Fat accretion and the regulation of insulin-mediated glycogen synthesis after puberty in rats

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Banerjee, Swati, Paul Saenger, Meizhu Hu, Wei Chen, and Nir Barzilai. Fat accretion and the regulation of insulin-mediated glycogen synthesis after puberty in rats. *Am. J. Physiol.* 273 (*Regulatory Integrative Comp. Physiol.* 42): R1534–R1539, 1997.—Peripheral insulin sensitivity decreases after puberty in both humans and rodents and can be explained mostly by a reduction in insulin-mediated glycogen synthesis. We tested the hypothesis that the increase in postpubertal fat mass (FM), reflecting an alternative energy store, regulates a decrease in the capacity to store muscle glycogen. We studied Sprague-Dawley rats (n = 21) before puberty (Pre) or after puberty (at 4 mo of age) in groups that were either ad libitum fed (Post) or moderately caloric restricted (CR). FM (by ^3H_2O isotope dilution technique) was decreased by >40% in CR compared with Post. Glucose uptake (R_d, by 18 mU·kg^{-1}·min^{-1} hyperinsulinemic clamp) was 63 ± 8 mg·kg^{-1}·min^{-1} in Pre and decreased to 39 ± 2 mg·kg^{-1}·min^{-1} in Post ($P < 0.001$). However, it increased in CR to 53 ± 2 mg·kg^{-1}·min^{-1} ($P < 0.001$ vs. Post). This increase in R_d was mainly accounted for by an increase in glycogen synthesis (R_d glycolysis determined by the rate of conversion of ^{3}H-labeled glucose to ^3H_2O) from 23 ± 2 in Post to 33 ± 2 mg·kg^{-1}·min^{-1} in CR ($P < 0.001$; 38 ± 7 mg·kg^{-1}·min^{-1} in Pre). Correction of glycogen synthesis in CR to near-prepubertal levels was further supported by directly assayed muscle glycogen content after insulin stimulation that was 45% higher and by a 35% enhanced accumulation of [^{3}H]glucose into glycogen. No changes in the enzyme kinetics of glycogen synthase or phosphorylase were observed. An additional group of 2-mo-old postpubertal ad libitum-fed rats was matched with CR for lean body mass but had more FM. This group demonstrated 25% lower rates of insulin-mediated glycogen synthesis compared with CR, further supporting the notion that a moderate reduction of FM prevents the decline in insulin responsiveness and glycogen synthesis occurring after puberty. These data suggest a cause-effect relationship between the increased deposition of fat and the reduced ability to store glucose in skeletal muscle after puberty.

calorie restriction; insulin responsiveness; glycolysis

DECREASED GLUCOSE TOLERANCE (2, 5), characterized by compensatory increases in basal and in glucose-stimulated insulin secretion (10, 21, 28), is seen during and after puberty. This suggests peripheral insulin resistance, which was further demonstrated by a decrease in insulin-mediated peripheral glucose uptake (R_d) by hyperinsulinemic clamp in postpubertal children (1). The reduction in insulin sensitivity during puberty has been related to increases in circulating hormones, and of particular importance is the rise in plasma growth hormone (GH) levels (1, 5). The insulin resistance was demonstrated to be restricted to glucose metabolism while enhancing the anabolic effect of the hyperinsulinemia on other substrates, such as amino acids, which is beneficial to support the tremendous growth requirements (1, 9). However, once puberty is completed, insulin sensitivity still remains decreased when compared with prepubertal values. This fact is puzzling because although an increase in GH levels may be responsible for the insulin resistance observed during puberty (3), a fall in the GH levels after puberty does not restore peripheral insulin action to prepubertal levels. Other typical changes occurring during puberty are the increases in body mass index, lean body mass (LBM), and fat mass (FM), which are also sustained when GH levels decline postpubertally. Because insulin resistance and decreases in glucose storage are common in human obesity (11), increased FM may be one of the mechanisms by which glucose homeostasis is affected postpubertally. The impact of the decrease in insulin-mediated R_d on the intracellular pathways of glucose disposal have not been characterized effectively in humans, and it is not clear whether glycogen storage is defective as commonly seen in other insulin-resistance states of adults.

As in humans, maturation and aging in rodents are also associated with an increase in plasma glucose and insulin levels (8, 13). Similarly, although GH has been implicated in the insulin resistance of puberty (15), few studies have addressed the postpubertal state in rats. We recently studied an established homogeneous rodent model (19, 30) to evaluate the changes in intracellular glucose metabolism occurring before and after puberty (19). We have demonstrated that peripheral insulin resistance after puberty is characterized mainly by a marked decrease in the ability of insulin to stimulate skeletal muscle glycogen storage. We postulate that fat accumulation is responsible for the postpubertal reduction in glycogen synthesis, thereby reflecting a switch in energy stores from carbohydrate to lipids. To verify our hypothesis, we prevented the fat accretion after puberty by carefully restricting the caloric intake of prepubertal rats and studied intracellular insulin-mediated pathways of R_d in the postpubertal animals.

MATERIAL AND METHODS

Animals. Weanling (~3 wk old) male Sprague-Dawley rats (Charles River Laboratories, Wilmington, MA) were used for this study. These rats are relatively obese and hyperphagic, and caloric restriction is used not as underfeeding but as normofeeding. Prepubertal rats (Pre) were studied within 1 wk (n = 5), and the remaining rats were divided into three groups. One group was fed ad libitum and studied postpu-

berty at 4 mo of age (Post, $n = 5$). The second group was kept on ~60% of the diet of the ad libitum-fed rats and was studied postpubertally at 4 mo of age (CR, $n = 6$). A third group was studied postpubertally at 2 mo of age with animals matched for LBM to CR (LBM-C, $n = 5$). All rats were fed a standard chow that contained 64% carbohydrates, 30% proteins, and 6% fats, with a physiological fuel value of 3.30 kcal/g chow, and vitamin supplements. The rats were sedentary and were housed in individual cages. They were subjected to a standard light (6 AM-6 PM)-dark (6 PM-6 AM) cycle. Pre animals were studied when they were <4 wk old and their body weights were <100 g, and prepuberty was confirmed by the lack of testicular descent (30). Post animals were identified by testicular descent and balano-preputial separation (30, 17). The animals that were moderately calorie restricted had an adjustment of food intake biweekly, maintained a steady weight gain, and completed puberty at the same age as the ad libitum-fed rats. Three to five days before the in vivo study, rats were anesthetized with an intraperitoneal injection of pentobarbital sodium (50 mg/kg body wt) and indwelling catheters were inserted in the right internal jugular vein and the left carotid artery. The venous catheter was extended to the level of the right atrium, and the arterial catheter was advanced to the level of the aortic arch (4, 22, 23, 25). Rats were studied when their body weights were within 3% of their preoperative weight.

Body composition. LBM and FM were calculated from the whole body volume of distribution of water estimated by tritiated water bolus injection in each experimental rat (4). On the morning of the study, 20 μCi of ^3H_2O (NEN, Boston, MA) was injected intra-arterially. Steady state for ^3H_2O specific activity in rats is generally achieved within 30–45 min, and 8 samples were collected between 1 and 1.5 h after injection.

The distribution space of water was obtained by dividing the total radioactivity injected by the steady-state specific activity of plasma water, which was assumed to be 93% of the total plasma volume. LBM was calculated from the whole body distribution space, divided by 0.73 (the percent water content of LBM). FM was calculated as the difference between total body weight and LBM. Because carcass protein was not changed in Pre, Post, and CR rats, we assumed similar distribution volume of water in all rats. Epididymal fat pads were dissected and weighed after the completion of the study as an additional index of adiposity.

Hyperinsulinemic euglycemic clamp. Studies were performed in awake, unstressed, chronically catheterized rats (4, 22, 24). All rats received a primed continuous (15–40 μCi bolus, 0.4 μCi/min) infusion of high-performance liquid chromatography-purified [3-^{3}H]glucose (NEN) throughout the study. A primed continuous infusion of insulin (18 mU·kg^{-1}·min^{-1}) and a variable infusion of a 25% glucose solution was started, and periodically adjusted, to clamp the plasma glucose concentration at the basal level for the 120 min of the clamp.

Plasma samples for determination of [3-^{3}H]glucose specific activity were obtained at 10-min intervals throughout the insulin infusion. Samples were also obtained for determination of plasma insulin, glycerol, and free fatty acid (FFA) concentrations every 30 min during the study. The total volume of blood withdrawn was ~2.0 ml/study; to prevent volume depletion and anemia, a solution (1:1 vol/vol) of ~2.0 ml of fresh blood (obtained by heart puncture from a littermate of the test animal) and heparinized saline (10 U/ml) was infused. At the end of the insulin infusion, rats were anesthetized (pentobarbital sodium, 60 mg/kg body wt iv), the abdomen was quickly opened, and the rectus abdominal muscle was freeze clamped in situ with aluminum tongs precooled in liquid nitrogen (4, 22, 24). The time from the injection of the anesthetic until freeze clamping of the muscle was <1 min. All tissue samples were stored at −80°C for subsequent analysis.

The study protocol was reviewed and approved by the Animal Care and Use Committee of the Albert Einstein College of Medicine.

Whole body glycolysis and glycogen synthesis. The rate of glycolysis was estimated from the rate of conversion of [3-^{3}H]glucose to ^3H_2O as previously described (4, 22, 24). Because tritium on the C-3 position of glucose is lost to water during glycolysis, it can be assumed that plasma tritium is present either in ^3H_2O or glucose. Plasma ^3H_2O specific activity was determined by liquid scintillation counting of the protein-free supernatant (Somogyi filtrate) before and after evaporation to dryness. Whole body glycogen synthesis was estimated by subtracting whole body glycolysis from whole body R_d.

Glycogen formation in vivo. Indexes for muscle glycogen synthesis were obtained by three independent methods. *1*) Glycogen synthesis was quantitated by subtracting the glycolytic rate from R_d. *2*) Glycogen synthesis was also quantitated by measuring the incorporation of [3-^{3}H]glucose counts into muscle glycogen. *3*) In addition, muscle glycogen after insulin infusion was determined after digestion with amyloglucosidase as previously described (4, 22, 24). The intra-assay and the interassay coefficients of variation were <10% (at 0.25 g/100 g tissue wt) when a muscle homogenate was assayed as multiple aliquots. Glycogen was precipitated by washing in 10 volumes of absolute ethanol and by incubation for 1 h at −20°C. The procedure was repeated three times, and then the precipitate was collected, dried down, and dissolved in water before scintillation counting.

Glycogen synthase activity. Muscle glycogen synthase (GSy) activity was measured by a modification (4, 22, 24) of the method of Thomas et al. (29) and is based on the measurement of the incorporation of radioactivity into glycogen from UDP-[U-^{14}C]glucose. Tissue samples (20–30 mg) were homogenized in 2.0 ml of tris(hydroxymethyl)aminomethane·HCl buffer, pH 7.8, containing (in mM) 10 EDTA, 5 dithiothreitol, and 50 NaF and 2.5 g/l rabbit liver glycogen type III. The homogenate was centrifuged at 2,000 revolutions/min for 15 min (at 4°C), and the supernatant was used for GSy assay by measuring the incorporation of UDP-[U-^{14}C]glucose into glycogen at 30°C. Synthase activity was measured in the presence of physiological 0.11 mM glucose 6-phosphate (G-6-*P*) to approximate the in vivo conditions. Total GSy activity was measured in the presence of 7.2 mM G-6-*P*. For the kinetic analysis, the assay was conducted at final concentrations of 0.003, 0.017, 0.033, 0.09, 0.33, and 1.4 mM UDP-glucose; the data were linearized as Eadie-Hofstee plots and were fitted using linear regression. The Michaelis constant (K_m) for UDP-glucose is the reciprocal of the slope, whereas maximal velocity (V_{max}) is the *y*-intercept divided by the slope.

Glycogen phosphorylase activity. Muscle glycogen phosphorylase (GP) activity was measured as previously described (4, 22, 24). This assay is based on the measurement of the incorporation of ^{14}C into glycogen from labeled glucose 1-phosphate (G-1-*P*). Glucose phosphate *a*, the active phosphorylated enzyme, was assayed in the absence of AMP in the tissue homogenates (20–30 mg), which were prepared as described in *Glycogen synthase activity*. The supernatant was used for GP assay by measuring the incorporation of ^{14}C-labeled G-1-*P* into glycogen at 30°C in a mixture containing 33 mM 2-(*N*-morpholino)ethanesulfonic acid, 200 mM KF,

0.45% mercaptoethanol, 15 mM G-1-*P* (50 μCi/mmol), and 3.4 mg/ml glycogen.

Analytic procedures. Plasma glucose was measured by the glucose oxidase method (Glucose Analyzer II, Beckman Instruments, Palo Alto, CA), and plasma insulin was measured by radioimmunoassay with the use of rat and porcine insulin standards. Plasma [^{3}H]glucose radioactivity was measured in duplicate on the supernatants of $Ba(OH)_2$ and $ZnSO_4$ precipitates of plasma samples before and after evaporation to dryness to eliminate tritiated water. We determined plasma nonesterified fatty acid concentrations using an enzymatic method with an automated kit according to the manufacturer's specifications (Waco Pure Chemical Industries, Osaka, Japan). Glycerol was determined using the GPO-Trinder reagents for the quantitative enzymatic determination of glycerol in plasma at 540 nm. (Sigma, St. Louis, MO).

Calculations. Rates of whole body glycolysis were estimated from the increment per unit time in tritiated water [disintegrations·min^{-1} (dpm)·ml^{-1}] × body water (ml)/[3-^{3}H]-glucose specific activity (dpm/mg). All values are presented as means + SE. Comparisons between groups were made with the use of repeated-measures analysis of variance where appropriate. Where *F* ratios were significant, further comparisons were made with the use of Student's *t*-tests.

RESULTS

Body composition and biochemical characteristics of experimental rats. Although CR animals attained ~75% of the weight of Post animals (Table 1), the total and epididymal FM of CR animals was reduced by approximately one-half, whereas their LBM was reduced by only ~20%. All groups had similar 6-h fasting glucose levels, whereas plasma insulin, FFA, and glycerol levels were very significantly lower in Pre. When CR is compared with Post, basal plasma insulin level was decreased by ~40% and plasma glycerol level was decreased by nearly one-third, whereas plasma FFA level was similar.

R_d, glycolysis, and glycogen synthesis. During the insulin clamp studies, insulin levels were increased to similar, maximally glucose-uptake stimulating levels (2,412 ± 250, 2,538 ± 128, and 2,921 ± 80 pM in Pre, Post, and CR, respectively; Fig. 1). Steady-state plasma levels of glucose were also similar in all groups (7.1 ± 0.5 mM). Hepatic glucose production was invariably suppressed to <2 mg·kg^{-1}·min^{-1} (controls 12–15 mg·kg^{-1}·min^{-1}), and plasma FFA level decreased by >60% in all groups. R_d (Fig. 1) was increased by 36% in CR to 53 ± 2 compared with 39 ± 2 mg·kg^{-1}·min^{-1} in Post ($P < 0.001$; 63 ± 8 mg·kg^{-1}·min^{-1} in Pre). Glycolysis in CR was mildly increased to 21 ± 1 from 16 ± 3 mg·kg^{-1}·min^{-1} in Post ($P < 0.01$; 25 ± 6 mg·kg^{-1}·min^{-1} in Pre). Glycogen synthesis (R_d-glycolysis) was increased to 33 ± 2 mg·kg^{-1}·min^{-1} in CR from 23 ± 2 mg·kg^{-1}·min^{-1} in Post ($P < 0.001$), similar to 38 ± 7 mg·kg^{-1}·min^{-1} in Pre. Thus more than two-thirds of the improvement in insulin-mediated R_d in the CR rats resulted from improvement in glycogen synthesis, whereas the rest of the improvement in R_d could be attributed to the increase in glycolysis.

Table 1. *Body composition and biochemical characteristics of Sprague-Dawley rats*

	Pre	Post	CR
Body composition			
Body weight, g	81 ± 8*	337 ± 25	258 ± 12†
FM, g	1.5 ± 1.0*	47.0 ± 5.6	27.3 ± 6.1†
LBM, g	80 ± 4*	299 ± 19	233 ± 11†
Epididymal fat, g	0.3 ± 0.1*	4.8 ± 0.5	2.2 ± 0.3†
Biochemical characteristics			
Glucose, mM	7.2 ± 0.3	7.4 ± 0.8	7.4 ± 0.6
Insulin, pM	139 ± 24*	348 ± 34	208 ± 5†
FFA, mM	0.33 ± 0.04*	1.05 ± 0.08	0.94 ± 0.07
Glycerol, μM	30 ± 7*	182 ± 18	125 ± 32†

Values are means ± SE. Body weight, fat mass (FM), lean body mass (LBM), epididymal fat pads, plasma glucose, insulin, free fatty acids (FFA), and glycerol levels in prepubertal (Pre), postpubertal (Post), and calorie-restricted (CR) rats. *$P < 0.001$ vs. Post and CR; †$P < 0.01$ vs. Post.

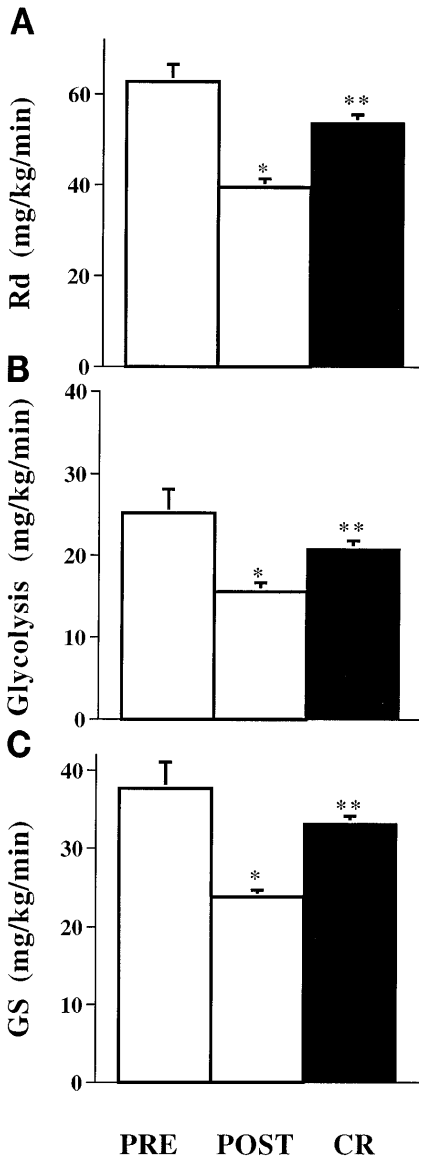

Fig. 1. Glucose disposal (R_d) and intracellular pathways in prepubertal (Pre), ad libitum-fed postpubertal (Post), and calorie-restricted (CR) postpubertal Sprague-Dawley rats. *A*: R_d measured during maximally stimulating insulin levels. *B*: glycolysis measured by generation of ^3H_2O from tritiated glucose. *C*: glycogen synthesis (GS) measured by subtracting glycolytic rates from R_d. *$P < 0.01$ vs. Pre and CR; **$P < 0.01$ vs. Pre.

Table 2. *Muscle glycogen, incorporation of [^{3}H]glucose into glycogen, GSy K_m and V_{max}, and GP*

	Pre	Post	CR
Glycogen, μmol/g wet wt	128 ± 18	80 ± 4*	118 ± 11
[^{3}H]glycogen, dpm·10^{-7}·g wet wt^{-1}	8.3 ± 2.6	4.4 ± 0.7*	5.9 ± 0.7†
GSy V_{max}, μmol·g wet wt^{-1}·min^{-1}	0.69 ± 0.08	0.68 ± 0.08	0.61 ± 0.09
GSy K_m, μmol·g wet wt^{-1}·min^{-1}	0.12 ± 0.05	0.11 ± 0.05	0.12 ± 0.03
GP V_{max}, μmol·g wet wt^{-1}·min^{-1}	21 ± 3	18 ± 2	19 ± 3

Values are means ± SE. Muscle was obtained in situ at end of insulin infusions in all rats. Glycogen synthase (GSy) K_m and V_{max} of muscle GSy are in presence of 0.11 mM physiological glucose 6-phosphate. Glycogen phosphorylase (GP) activity is AMP-independent form. dpm, disintegrations per minute. *$P < 0.001$ vs. Pre and CR; †$P < 0.001$ vs. Pre.

Glycogen synthesis was also determined by two other methods (Table 2). Muscle glycogen stores were ~45% higher in the CR compared with Post. Maintenance of prepubertal glycogen synthesis was further supported by enhanced insulin-stimulated accumulation of [^{3}H]glucose into glycogen, with CR incorporating ~35% more than Post.

Muscle GSy and GP activities. No differences were noted in the V_{max} for UDP-glucose of GSy (Table 2). Insulin-activated (decrease in K_m for UDP-glucose) GSy activity in all groups was comparable. No differences were noted in the AMP-independent form of GP activity.

Comparison with LBM-matched control. Because CR resulted in a decrease in LBM compared with Post (Table 3), we compared a subgroup of CR rats ($n = 4$) with an additional group of ad libitum-fed 2-mo-old rats ($n = 5$) with identical LBM (LBM-C). CR had similar body weight but ~30% less FM compared with LBM-C. Although the glucose and FFA levels were comparable, the insulin levels were ~30% higher in LBM-C. Insulin-mediated R_d was increased in CR by 18%, and although glycolysis was unchanged, most of the improvement in R_d resulted from the increase in glycogen synthesis. The lower glycogen synthesis in the LBM-C was further confirmed by an ~40 decrease in muscle glycogen (74 ± 6 μmol/g wet wt) as well as by a milder decreased incorporation of [^{3}H]glucose into glycogen (4.9 ± 1.4 × 10^7 dpm/g muscle).

Table 3. *Major characteristics of 4-mo-old CR and 2-mo-old ad libitum-fed rats matched for LBM*

	CR	LBM-C
LBM, g	226 ± 5	223 ± 11
FM, g	25.2 ± 2.7	35.4 ± 3.3*
Insulin, pM	212 ± 6	273 ± 18*
R_d, mg·kg^{-1}·min^{-1}	54 ± 2	46 ± 2*
Glycolysis, mg·kg^{-1}·min^{-1}	21 ± 1	20 ± 3
GSy, mg·kg^{-1}·min^{-1}	33 ± 1	26 ± 3*

Values are means ± SE. LBM-C, 2-mo-old ad libitum-fed rats matched with CR for LBM. Rd, glucose uptake. *$P < 0.01$ vs. CR.

DISCUSSION

In this study we used caloric restriction as a specific probe to examine the effects of increase in postpubertal FM on peripheral insulin action. We have demonstrated that by partially preventing the fat accumulation that occurs during and after puberty, we were able to maintain insulin-mediated R_d and glycogen synthesis at near prepubertal levels. This improvement occurred when compared with both ad libitum-fed age-matched animals and ad libitum-fed younger but LBM-matched controls.

Fat stores were significantly reduced by careful caloric restriction without affecting puberty as determined by testicular descent and balano-preputial separation (17). Furthermore, steady weight gain was preserved, ensuring a steady increase in LBM in the animals studied. Reduction of fat stores was demonstrated by radioisotope techniques measuring LBM and by dissecting out the discrete epididymal, perinephric, and mesenteric fat pads, all of which decreased proportionately. However, although the Pre animals had negligible amounts of visceral and subcutaneous fat, fat deposition was only partially prevented to ~40% of age-matched Post.

Although basal glucose levels were similar in all animals, postpubertal plasma insulin levels were decreased by ~40% in the CR compared with the Post group. In addition, CR rats had a decreased plasma glycerol level, indicating a decrease in FM and lipolysis. Although FFA levels were similar in the CR group compared with the Post group, the rate of lipid oxidation throughout human puberty is dissociated from the rate of lipolysis (3), suggesting that plasma FFA level may be a poor indicator of FFA fluxes. Thus, although elevated concentrations of circulating FFA may contribute to insulin resistance, normalization of these levels does not appear to be required to restore insulin sensitivity. Indeed, on the basis of the lower FM and plasma insulin and glycerol levels in CR, we expected this model to partially restore the impaired insulin-mediated R_d to prepubertal levels. In fact, insulin-mediated R_d was decreased after puberty in the CR to near prepubertal levels compared with Post. Because it could be argued that CR rats had lower LBM when compared with age-matched controls, we further matched LBM of CR with that of younger Post rats and still maintained higher insulin-mediated R_d. In fact, the difference between these groups (Table 3) resulted from an increased FM in the 2-mo-old rats, further suggesting that FM plays a role in establishing insulin sensitivity postpubertally.

Several lines of evidence suggest that the improvement in R_d seen in the CR rats was largely a result of an improvement in glycogen synthesis. When calculated as the difference between R_d and glycolysis, glycogen synthesis rates were significantly improved in the CR compared with Post and LBM-C (Fig. 1 and Table 3). In addition, the [^{3}H]glucose incorporation in muscle glycogen was increased in the CR group compared with the controls. Finally, the insulin-induced increase in muscle

glycogen was also higher in the CR rats (Table 2). This occurred with no changes in the kinetics of muscle GSy or in the activity of GP. Thus the improvement in the ability to store muscle glycogen by CR supports the hypothesis that the decreased glycogen synthesis and insulin resistance in Post rats is predominantly a function of the fat accumulation seen after puberty. Earlier studies in humans have characterized the insulin resistance of type II diabetes and suggested defective glycogen storage. These studies, with the use of indirect calorimetry or ^{13}C nuclear magnetic resonance, have demonstrated a major decline in insulin-mediated glycogen synthesis (27). Moreover, such a decrease was demonstrated in the insulin resistance of normoglycemic obese subjects (11). Such a defect in insulin-mediated glycogen synthesis has also been demonstrated directly in diabetic (22) and obese (4) rats. Our data suggest that this defect in glycogen storage, which is typical in other insulin resistance states, is initiated early in life with the appearance of FM. A milder decrease in the rate of glycolysis was demonstrated in this study and has been previously demonstrated in humans during puberty by indirect calorimetry (3). A mild decrease in glycolysis was also demonstrated in diabetic and obese humans (11) and in obese rats (4). Thus, whereas glycogen synthesis accounts for much of the decrease in insulin-mediated R_d after puberty, postpubertal fat accretion or maturation may also substantially affect the glycolytic and glucose oxidative pathways.

One mechanism that may signal the muscle to decrease its capacity to store glycogen is an increase in substrates such as FFA and glycerol, which are associated with increased FM. Acute increase in plasma FFA levels in humans is associated with a reduction in glucose oxidation (31), supporting the Randle hypothesis that glucose-FFA substrate competition is the initial event that leads to insulin resistance (20). However, intralipid infusion did not consistently cause a decrease in insulin-mediated R_d or produce changes in the activities of important enzymes that should be impaired as a result of glucose-FFA substrate competition (26). Recent evidence suggests that, although an experimentally induced elevation in plasma FFA levels promptly decreases the rate of glucose oxidation, it took >4 h to significantly impair insulin-mediated R_d and glycogen synthesis (6, 7). Thus the downregulation of insulin action requires additional "downstream" intracellular steps to sense increased energy stores in fat.

In summary, the transition to puberty results in an increased deposition of fat and a decrease in insulin sensitivity. We demonstrated that this FM-mediated insulin resistance is characterized by a marked decrease in the ability to store muscle glycogen. This model demonstrates a cause-effect relationship between the increased deposition of fat and the reduced ability to store glucose in muscle in postpubertal rats.

Perspectives

If FM accretion is associated with a decrease in the muscle capacity to store glycogen in response to insulin, what are the mechanisms by which fat tissue "cross talks" with muscle? To deliver such a message to the muscle, candidates should ideally be secreted from fat, their plasma levels should reflect FM, and they should impair insulin action on glucose storage. The most commonly accepted candidate is the substrate FFA, and, although it was generally accepted that the acute increase of FFA causes an immediate "switch" from glucose to lipid oxidation (20), its effect on the decrease of insulin-mediated glycogen synthesis is markedly delayed. A recent hypothesis suggests that the hexosamine pathway acts as a sensor that reacts to an increase in intracellular nutrients such as FFA by decreasing insulin sensitivity (23). Indeed, prolonged FFA infusion to rats increased the accumulation of the end products of the glucosamine biosynthetic pathway, followed by the development of insulin resistance and a decrease in the rate of glycogen synthesis (14). In addition to substrates, fat is becoming increasingly recognized as a metabolically active endocrine tissue that secretes bioactive peptides and may contribute to the changes in the R_d with enhanced fat stores. For example, impairment in tyrosine kinase-dependent insulin signaling and the development of peripheral and hepatic insulin resistance were shown to be induced by tumor necrosis factor-α (12, 16). Although leptin is secreted from fat in proportion to body weight and may play a role in the control of body composition and glucose tolerance, it has been suggested that some obese subjects are resistant to the action of leptin (18). This latter example suggests that a mechanism of failure to communicate between fat and muscle may result in obesity and insulin resistance. These mechanisms, which may be involved in the cross talk between FM and skeletal muscle and which are causing a decrease in insulin action on R_d and glycogen storage, are targets for further investigation.

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