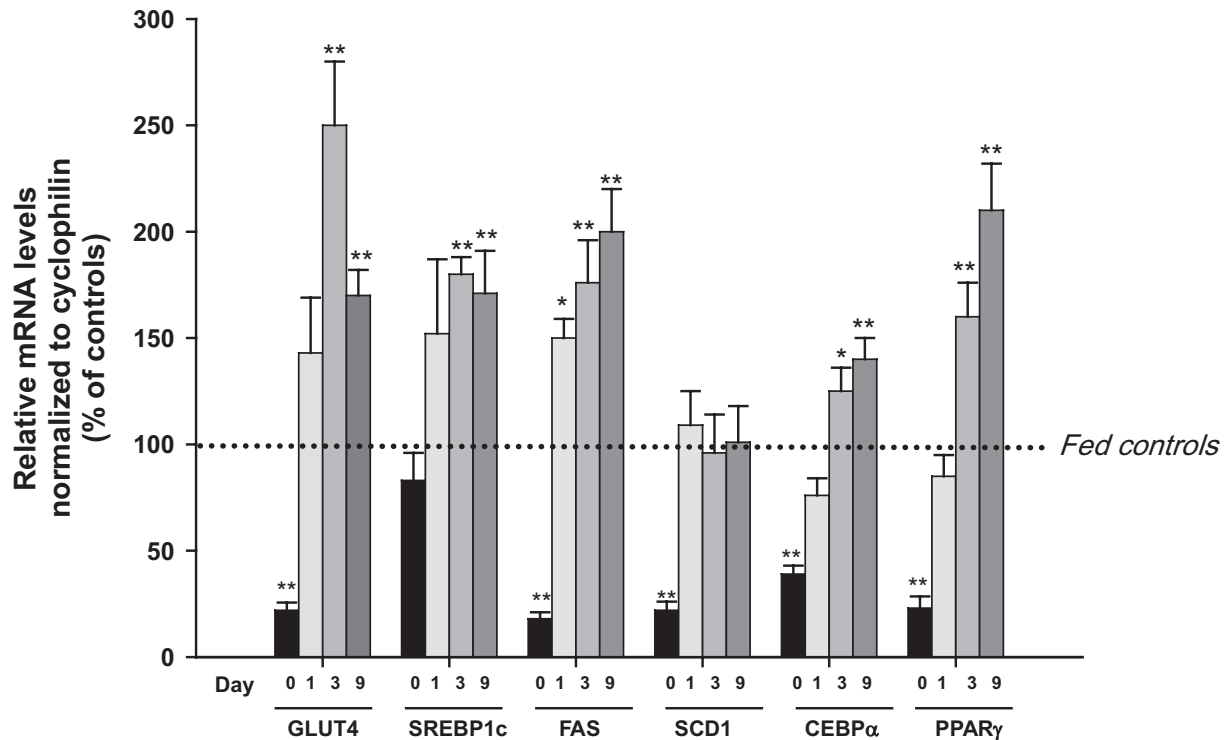
FIG. 7. Time-course gene expression analysis of key genes implicated in adipogenesis and in glucose flux toward lipogenesis during the course of catch-up fat, namely at the end of semistarvation (day 0) and on days 1, 3, and 9 of refeeding. The data in EWAT from semistarved (day 0) or refed animals (days 1, 3, and 9) are expressed as a percentage of that of the respective fed controls (represented as 100%; dotted line). Values are means ± SE ($n = 6–8$); for each time-point, statistical significance of differences are relative to fed controls and indicated as follows: *$P < 0.05$; **$P < 0.01$ as assessed by unpaired t test.

known. However, a state of whole-body insulin resistance is already evident on day 1 of refeeding, as judged by a higher insulin response curve but normal glucose tolerance in refed animals relative to controls following administration of a glucose load (Fig. 6). Whether this hyperinsulinemia can be explained entirely by peripheral muscle insulin resistance or whether it may also be contributed by pancreatic β-cell hyperresponsiveness to glucose is not known and is an interesting avenue for future investigations. Nonetheless, the data obtained here on day 1 of catch-up fat showing rapid induction in the expression of genes controlling de novo lipogenesis in WAT concomitant to a state of whole-body insulin resistance suggest that hyperinsulinemia is an early event and could therefore be a causal factor in the induction of de novo lipogenesis in adipose tissue at the onset of refeeding. As this hyperinsulemia persists during the course of catch-up fat, it may also be important in maintaining the high rate of de novo lipogenesis, as reflected by the marked upregulation of lipogenic genes and/or high FAS activity between days 3 and 10 of refeeding. However, while increased insulin is undeniably a major factor in initiating and sustaining enhancing glucose uptake and de novo lipogenesis, the results of our previous hyperinsu-linemic-euglycemic clamp studies (13) showing enhanced glucose utilization in adipose tissue on day 7 of catch-up fat implies increased insulin-dependent glucose utilization by adipocytes for the same insulin stimulation. Consequently, it can be speculated that the marked increases in de novo lipogenic capacity observed here between days 3 and 10 of catch up fat could also be contributing to the increased glucose uptake in adipose tissue. This concept, which embodies interactions between insulin-stimulated

glucose uptake and de novo lipogenesis in adipose tissue (depicted in Fig. 8), is consistent with studies showing that pharmacological inhibitors of de novo lipogenesis decrease the ability of insulin to stimulate both the pentose shunt glucose oxidation and overall glucose utilization but not Krebs cycle or glyceride-glycerol synthesis (18,20). In other words, 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 led to the proposal that the enzymatic capacity for fatty acid synthesis is an important factor in determining insulin-stimulated glucose utilization in WAT (18,20).

Conclusions. We show here that the process of catch-up fat involves an early and sustained induction of hyperinsulinemia, increased glucose flux toward lipogenesis, increased SFAs specifically channelled to the triglyceride stores and not to membrane phospholipids and that the recovery of adipocyte size is accompanied by enhanced adipogenesis, thereby limiting adipocyte hypertrophy. Such adipose tissue plasticity suggest that WAT plays an active role in glucose redistribution toward catch-up fat during catch-up growth. Impairment in adipose tissue plasticity may underlie some of the pathophysiological consequences of catch-up growth. Indeed, enhanced lipogenesis in the absence of hyperplasia was recently reported in a murine model of postnatal catch-up growth characterized by hypertrophic adipocytes and glucose intolerance (42). However, as underscored by apparently conflicting findings that postnatal catch-up growth after protein malnutrition programs proliferation of preadipocytes in rats (43), it is likely that genetics and epigenetics-
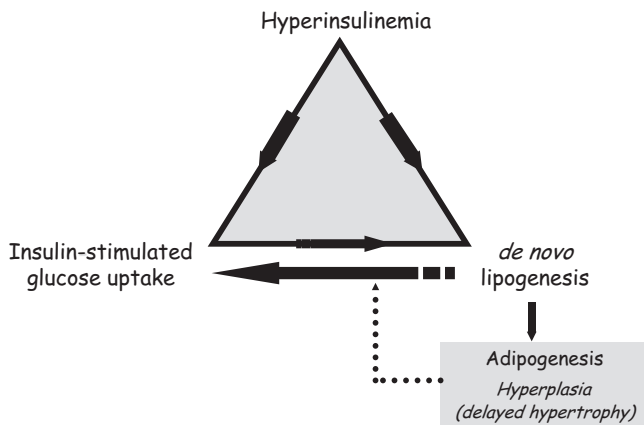
FIG. 8. A model integrating our previous (13) and current findings based on the interrelationships between hyperinsulinemia, insulin-dependent glucose uptake, and de novo lipogenesis. In this model, hyperinsulinemia on day 1 of refeeding plays an important role in initiating and sustaining catch-up fat through the stimulation of glucose uptake and de novo lipogenesis in adipocytes. Once the machinery for de novo lipogenesis is fully activated, the increased glucose metabolism to de novo lipogenesis will contribute to the enhanced insulin-dependent glucose uptake observed in adipose tissue during catch-up fat. According to this model the enzymatic capacity for fatty acid synthesis is hence an important factor in determining insulin-stimulated glucose utilization in WAT. The newly synthesized lipids will lead to a moderate adipocytes hypertrophic growth since hyperplastic growth will channel part of the lipid pool in newly formed adipocytes. The net effect is a delayed adipocyte size restoration, which could possibly play a role in maintaining the high lipogenic activity and insulin sensitivity observed in adipose tissue during catch-up fat.

environment interactions play a critical role in defining adipose tissue plasticity during catch-up growth (44).

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