A Role for Suppressed Thermogenesis Favoring Catch-Up Fat in the Pathophysiology of Catch-Up Growth

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Catch-up growth is a risk factor for later obesity, type 2 diabetes, and cardiovascular diseases. We show here that after growth arrest by semistarvation, rats refed the same amount of a low-fat diet as controls show *1*) lower energy expenditure due to diminished thermogenesis that favors accelerated fat deposition or catch-up fat and *2*) normal glucose tolerance but higher plasma insulin after a glucose load at a time point when their body fat and plasma free fatty acids (FFAs) have not exceeded those of controls. Isocaloric refeeding on a high-fat diet resulted in even lower energy expenditure and thermogenesis and increased fat deposition and led to even higher plasma insulin and elevated plasma glucose after a glucose load. Stepwise regression analysis showed that plasma insulin and insulin-to-glucose ratio after the glucose load are predicted by variations in efficiency of energy use (i.e., in thermogenesis) rather than by the absolute amount of body fat or plasma FFAs. These studies suggest that suppression of thermogenesis per se may have a primary role in the development of hyperinsulinemia and insulin resistance during catch-up growth and underscore a role for suppressed thermogenesis directed specifically at catch-up fat in the link between catch-up growth and chronic metabolic diseases. *Diabetes* 52:1090–1097, 2003

Catch-up growth is generally considered a physiological adaptation that allows humans and other higher animals to return to their genetically programmed growth trajectory after a period of growth retardation. There is, however, an impressive body of epidemiological evidence suggesting that catch-up growth also has long-term pathophysiological consequences (1–8). These studies suggest that people who had low birth weight or who were stunted during infancy and childhood, but who subsequently showed catch-up growth, had higher susceptibility for central obesity, impaired glucose tolerance, diabetes, and cardiovascular diseases later in life (1,2,5–7). Although there is at present no direct evidence for a cause-and-effect relation between catch-up growth and these chronic metabolic diseases, nutritional rehabilitation studies conducted in malnourished infants and children often report excessive fat accumulation (8–12) or a higher insulin response to a glucose load (13) during catch-up growth. In several other mammalian species, including rats and pigs, a preferential recovery of body fat, rather than protein mass, has also been observed during catch-up growth (14,15) and is accompanied by glucose intolerance, hyperinsulinemia, and/or higher blood pressure (16–20).

Therefore, the question that arises is why should the phase of catch-up growth be particularly susceptible toward the accumulation of body fat, development of insulin-related metabolic abnormalities, and high risk for cardiovascular diseases? The most common explanations center on the impact of an exaggerated compensatory increase in energy intake (particularly during catch-up growth when refed energy-dense fatty foods), on energy-dense fatty foods, and on the development of excess adiposity, insulin resistance, hyperinsulinemia, and an overactive sympathetic nervous system.

However, a disproportionately high rate of fat deposition (or catch-up fat) during catch-up growth still occurs in the absence of hyperphagia (21–23), which hence underscores an elevated efficiency of body fat recovery as a fundamental physiological reaction to growth retardation (24–27). It is viewed as a control system that operates as a feedback loop between depletion or delayed expansion of the fat stores and suppressed thermogenesis and has been referred to as an adipose-specific control of thermogenesis (28), whose sustained suppression during refeeding favors catch-up fat. Given these close associations between catch-up growth, a high metabolic efficiency underlying catch-up fat, and the development of chronic metabolic diseases later in life, the possibility therefore arises that a sustained reduction in energy expenditure per se (due to suppressed thermogenesis in certain organs/tissues), for the purpose of enhancing the efficiency of fat deposition during catch-up growth, may also be involved in the pathogenesis of these chronic metabolic diseases.

To test this hypothesis, we have used, in the growing rat, an approach that allows suppressed thermogenesis specific for fat recovery to be studied in isolation from the effects of other confounding variables (such as body size,

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AM, age-matched; FFA, free fatty acid; ME, metabolizable energy; RF, refed; WM, weight-matched.

food intake, and differential rates of protein gain) on energy expenditure. Using this validated model of weight recovery during calorie-controlled refeeding (25–27), we report here studies that investigate the impact of suppressed thermogenesis favoring catch-up fat on early risk factors for chronic metabolic diseases.

RESEARCH DESIGN AND METHODS

General study design. Male Sprague-Dawley rats, age 6 weeks, caged singly in a temperature-controlled room (22 ± 1°C) with a 12-h light/dark cycle, were maintained on a commercial pelleted diet (Kliba, Cossonay, Switzerland) consisting, by energy, of 24% protein, 66% carbohydrates, and 10% fat and had free access to tap water. Animals used in the present studies were maintained in accordance with our institute's regulations and guide for the care and use of laboratory animals. Experiments described here used a design similar to that previously described in establishing a rat model for studying adjustments in energy expenditure specific for accelerating fat deposition during refeeding (25–27).

Study 1: catch-up fat on low-fat diet. In a first set of experiments (study 1), the specific design of which is presented in Fig. 1, groups of rats ($n = 6$) were either fed ad libitum on a pelleted diet or food restricted for 2 weeks at ~50% of the diet intake of ad libitum–fed rats. At the end of this semistarvation period (corresponding to day 0 of refeeding), one group of semistarved rats as well as one group of ad libitum–fed rats were killed by decapitation. Whereas another group of ad libitum–fed rats, referred to as age-matched (AM) controls, continued to be fed ad libitum, another group of semistarved rats were refed the pelleted diet at a level approximately equal in metabolizable energy (ME) content to the spontaneous food intake of rats matched for weight at the onset of refeeding. The refed (RF) group therefore consumed, on a day-to-day basis, the same amount of food energy as their weight-matched (WM) controls fed ad libitum. At the end of the 2-week period of controlled refeeding, animals in all three groups (RF, WM, and AM) were killed by decapitation. Changes in body energy content, body composition (fat and protein), and energetic efficiency were determined during the 2-week energy balance study from the groups killed on day 0 and 14. Glucose tolerance tests were performed on all groups on the last day of semistarvation (i.e., day 0 of refeeding) and on day 7 of refeeding. In parallel studies, rats were instrumented for measurements of 24-h blood pressure, which were compared in RF and control groups fed a pelleted diet.

Study 2: catch-up fat on high-fat diet. In a second set of experiments, the specific design of which is shown in Fig. 2, groups of rats ($n = 6$), referred to as fed controls, were fed either a low-fat diet or an isocaloric amount of a high-fat diet (rich in lard) for a period of 2 weeks; details of composition of the diets and measurements of ME intake have been reported previously (29). During this same 2-week period, another two groups of rats were semistarved as described above, and during subsequent refeeding lasting for 2 weeks, one group was refed on the low-fat diet and the other group on isocaloric amounts of the high-fat diet. All groups were thus provided with the same amount of ME intake, which corresponds to that consumed during spontaneous food intake on pelleted diet. Changes in body energy content, body composition (fat and protein), and energetic efficiency were determined over the 2-week energy balance study in fed and RF groups. Glucose tolerance tests were performed on all groups on days 12–13 of feeding or refeeding. In parallel studies, rats were instrumented for measurements of 24-h blood pressure, which were compared in groups refed on isocaloric amounts of the low-fat and high-fat diet.

Determination of body composition. After the animals were killed by decapitation, the skull, thorax, and abdominal cavity were incised, and the gut was cleaned of undigested food. The whole carcasses were dried to a constant weight in an oven maintained at 70°C and were subsequently homogenized. Triplicate samples of the homogenized carcass were analyzed for energy content by bomb calorimetry (30) and for fat content by the Soxhlet extraction method (31). Body protein was determined from a general formula relating energy derived from fat, total energy value of the carcass, and energy derived from protein (25); the caloric values for body fat and protein were taken as 38.6 and 22.7 kJ/g, respectively.

Determination of energy balance and energetic efficiency. Energy balance measurements were conducted during refeeding, as previously described (25–27), by the comparative carcass technique over 2 weeks, during which ME intake was monitored, as detailed previously (29). Energy expenditure was determined as the difference between energy gain and ME intake, and the energetic efficiency was calculated as the percentage of total energy gain per ME intake.

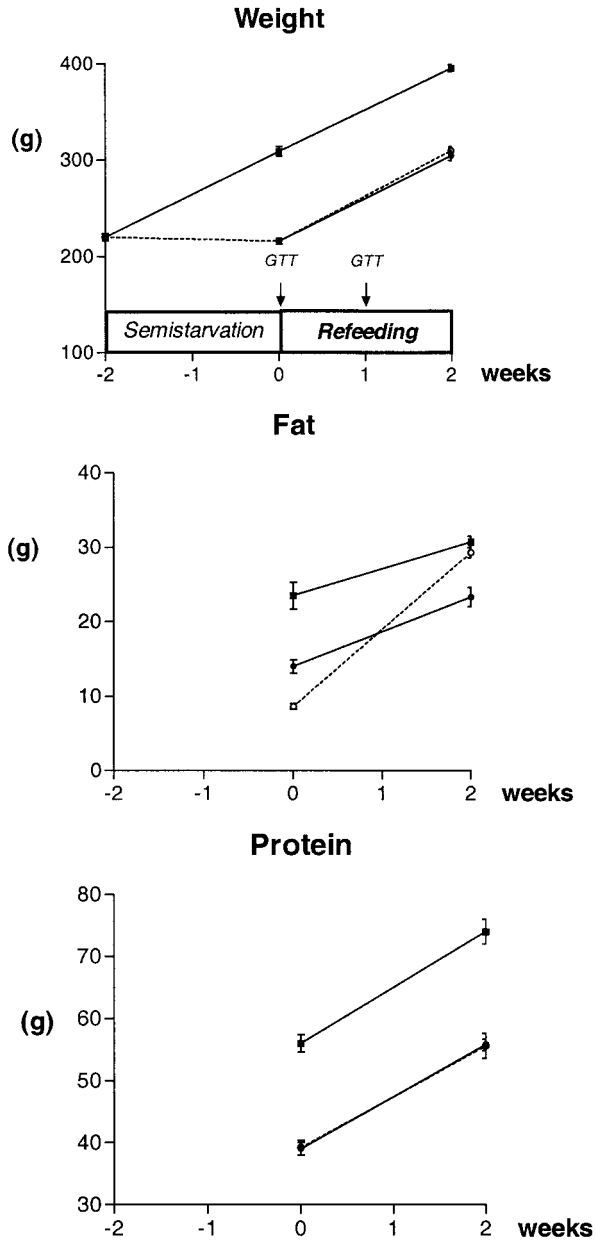

FIG. 1. Body weight, fat, and protein at the onset and end of 2 weeks of controlled refeeding on a pelleted (low-fat) diet after 2 weeks of growth arrest by semistarvation. The various groups are as follows: RF, WM controls, and AM controls. All values are means ± SE ($n = 6$). The arrows indicate the specific days on which glucose tolerance tests (GTTs) were performed. ■, AM; ○, RF; ●, WM.

Blood parameters and glucose tolerance test. Glucose tolerance tests were performed according to the protocol described previously (32). From blood samples collected before the glucose load (time point 0), the plasma levels of nonesterified fatty acids and leptin were also measured in both studies 1 and 2. Sufficient plasma in study 2 was also available for assays of adiponectin and corticosterone. Plasma glucose was determined using a Beckman glucose analyzer (Beckman Instruments, Palo Alto, CA), plasma free fatty acids (FFAs) using an NEFA C kit (Wako, Neuss, Germany), and plasma immunoreactive insulin by radioimmunoassay according to the method of Herbert et al. (33). Plasma leptin and adiponectin were measured using radioimmunoassay kits from Linco (St. Charles, MD).

Blood pressure measurements. In study 1, 24-h mean arterial blood pressure and heart rate were determined using implanted catheters, as described by Wang et al. (34). In study 2, they were measured using an implantable transducer and radiotransmitter (model TA11PA-C40; DSI, St. Paul, MN), as detailed previously (35).

Data analysis and statistics. The data were analyzed by unpaired t test for comparisons between two groups. For comparisons across three groups,

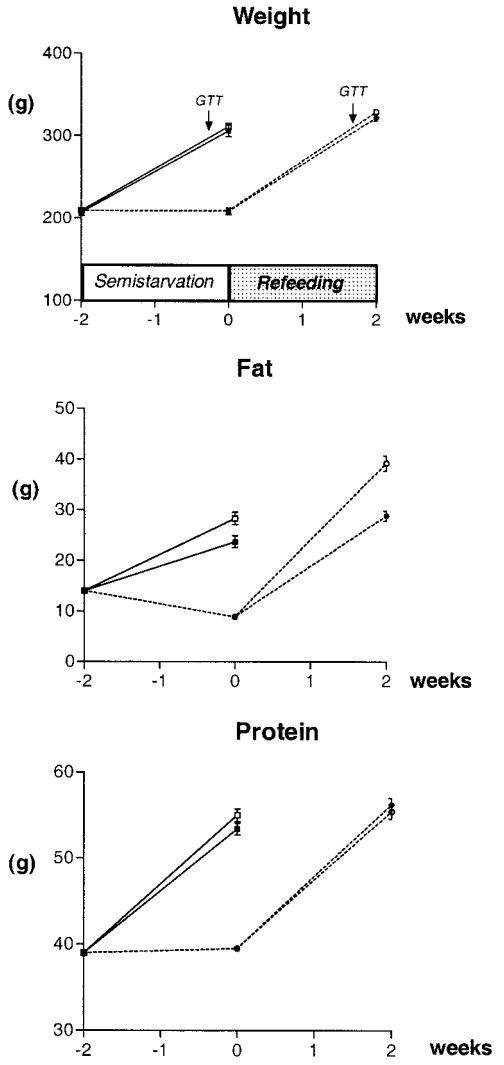

FIG. 2. Body weight, fat, and protein at the onset and end of 2 weeks on a low-fat (LF) or high-fat (HF) diet during feeding in controls (C) or during refeeding in RF animals after growth arrest due to semistarvation. All four groups were fed isocalorically, with similar ME intake. All values are means ± SE (n = 6). The arrows indicate specific days at which glucose tolerance tests (GTTs) were performed. ■, C-LF; □, C-HF; ○, RF-HF; ●, RF-LF.

one-factor ANOVA was used, followed by post hoc pairwise comparisons by the Tukey's test after ANOVA established significant differences. In study 2, the data were analyzed *1*) by using two-factor ANOVA for the main effects of group (controls gaining weight vs. RF regaining weight) and diet (low-fat vs. high-fat), as well as for the group × diet interaction, and *2*) by unpaired t test for between-diet comparisons (high-fat vs. low-fat) within either fed or RF groups. The statistical treatment of data (as well as stepwise regression analyses) was performed using the computer software STATISTIK, version 4.0 (Analytical Software, St. Paul, MN).

RESULTS

Study 1: catch-up fat on a low-fat diet

Energetics. The data on body weight, body fat, and body protein in ad libitum–fed, semistarved, and RF groups, all fed on pelleted (low-fat) diet, are presented in Fig. 1. At the end of the semistarvation period, during which growth was arrested, body weight, protein, and fat were all lower in the semistarved group compared with AM controls; however, compared with their WM controls, only body fat was significantly lower (8.6 vs. 14 g, $P < 0.001$). After 2 weeks of refeeding, during which the RF group consumed the same amount of diet as WM controls, the final body weight and protein were still not significantly different between the RF and WM control groups. Body fat, by contrast, was significantly higher in the RF group relative to WM controls, reaching values similar to those observed in the AM controls. As shown in Table 1, the gain in body weight and protein was not significantly different in RF compared with WM or AM controls, but body fat gain was more than twofold greater in the RF group than in either control group. This specific catch-up in body fat despite the fact that energy intake was either similar or lower relative to WM and AM controls, respectively, is due to a significantly lower energy expenditure (or higher energetic efficiency) in the RF group than in the control groups. Relative to WM controls, energy expenditure in the RF group was lower by ~12% ($P < 0.001$) and energetic efficiency was higher by ~60% ($P < 0.001$).

TABLE 1
Energy balance and changes in body weight and body energy stores during 2 weeks of refeeding after semistarvation in RF, WM, and AM controls

	AM	WM	RF	ANOVA
Weight gain (g)	87 ± 3	88 ± 3	93 ± 2	NS
Fat gain (g)	7.2 ± 0.8[a]	9.3 ± 1.3[a]	20.7 ± 0.7[b]	$P < 0.001$
Protein gain (g)	17.5 ± 0.4	16.8 ± 0.9	16.4 ± 0.9	NS
Energy gain (kJ)	677 ± 33[a]	740 ± 68[a]	1,171 ± 19[b]	$P < 0.001$
ME intake (kJ)	5,158 ± 119[a]	4,706 ± 144[b]	4,662 ± 20[b]	$P < 0.01$
Energy expenditure (kJ)	4,481 ± 91[a]	3,965 ± 82[b]	3,491 ± 22[c]	$P < 0.001$
Efficiency (%)	13.1 ± 0.4[a]	15.6 ± 1.0[b]	25.1 ± 0.4[c]	$P < 0.001$

Data are means ± SE. Values not sharing the same superscript (a, b, c) are significantly different from each other ($P < 0.05$). ME intake refers to metabolizable energy intake and was determined from differences between gross energy intake and the energy lost through feces and urine, as reported previously (29).

Circulating substrates and hormones. The data on the basal (postabsorptive) level of plasma glucose, FFA, insulin, and leptin are provided in Table 2. At the end of semistarvation, plasma glucose was the same in both semistarved and ad libitum–fed controls, but plasma insulin, leptin, and FFA were all markedly lower in the semistarved group. On day 7 of refeeding, no significant differences were observed between RF and control groups in plasma FFA and glucose, although there was a tendency for glucose to be higher in the RF group than in WM controls. Similarly, postabsorptive plasma insulin tended to be higher in the RF group than in controls, a difference that was not far from reaching statistical significance ($P < 0.1$). Basal plasma leptin was slightly higher (nonsignificantly) in RF and WM controls but was significantly higher in AM controls than in the other two groups.

Glucose tolerance tests and blood pressure. The results of the glucose tolerance tests conducted at the end of semistarvation and on day 7 of refeeding are presented in Fig. 3. No significant differences were observed in glucose tolerance curves between semistarved and control groups or between RF and control groups; there was, however, a tendency for plasma glucose to be higher in RF than in WM controls between 0 and 60 min. By contrast, after food restriction, the insulin response to glucose administration was considerably less marked in the semistarved group relative to controls, whereas during refeed-

TABLE 2
Basal (postabsorptive) levels of plasma glucose, FFA, insulin, and leptin at the end of semistarvation and on day 7 of refeeding

	Semistarvation			Refeeding			
	AM	FR	*t* test	AM	WM	RF	ANOVA
Glucose (mg/100 ml)	83 ± 2	80 ± 4	NS	96 ± 7	93 ± 9	97 ± 5	NS
FFA (mmol/l)	0.45 ± 0.03	0.29 ± 0.05	$P < 0.05$	0.36 ± 0.05	0.40 ± 0.05	0.34 ± 0.05	NS
Insulin (ng/ml)	1.98 ± 0.3	1.29 ± 0.19	$P < 0.02$	2.36 ± 0.31	2.13 ± 0.18	3.04 ± 0.3	NS ($P = 0.1$)
Leptin (ng/ml)	1.83 ± 0.23	0.46 ± 0.11	$P < 0.01$	3.17 ± 0.37[a]	2.41 ± 0.52[b]	2.60 ± 0.34[b]	$P < 0.02$

Data are means ± SE. FR, food-restricted during semistarvation. For pairwise comparisons during refeeding, values not sharing the same superscript (a, b) are significantly different from each other ($P < 0.05$).

ing, the insulin response was significantly higher in peak values and, thereafter, remained significantly higher in the RF group than in the control group (WM or AM). Thus, the results of the glucose tolerance tests indicate that whereas semistarvation is characterized by increased insulin sensitivity (i.e., normal glucose tolerance but reduced plasma insulin), refeeding on a low-fat diet is characterized by hyperinsulinemia and insulin resistance, as judged by normal glucose tolerance but elevated plasma insulin. No significant differences were observed in 24-h mean blood pressure (101 ± 4 vs. 100 ± 3 mmHg) or in heart rate (388 ± 6 vs. 380 ± 7 beats/min) between the control and RF groups, respectively.

Study 2: catch-up fat on a high-fat diet

Energetics. The data on body weight, body composition, and energy balance in this study are shown in Fig. 2 and Table 3. Comparison between RF and fed groups on the low-fat diet (i.e., RF-LF vs. C-LF in Table 3) indicates that, as in study 1, the RF animals showed higher body fat gain because they experienced lower energy expenditure and higher energetic efficiency than the controls. Within either fed or RF groups (Fig. 2), the animals receiving the high-fat diet showed no significant differences in final body weight and body protein but had more body fat than those fed isocaloric amounts of the low-fat diet; however, this between-diet difference in body fat was more marked in the RF animals (39.2 vs. 28.8 g, $P < 0.001$) than in the control animals (28.3 vs. 23.7 g, $P < 0.05$). This more pronounced effect of dietary fat on total body fat in RF and fed states is reflected in the data (presented in Table 3 on body fat gain and body energy gain), which were higher in the RF animals, despite similar ME intake and protein gain across all four groups. Whereas energy expenditure was found to be lower in RF than in fed groups independently of diet, the effect of the high-fat diet on energy expenditure in the fed controls (−4% vs. low-fat–fed group) was much less than its effect during refeeding (−12% vs. low-fat–RF group). This more pronounced effect of the high-fat diet than the low-fat diet on body fat gain, body energy gain, energy expenditure, and energetic efficiency is underscored by ANOVA, which indicates a statistically significant group × diet interaction for these parameters (Table 3). As also shown in Table 3, this between-diet difference in energy expenditure between these two RF groups remains statistically significant after adjustment for the differential energy costs for body fat gain on low-fat and high-fat diets (i.e., 0.36 and 0.16 kJ/kJ fat gained, respectively).

Circulating substrates and hormones. The data on basal (postabsorptive) level of plasma substrates and

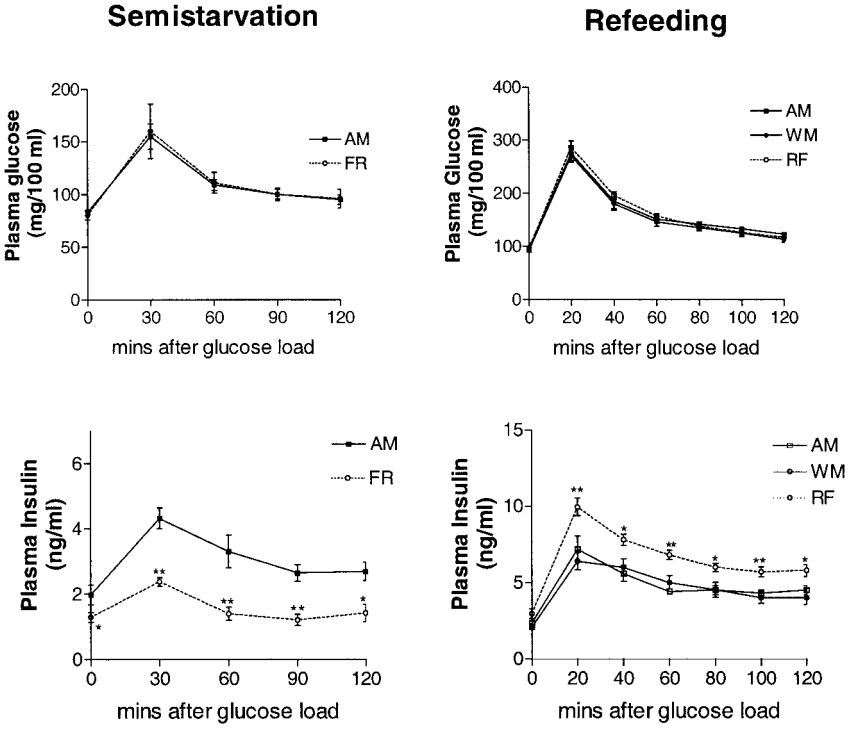

FIG. 3. Plasma glucose and plasma insulin before (time point = 0) and at 20- or 30-min intervals for 2 h after intraperitoneal administration of glucose (2 g/kg body wt) at the end of semistarvation and at day 7 of refeeding diet. All values are means ± SE ($n = 6$). The level of statistical significance of differences relative to controls is indicated as follows: *$P < 0.05$, **$P < 0.01$.

TABLE 3
Energy balance and changes in body weight and body energy stores during 2 weeks of refeeding on isocaloric amounts of low-fat and high-fat diets

					ANOVA		
	C-LF	C-HF	RF-LF	RF-HF	Group	Diet	Group × diet
Weight gain (g)	99 ± 9	102 ± 5	114 ± 6	120 ± 2	$P < 0.02$	NS	NS
Fat gain (g)	9.7 ± 1.2	14.3 ± 1.2*	19.8 ± 1.0	30.3 ± 1.5†	$P < 0.001$	$P < 0.001$	$P < 0.05$
Protein gain (g)	15.3 ± 0.7	16.0 ± 0.7	16.7 ± 0.8	16.3 ± 0.8	NS	NS	NS
Energy gain (kJ)	722 ± 41	914 ± 56*	1,144 ± 38	1,540 ± 58†	$P < 0.001$	$P < 0.001$	$P < 0.05$
ME intake (kJ)	4,632 ± 32	4,652 ± 15	4,641 ± 25	4,624 ± 32	NS	NS	NS
Energy expenditure (kJ)	3910 ± 29	3,738 ± 48*	3,497 ± 27	3,084 ± 45	$P < 0.001$	$P < 0.001$	$P < 0.01$†
Efficiency (%)	15.6 ± 0.8	19.6 ± 1.2*	24.6 ± 0.7	33.3 ± 1.1†	$P < 0.001$	$P < 0.001$	$P < 0.05$
Adjusted energy expenditure (kJ)	3,775 ± 41	3,649 ± 55	3,217 ± 38	2,895 ± 52‡	$P < 0.001$	$P < 0.001$	$P = 0.05$

Data are means ± SE. C-LF and C-HF, control groups fed a low-fat or high-fat diet, respectively; RF-LF and RF-HF, RF groups consuming a low-fat or high-fat diet, respectively. ME intake refers to metabolizable energy intake and was determined from differences between gross energy intake and the energy lost through feces and urine, as reported previously (29). Adjusted energy expenditure is calculated as the difference between energy expenditure and the energy cost of fat gain on a low-fat diet (0.36 kJ/kJ fat gain) or high-fat diet (0.16 kJ/kJ fat gain). Within controls or RF groups, statistical differences between groups consuming low-fat or high-fat diets are indicated as follows: *$P <$ 0.05; †$P < 0.001$; ‡$P < 0.01$.

hormones are presented in Table 4. Plasma glucose is slightly elevated in RF groups relative to the low-fat–fed controls. However, the ANOVA test indicates no significant differences due to the effect of groups or diet on plasma glucose. By contrast, there is a significant effect of group (but not of diet) for plasma insulin and adiponectin (i.e., both are higher during refeeding), as well as a tendency for increased plasma corticosterone and leptin during refeeding (group effect: $P = 0.06$). There is also a significant effect of diet on plasma FFA, leptin, and corticosterone; these values are higher on a high-fat diet than a low-fat diet in both fed and RF groups.

Glucose tolerance tests and blood pressure. The results of glucose tolerance tests after feeding and during refeeding of the low-fat or high-fat diets are presented in Fig. 4. Although no significant differences are observed for peak plasma glucose (Fig. 4*A*), ANOVA indicates a significant effect of diet for mean plasma glucose during the second hour after the glucose load, as well as for the total area under the curve over 2 h after glucose administration. However, it is only in the RF group, and not in the fed group, that a marked and significant effect of high-fat diet is observed for mean plasma glucose during the second hour after the glucose load (+35%, $P < 0.05$) or when assessed as the total area under the glucose curve (plus twofold, $P < 0.05$). Independently of diet, the plasma insulin and plasma insulin-to-glucose ratio after the glucose load are higher in RF than in fed animals, with statistically significant differences appearing in the second hour after the glucose load (Fig. 4*B* and *C*). For both plasma insulin and insulin-to-glucose ratio after the glucose load, no significant differences were detected between diets within fed or RF groups, and ANOVA indicated only a significant group effect (RF vs. fed) but not a diet effect (high-fat vs. low-fat) or group × diet interaction effect. Thus, the results of this study *1*) are consistent with those found in study 1, which show that refeeding on a low-fat diet results in hyperinsulinemia with normal glucose tolerance, and *2*) furthermore indicate that isocaloric refeeding on the high-fat diet leads to both glucose intolerance and hyperinsulinemia. No significant differences were observed in 24-h mean blood pressure (105 ± 3 vs. 109 ± 3 mmHg) or in heart rate (385 ± 8 vs. 390 ± 10 beats/min) between groups refed the low-fat or high-fat diets, respectively.

Predictors of plasma insulin and insulin-to-glucose ratio. An important issue in the present studies about the pathophysiology of catch-up growth is whether the greater susceptibility of the phase of weight regain toward hyperinsulinemia and insulin resistance resides in the higher efficiency of fat recovery per se (i.e., due to suppressed thermogenesis) or whether it is secondary to the greater absolute amount of body fat and/or elevated circulating FFAs in the RF groups. Further analysis of our data from

TABLE 4
Basal (postabsorptive) levels of plasma glucose, FFA, insulin, and leptin on days 12–13 of refeeding on isocaloric amounts of low-fat and high-fat diets

					ANOVA		
	C-LF	C-HF	RF-LF	RF-HF	Group	Diet	Group × diet
Glucose (mg/100 ml)	104 ± 4	107 ± 5	110 ± 5	112 ± 4	NS	NS	NS
FFA (mmol/l)	0.31 ± 0.02	0.47 ± 0.03*	0.37 ± 0.06	0.54 ± 0.06†	NS	$P < 0.01$	NS
Insulin (ng/ml)	2.13 ± 0.53	2.92 ± 0.32	3.23 ± 0.34	3.51 ± 0.26	$P < 0.05$	NS	NS
Corticosterone (ng/ml)	59 ± 6	129 ± 10‡	115 ± 22	141 ± 22	$P = 0.06$	$P < 0.01$	NS
Leptin (ng/ml)	2.34 ± 0.31	3.23 ± 0.35†	2.69 ± 0.29	4.10 ± 29*	$P = 0.06$	$P < 0.01$	NS
Adiponectin (μg/ml)	8.14 ± 0.45	7.77 ± 0.70	10.7 ± 0.67	10.6 ± 1.88	$P < 0.02$	NS	NS

Data are means ± SE. C-LF and C-HF, control groups fed a low-fat or high-fat diet, respectively; RF-LF and RF-HF, RF groups consuming a low-fat or high-fat diet, respectively. Within controls or RF groups, statistical differences between groups consuming a low-fat or high-fat diets are indicated as follows: *$P < 0.01$; †$P < 0.05$; ‡$P < 0.001$.

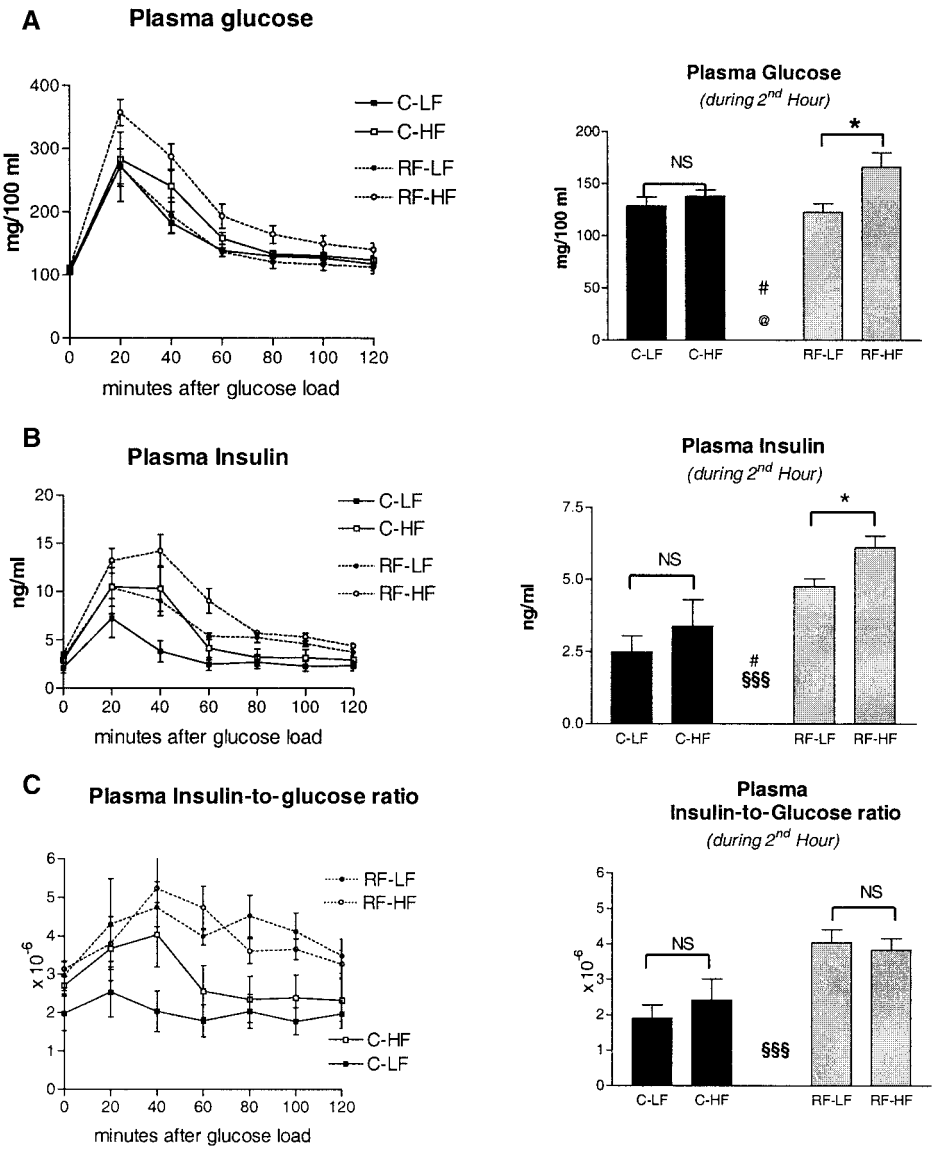

FIG. 4. Plasma glucose, plasma insulin, and plasma insulin-to-glucose ratio before (time point = 0) and at 20-min intervals for 2 h after intraperitoneal administration of glucose (2 g/kg body wt). The bar charts for plasma glucose, plasma insulin, and insulin-to-glucose ratio represent mean absolute values during the second hour after the glucose load. All values are means ± SE (n = 6). The results of two-factor ANOVA are indicated as follows: §§§significant effect of groups, fed vs. RF (P < 0.001); #significant effect of diet, low-fat vs. high-fat (P < 0.05); @significant group × diet interaction (P < 0.05). Within controls or RF groups, statistical differences between groups consuming low-fat or high-fat diets are indicated as follows: *P < 0.05; NS, not significant.

both energy balance studies can be shown to support the explanation based on increased efficiency of fat deposition. First, in study 1 comparing RF and control groups consuming a low-fat diet, the glucose tolerance test was performed 1 week after the onset of refeeding—a time point at which we showed in previously reported studies (27) that total body fat in the RF group does not yet exceed that in the control group. Second, in our study 2 comparing groups refed the high-fat and low-fat diets, by contrast, the glucose tolerance test was performed near the end of the study and, hence, at a time point when total body fat in the RF group had clearly exceeded that of the respective controls. However, using a stepwise regression analysis, it can be shown that it is the efficiency of fat deposition (i.e., variations in thermogenesis), and not total body fat or elevated circulating FFAs, that is the primary predictor of plasma insulin ($r^2 = 0.64, P < 0.001$) and insulin-to-glucose ratio ($r^2 = 0.39, P < 0.01$) after the glucose load.

DISCUSSION

In the studies presented here, evidence is provided that suggests that the state of hyperinsulinemia and insulin resistance during catch-up growth is an early event that can be attributed to diminished energy expenditure per se (due to suppressed thermogenesis that leads to accelerated fat recovery or catch-up fat) rather than to hyperphagia, increased blood pressure, excess fat mass, and/or an elevation in circulating FFAs.

Catch-up fat on a low-fat diet. By pair-feeding RF animals to ad libitum–fed controls matched for similar body weight and lean tissue mass at the onset of refeeding (i.e., to WM controls), our experimental approach bypasses the problems associated with a comparison between animals of different body sizes and, hence, provides a means of assessing the contribution of regulatory adjustments in energy expenditure during weight regain. As shown here, when rats rehabilitated from semistarvation are refed the same amount of diet as WM controls (i.e., in absence of hyperphagia), the rate of protein deposition is the same as in controls, but that of fat deposition is increased by more than twofold because of diminished energy expenditure (by ~12%) in the RF group compared with the controls. A number of factors that could theoretically contribute to this difference in energetics between

the RF and WM controls (namely age, level of physical activity, and size of organs) have also been previously evaluated and were shown to have little or no impact on the difference in energy expenditure between the two groups (24–26). Consequently, under conditions of our refeeding studies, the lower energy expenditure in the RF group compared with the controls is explained essentially by the energy spared as a result of a sustained suppression of thermogenesis for the purpose of catch-up fat.

Similarly, the elevated plasma insulin after the glucose load during refeeding cannot be explained by the 2-week age difference between the RF and WM controls, because in response to glucose administration, both AM and WM controls showed insulin response curves that were almost superimposable (and that were lower than response curves in the RF group). Taken together, the data comparing the RF and WM controls consuming the same amount of a low-fat diet therefore support a role for suppressed thermogenesis in the phenomenon of catch-up fat and in the development of hyperinsulinemia and insulin resistance during weight recovery after growth arrest induced by semistarvation.

Catch-up fat on a high-fat diet. The present study also reveals that isocaloric refeeding on a high-fat diet resulted in further elevation in the efficiency of fat deposition, in an exacerbation of hyperinsulinemia, and in hyperglycemia. These more pronounced effects of high-fat diet during weight regain upon refeeding than during weight gain in fed controls raise the possibility that the effect of dietary fat in suppressing "facultative" thermogenesis depends on the existence of an underlying suppression of thermogenesis for catch-up fat. Similarly, this greater susceptibility of the weight regain phase pertaining to the effect of dietary fat in inducing both hyperinsulinemia and glucose intolerance, compared with less marked hyperinsulinemia and normal glucose tolerance during low-fat refeeding, can thus be linked to the exacerbated suppression of thermogenesis on the high-fat diet.

Catch-up fat and insulin resistance. The important demonstration here is that the efficiency of fat deposition, and not total body fat or elevated circulating FFAs, is the primary predictor of plasma insulin and insulin-to-glucose ratio after the glucose load. Because (as discussed above) the higher energetic efficiencies (hence the rate) of fat deposition in RF groups is the result of suppressed thermogenesis, the implication of these findings is that the suppression of thermogenesis favoring catch-up fat, rather than total body fat or elevated circulating FFAs, is the prime early determinant of the hyperinsulinemic and insulin-resistant state of catch-up growth.

The other neurohormonal systems that are implicated in the regulation of catch-up fat are still unclear. A role for diminished sympathetic nervous system activity in the suppression of thermogenesis during refeeding is, however, unlikely. This is because the well-known reduction in sympathetic nervous system activity during starvation is rapidly restored to fed levels within a few days of refeeding (36) and, hence, contrasts with kinetics of suppressed thermogenesis during refeeding that lasts for >2 weeks (28). By contrast, a role for corticosterone has been suggested by our previous demonstration that adrenalectomy before refeeding was partially effective in preventing the diminished energy expenditure and excess fat deposition during refeeding (37). Indeed, data presented here showing that plasma corticosterone is higher (by about twofold) in the RF group than in the control group consuming the low-fat diet would be consistent with a role for corticosterone in suppressed thermogenesis and insulin resistance underlying catch-up fat. However, plasma corticosterone is also found to be higher (by twofold) in the controls on high-fat diets than on low-fat diets, despite no between-diet differences in energy expenditure (after adjusting for energy cost of fat deposition) or in plasma glucose, insulin, and insulin-to-glucose ratio after the glucose load. It therefore follows that an elevation in circulating corticosterone alone cannot account for the pathogenesis of catch-up fat. Because of recent evidence implicating the adipocyte-secreted hormones leptin and adiponectin in skeletal muscle thermogenesis and in protection against skeletal muscle insulin resistance by influencing fuel substrate metabolism (38–40), we also examined whether changes in plasma levels of these hormones could be linked to the sustained suppression of thermogenesis favoring catch-up fat and insulin resistance during refeeding. Our results indicate, however, higher (rather than lower) plasma leptin and adiponectin during refeeding, which might be reflecting an attempt of the body to counteract or limit the development of insulin resistance consequential to suppressed thermogenesis favoring catch-up fat.

In conclusion, the current studies suggest a primary role for suppressed thermogenesis per se in the development of hyperinsulinemia and insulin resistance during catch-up growth, by demonstrating that this link can be delineated from hyperphagia, elevated body fat, or elevated circulating FFAs. This model of catch-up fat due to suppressed thermogenesis therefore provides a window in time that reproduces the same high-risk factors for obesity, type 2 diabetes, and cardiovascular diseases found in human adults who showed catch-up growth after exposure to famine early in life. Elucidation of the mechanisms that regulate catch-up fat through suppression of thermogenesis; how they are modulated by diets high in fat to result in oversuppression of thermogenesis, hyperinsulinemia, and glucose intolerance; and whether they are hypersensitized by repeated periods of growth retardation and catch-up growth or by fetal and neonatal programming are crucial steps toward understanding the complex routes by which early growth retardation enhances human susceptibility toward chronic metabolic diseases.

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