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Glycogen synthase activation in the epididymal adipose tissue from chronic hyperinsulinemic/obese rats

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Rat pups that received a high carbohydrate (HC) milk formula in their neonatal period became chronically hyperinsulinemic and obese in their adulthood. Pups born to HC female rats spontaneously became chronically hyperinsulinemic and obese. Basal activities of mitogen-activated protein kinase (MAPK), 90-kD ribosomal S6 kinase ($pp90^{rsk}$), protein phosphatase-1 (PP-1), glycogen synthase, and protein kinase A (PKA) were measured in the epididymal adipose tissue of 100-day-old male HC rats (born to HC females) and compared with the activities in the epididymal adipose tissue of rats born to mother-fed females (MF). Basal activities of MAPK, $pp90^{rsk}$, PP-1, and glycogen synthase were increased in the epididymal adipose of HC rats while the basal activity of PKA was reduced. Insulin-stimulated activities of MAPK and PP-1 and glucose uptake were also studied in adipocytes from these HC and MF rats. Although the basal activities of MAPK and PP-1 and glucose uptake were higher in adipocytes of HC rats, the ability of insulin to stimulate these processes in vitro above basal levels was less in these adipocytes compared with adipocytes from MF rats. It is possible that circulating higher levels of insulin in HC rats sustain the increased activities of MAPK, $pp90^{rsk}$, PP-1, and glycogen synthase in the epididymal adipose tissue of HC rats and the reduced ability of insulin to further activate MAPK, PP-1, and glucose uptake above basal levels in adipocytes in HC rats may be a compensatory mechanism for the observed effects of chronic hyperinsulinemia in HC rats. (J. Nutr. Biochem. 9:81–87, 1998) © *Elsevier Science Inc. 1998*

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Introduction

An artificial rearing technique has been adopted in this laboratory by which 4-day-old rat pups are reared on a high carbohydrate milk formula (HC) up to day 24 and then weaned onto lab chow.[1] This nutritional intervention in the immediate postnatal period (suckling period) causes the early onset of hyperinsulinemia, persistence of hyperinsulinemia into adult life, and adult onset obesity.[2] These traits were transmitted spontaneously to the progeny by female rats (HC female rats) that underwent the dietary modification in their suckling period.[3] Thus, these progeny constitute a model for chronic hyperinsulinemia and adult onset obesity without themselves undergoing any dietary treatment in their immediate postnatal life.

Hyperinsulinemia and insulin resistance are pathological states associated with a number of clinical conditions including obesity. In obesity, hyperinsulinemia and insulin resistance predispose the individual to cardiovascular complications and non-insulin dependent diabetes mellitus (NIDDM). Insulin exerts pleiotropic effects in target tissues.[4] The initial event in the sequence of insulin action is the interaction of the hormone with high affinity receptors on the plasma membrane of target cells.[4] This binding results in the activation of the intrinsic tyrosine kinase activity of the insulin receptor leading to cellular events mediated by net increases or decreases in Ser/Thr phosphorylation of multiple proteins. This is a consequence of the activation of specific kinases such as the p70 and p90 S6 kinases ($pp70^{S6k}$, $pp90^{rsk}$), mitogen-activated protein kinase

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(MAPK), and protein phosphatase-1 (PP-1) by insulin.[5,6] Studies on rabbit skeletal muscle have indicated that MAPK activates $pp90^{rsk}$ also termed insulin-stimulated protein kinase or S6 kinase II, which subsequently activates PP-1. PP-1 in the phosphorylated form dephosphorylates and activates glycogen synthase, the rate limiting step in glycogen synthesis.[7]

Adipose tissue is an important target tissue for insulin action. Reports on glycogen synthase in adipose tissue in a chronic hyperinulinemic/obesity state are few. In isolated adipocytes from type II diabetic and obese subjects, activation of glycogen synthase (active form) by insulin has been reported to be comparable to that in control subjects.[8] In contrast, a reduction was observed in the in vitro stimulation of glycogen synthase (active form) in isolated adipocytes from hyperinsulinemic and type II diabetic rhesus monkeys.[9] Despite the physiologic importance of MAPK, $pp90^{rsk}$, and PP-1, little is known about the effects of chronic hyperinsulinemia/obesity on their activities in the adipose tissue and their role in the regulation of glycogen synthase as its upstream activators. Because the progeny of HC females are characterized by chronic hyperinsulinemia and adult onset obesity, the basal activities of some of the kinases and the phosphatase involved in the activation of glycogen synthase in the epididymal adipose tissue of these rats were investigated. We observed that basal activities of MAPK, $pp90^{rsk}$, PP-1, and glycogen synthase were increased in epididymal adipose tissue of the hyperinsulinemic HC rats. Although it is known that insulin stimulates MAPK, PP-1, and glucose uptake in adipocytes from normal rats,[10,11] very little information is available on the effects of chronic hyperinsulinemia on the activation of these processes by insulin in the epididymal adipocytes. In adipocytes isolated from the epididymal adipose tissue of HC rats although the basal activities of MAPK, PP-1, and glucose transport were higher compared with adipocytes from MF rats, under in vitro conditions insulin activated these processes to a lesser extent in these adipocytes compared to adipocytes from MF rats.

Methods and materials

Materials

[γ^{32}P]-ATP, UDP-[U-^{14}C]-glucose and [^{3}H]-2-deoxyglucose were purchased from DuPont-NEN (Boston, MA, USA). Phosphorylase a, phosphorylase kinase, myelin basic protein, $pp90^{rsk}$ substrate peptide (32mer), okadaic acid, protein kinase A inhibitor (PKI), phosphocellulose disc sheets, and protein kinase A assay system were purchased from Life Technologies (Gaithersburg, MD, USA). Glut-4 antibody was from East Acres Biologicals (Southbridge, MA, USA). Antibody to the C subunit of PP-1 was from UBI (Lake Placid, NY, USA) and antibody to the G subunit of PP-1 was a kind gift from Dr. D.L. Brautigan (Markey Center for Cell Signaling, Charlottesville, VA, USA). Chemiluminesence reagents were from DuPont (Boston, MA, USA). All other chemicals used were of reagent grade.

Animal protocols

All animal protocols were approved by the Institutional Laboratory Animal Care and Use Committee, State University of New York at Buffalo. Timed-pregnant Sprague-Dawley rats were purchased from Zivic-Miller (Zellenople, PA, USA). Newborn pups were nursed by their mothers until day 4, when the pups were assigned randomly to one of two groups: (1) a mother-fed control group (MF) and (2) a group of animals artificially reared on a milk formula high in carbohydrate (HC; 56% carbohydrate, 20% fat, and 24% protein) but isocaloric compared with rat milk as described in detail previously.[2] Briefly, the HC pups underwent gastrostomy and were fed through a cannula until day 18, when they were allowed oral access to the same formula until day 24, at which time they were weaned onto a standard lab diet (PROLAB RMH 1000; Agway, Syracuse, NY, USA). MF pups were also weaned onto a standard lab diet on day 24. Food and water were provided ad libitum until the time of killing. Animals were maintained on a 12–12 h photoperiod.

On day 60 females and males from the same dietary group were bred (MF males versus MF females and HC males versus HC females) and the resulting litters (adjusted to 12 pups per dam) reared by their natural mothers were weaned onto the standard lab diet on day 24. These rats constituted the second generation HC and MF animals. Plasma insulin and glucose levels of the second generation animals were determined on day 45, 65, and 100 and body weights were recorded at 8 to 10 day intervals until animals were killed in fed condition on day 100 between 0900 and 1000 hr. The epididymal adipose tissue was quickly removed, frozen in liquid nitrogen, and stored at −80°C.

The data presented in this paper were generated from the epididymal adipose tissue from 100-day-old second generation male HC and MF rats used in an earlier study.[3] The HC rats were characterized by a marked increase in the body weight (710 ± 18 g) compared with age-matched MF controls (577 ± 30 g). A significant increase in plasma insulin levels was observed in HC rats compared with MF controls (600 pmol/l in HC vs 321 pmol/l in MF rats on day 65). No significant change in plasma glucose levels was observed between the two groups of animals up to 100 postnatal days.[3]

Glycogen synthase assay

Epididymal adipose tissue from MF and HC rats was homogenized in 5× volume of 50 mM Tris-HCl, pH 7.8 containing 100 mM sodium fluoride, 10 mM EDTA and 10 μg/mL of each of the protease inhibitors (aprotinin, leupeptin, antipain, soybean trypsin inhibitor, and pepstatin-A) and were centrifuged at 1500 × g for 5 min. Glycogen synthase activity was determined in the supernatants using UDP-[U-^{14}C]-glucose, in the absence and presence of 6.7 mM glucose-6-phosphate as described by Thomas et al.[12] Active and total forms of the enzyme are the activities measured in the absence and presence of glucose-6-phosphate respectively, expressed as nmol of UDP-glucose incorporated/min/mg protein. Activity ratio is ratio of the active to total form of the enzyme.

Protein phosphatase-1 (PP-1) assay

Epididymal adipose tissue samples were homogenized in 10× volume of extraction buffer containing 40 mM imidazole hydrochloride buffer (pH 7.2), 2 mM EDTA, 0.2% β-mercaptoethanol, 2 mg glycogen/mL, 1 mM benzamidine, 100 μM phenylmethylsulfonyl fluoride (PMSF), and the cocktail of protease inhibitors (as described above). The extracts were centrifuged at 100,000 × g for 30 min. PP-1 activity was measured in the 100,000 × g supernatant in the presence of 3 nM okadaic acid according to Cohen et al.[13] using purified ^{32}P-labeled phosphorylase a as substrate. The latter was prepared by incubating purified phosphorylase b with [γ^{32}]-ATP and phosphorylase kinase.[14] Activity is expressed as nmoles of Pi released/min/mg protein at 30°C.

Assay of PP-1 activity associated with the glycogen protein particle (PP-1G)

The protocol for the isolation of the glycogen protein particle was adapted from Stralfors et al.[15] Epididymal adipose tissue was homogenized in 10× volume of 2 mM EDTA, 2 mM EGTA, pH 7.0, and the extracts spun at 4200 × g for 30 min. The pH of the supernatant was adjusted to 6.1, allowed to stand on ice for 15 min and centrifuged at 4200 × g for 30 min. The pellet was resuspended in 50 mM Tris-HCl buffer pH 7.0 containing 100 μM EDTA and protease inhibitors, and centrifuged at 80,000 × g for 90 min. This pellet was resuspended in PP-1 extraction buffer and PP-1 activity determined in the presence of 3 nM okadaic acid as described above.

Assay of trypsin-released PP-1 activity

Because PP-1 activity is also present in latent forms, trypsin-released PP-1 activity was determined by incubation of the homogenate with TPCK-treated trypsin (40 μg/mL, for 5 min at 37°C). The reaction was stopped by the addition of soybean trypsin inhibitor (100 μg/mL) followed by centrifugation and assay of PP-1 activity in the supernatants as described above.

Assay of MAPK

Epididymal adipose tissue was homogenized in 10× volume of buffer containing 100 mM β-glycerophosphate (pH 7.2), 1 mM EGTA, 1 mM $MgCl_2$, 100 μM orthovanadate, 1 mM DTT, 1 mM PMSF, and 10 μg/mL of each of the protease inhibitors (as described above). MAPK activity in the 100,000 × g supernatant was assayed as described previously.[16] Activity is expressed as CPM/min/mg protein at 30°C.

Assay of insulin-stimulated protein kinase ($pp90^{rsk}$)

Epididymal adipose tissue was homogenized in 10× volume of buffer containing 25 mM morpholinopropane sulfonic acid (MOPS), pH 7.0, 200 μM EDTA, 2 mM EGTA, 50 mM sodium fluoride, 0.1% β-mercaptoethanol, 10 μg/mL leupeptin, 10 μg/mL soybean trypsin inhibitor, 100 μM PMSF, and 1 mM benzamidine. The homogenate was centrifuged at 4000 × g for 30 min and activity was determined in the supernatant as described by Dent et al.[7] Activity is expressed as CPM/min/mg protein at 30°C.

Assay of protein kinase A (PKA)

Epididymal adipose tissue samples were homogenized in 10× volume of 5 mM Tris-HCl, pH 7.5 containing 5 mM EDTA. After centrifugation at 2000 × g for 10 min the supernatant was assayed using the PKA (cAMP-dependent protein kinase) assay system from Life Technologies. Assays were performed ± cAMP (activator) and ± PKI to determine total PKA activity and proportion of PKA activated in these samples. Activity is expressed as pmol of ^{32}P incorporated/min/mg protein at 30°C. Activity ratio is the ratio of active to total form of the enzyme.

Isolation of adipocytes from the epididymal adipose tissue

Adipocytes were isolated from the epididymal fat pads of MF and HC rats by collagenase digestion as described by Rodbell.[17]

Measurement of 2-deoxyglucose transport

The method of Draznin et al. was adopted for glucose uptake studies using an insulin concentration of 9 nM for 30 min.[11] Glucose uptake is expressed as nmoles of deoxy glucose/3 min/mg protein.

Activation by insulin of PP-1 and MAPK activities in isolated adipocytes

Adipocytes from MF and HC rats were incubated with and without insulin (10 nM for 10 min at 37°C). At the end of the incubation period the adipocytes were centrifuged, washed with PBS, and homogenized in extraction buffers for either PP-1 or MAPK. PP-1 and MAPK activities were determined as described above.

Determination of protein content of Glut-4, and C and G subunits of PP-1

For the determination of Glut-4 protein content, epididymal adipose tissue of MF and HC rats was extracted in phosphate buffered saline (PBS) containing protease inhibitors and centrifuged at 500 × g for 2 min at 4°C and the fat cake removed. The total Glut-4 content was measured in the homogenate. For the determination of the concentration of PP-1 subunits epididymal adipose tissues of MF and HC rats were homogenized in buffer containing 20 mM MOPS, pH 7.5, 10 mM EDTA, 10 mM EGTA, 0.3% β-mercaptoethanol, 1 mM DTT, and protease inhibitors and centrifuged at 10,000 × g for 20 min. The fat cake was removed and the resulting homogenate was used for determination of PP-1 C and G subunit content. Equal amounts of protein were separated by electrophoresis on a 7.5% gel for Glut-4 and PP-1 C subunit and 10% gel for PP-1 G subunit, transferred to nitrocellulose membrane and probed with the respective antibodies. Protein bands were visualized by chemiluminescence. The immunoblots were scanned using a densitometric scanner.

Measurement of protein

The protein concentration of the extracts was determined colorimetrically based on the Bradford dye-binding procedure.[18]

Statistical analysis

Results are the means ± SEM of six animals per treatment. Student's *t* test was used to evaluate the significance of the results and $P < 0.05$ was considered as significant.

Results

The first objective of this study was to evaluate the effects of chronic hyperinsulinemia on the basal activities of some of the kinases and protein phosphatase-1 in the pathway of insulin action that activates glycogen synthase. Basal activities of the active and total forms and activity ratio of glycogen synthase in epididymal adipose tissue of HC and MF rats were measured (*Figure 1*). A 2.4-fold increase in the active form of the enzyme was observed in HC rats compared with MF controls. Activity of the total form of the enzyme also increased (1.6 fold) in HC rats (*Figure 1*). The activity ratio of glycogen synthase was also significantly increased in the HC rats compared with MF controls (*Figure 1*).

PP-1 is the immediate upstream activator of glycogen synthase. Basal PP-1 activity in homogenates of epididymal adipose tissue of hyperinsulinemic HC rats was significantly higher compared to MF values (*Figure 2*, column A). C subunit of PP-1 is bound to different regulatory subunits that target the enzyme to different locations within the

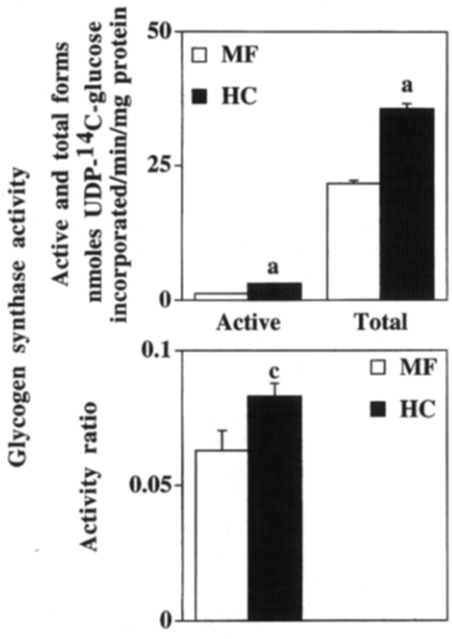

Figure 1 Activities of the active form of glycogen synthase, its total activity (upper panel) and activity ratio (lower panel) in epididymal adipose tissue of MF and HC rats. Values are means ± SEM of six animals. (a) $P < 0.001$, (c) $P < 0.05$ compared with MF.

cell.[19] The G subunit is primarily responsible for glycogen synthase activation and exists in association with the C subunit in the glycogen protein particle. PP-1 activity in the glycogen protein particle was higher in HC rats compared with MF rats (*Figure 2*, column B). C subunit of PP-1 also binds to heat stable protein inhibitors in the cytosol and is released from these inhibitors by proteolytic treatment with trypsin. The trypsin-released PP-1 activity was significantly less in the HC rats compared with MF rats (*Figure 2*, column C).

In the pathway of insulin action, regulating glycogen synthesis, $pp90^{rsk}$ phosphorylates and activates PP-1 and is itself phosphorylated and activated by MAPK.[4] The activities of both $pp90^{rsk}$ and MAPK were significantly higher in

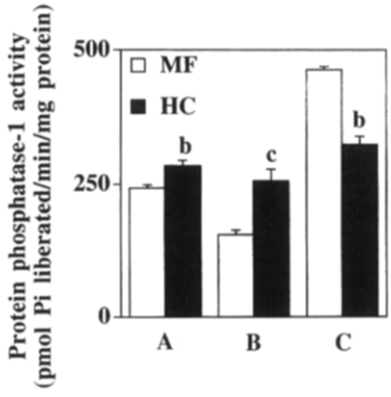

Figure 2. PP-1 activity in epididymal adipose tissue of MF and HC rats. Column A: Basal PP-1 activity in the homogenate; Column B: PP-1 activity in the glycogen protein particle; Column C: PP-1 activity in the homogenate after trypsin treatment. Values are means ± SEM of six animals. (b) $P < 0.01$, (c) $P < 0.05$ compared with MF.

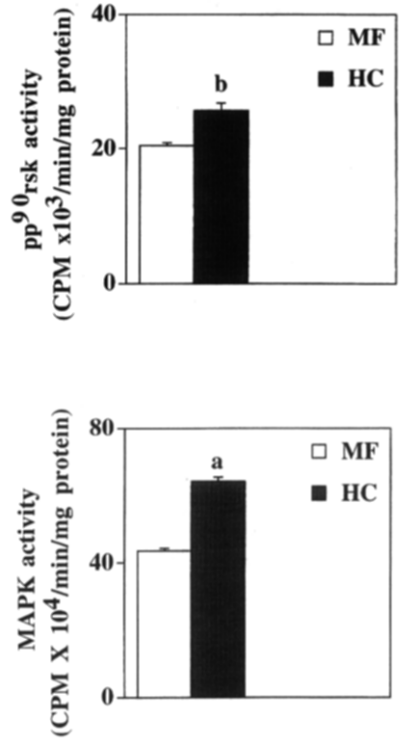

Figure 3 $pp90^{rsk}$ activity (upper panel) and mitogen-activated protein kinase (MAPK) activity (lower panel) in epididymal adipose tissue of MF and HC rats. Values are means ± SEM of six animals. (a) $P < 0.001$, (b) $P < 0.01$ compared to MF.

the epididymal adipose tissue of HC rats compared with MF controls (*Figure 3*).

PKA functions in an antagonistic fashion to insulin with respect to glycogen metabolism. In view of its importance in the regulation of glycogen synthesis PKA activity was measured in epididymal adipose tissue of HC and MF rats. The active form of the enzyme was significantly decreased in HC rats compared to MF rats (*Figure 4*). A concomitant decrease in activity ratio was also observed in HC rats. The total PKA activity was higher in HC rats compared with MF rats (data not shown).

The second objective of this study was to investigate the biological actions of insulin in vitro in epididymal adipocytes isolated from HC and MF rats. Glucose uptake and activation of PP-1 and MAPK, processes known to be activated by insulin in the adipose tissue, were evaluated in epididymal adipocytes for their insulin responsiveness. Although the basal glucose uptake was 218% higher in adipocytes from HC rats compared with adiopocytes from MF rats, insulin did not stimulate this process to the same extent as it did in adipocytes from MF rats (approximately 125% increase over basal in HC adipocytes compared with approximately 397% increase over basal in MF adipocytes; *Figure 5*). Similarly, although the basal activities of PP-1 and MAPK were 55% and 49% higher in adipocytes from HC rats, the ability of insulin to activate these processes was reduced in adipocytes from HC rats compared to adipocytes from MF rats (*Figure 5*).

Our final objective was to see if the protein content of the subunits of PP-1 and Glut-4 paralleled the changes in the

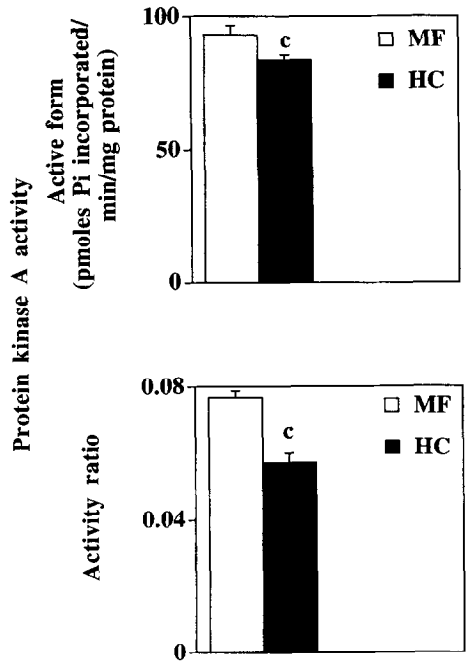

Figure 4. Activity of the active form of PKA (upper panel) and its activity ratio (lower panel), in epididymal adipose tissue of MF and HC rats. Values are means ± SEM of six animals. (c) $P < 0.05$ compared to MF.

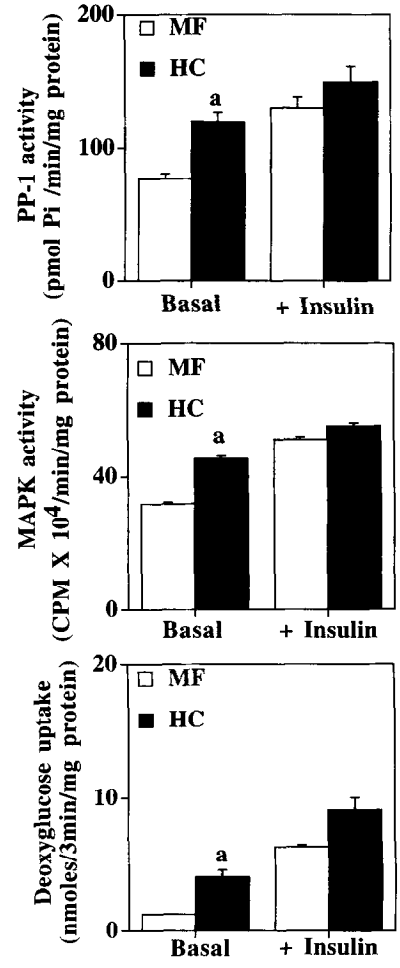

Figure 5. Stimulation of PP-1 and MAPK activities and glucose uptake by insulin in adipocytes from HC and MF rats. Values are means ± SEM of six animals. (a) $P < 0.001$ compared with MF.

activity patterns that were observed. For this purpose western blot analyses of Glut-4 and C and G subunits of PP-1 were performed on homogenates of epididymal adipose tissue of HC and MF rats. The content of the C subunit of PP-1 was increased (1.85 fold; $P < 0.001$) in HC epididymal adipocytes compared to MF controls (*Figure 6*). There was no significant change in the G subunit content between the two groups of rats (results not shown). The total Glut-4 content in HC adipose tissue was significantly increased compared to MF adipose tissue ($P < 0.01$) (*Figure 6*).

Discussion

Chronic hyperinsulinemia and adult onset obesity are the salient features of the rat model used in this study.[2] These rats did not undergo any dietary modification in their neonatal period but were born to HC female rats that were reared on an HC formula in their suckling period. Chronic hyperinsulinemia and adult onset obesity were transmitted spontaneously from HC females to their progeny.[3] We have used this model to study the effects of chronic hyperinsulinemia/obesity on the basal activities of MAPK, $pp90^{rsk}$, PP-1, glycogen synthase, and PKA in the epididymal adipose tissue. In addition the stimulatory effect of insulin on glucose uptake and MAPK and PP-1 activities in isolated adipocytes was also studied.

Insulin promotes several anabolic effects in the body. With respect to glucose homeostasis, liver, muscle, and adipose tissue are the specific target tissues. Activation of glycogen synthesis and inhibition of glycogenolysis are important metabolic actions of insulin. In our earlier study on the pathway of insulin action activating glycogen synthesis, we demonstrated that the basal activities of MAPK, $pp90^{rsk}$, PP-1, and glycogen synthase were reduced in the liver and skeletal muscle of HC rats compared with MF controls, whereas the activity of the counter-regulatory enzyme PKA was elevated.[20] In the present study we show that these effects are reversed in epididymal adipose tissue compared to liver and muscle. Under normal conditions insulin promotes glycogen synthesis in both muscle and adipose tissue. Glycogen storage has a different role in adipose tissue compared with its function in the muscle. In the adipose tissue glycogen has been reported to serve as a source of glycerol-3-phosphate which is necessary for the esterification of fatty acids.[21] The increased activation of the insulin signaling cascade leading to enhanced glycogen synthesis in epididymal adipose tissue in the HC rat correlates well with the increased rate of lipogenesis (and increased storage of triglycerides) reported in the hyperinsulinemic HC rat.[2] In this rat model for chronic hyperinsulinemia/obesity it is possible that the elevated levels of insulin promote the storage of both triglycerides and glycogen in the adipose tissue. This study clearly shows that the same process is regulated differently in different tissues in

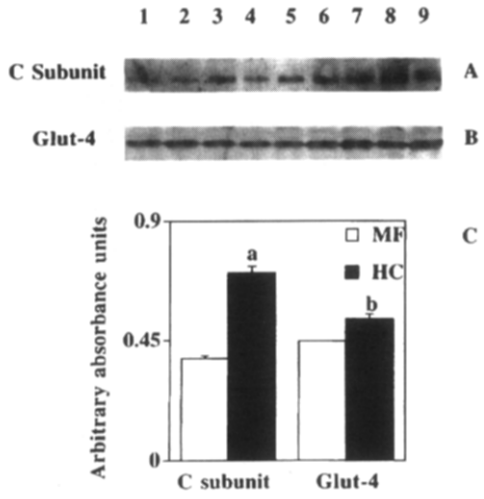

Figure 6. Western blot analyses of PP-1 and Glut-4 in the homogenate from epididymal adipose tissue of MF and HC rats. A: C subunit of PP-1; lanes 1–5 MF and lanes 6–9 HC. B: Glut-4; lanes 1–4 MF and lanes 5–9 HC. C: Arbitrary absorbance units for Glut-4 and C subunit protein contents in epididymal adipose tissue of MF and HC rats. Values are means ± SEM of six animals. (a) $P < 0.001$, (b) $P < 0.01$ compared to MF.

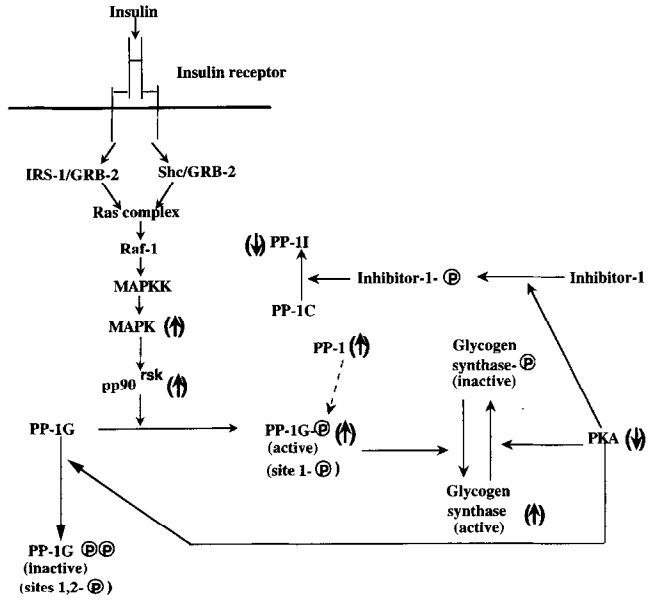

Figure 7 Insulin mediated signal transduction pathway for activation of glycogen synthesis (adapted from References 4 and 19). IRS-1, Insulin receptor substrate-1; MAPKK, mitogen-activated protein kinase kinase; pp90 ^{rsk}, insulin-stimulated protein kinase; PP-1, protein phosphatase-1; PP-1G, protein phosphatase-1 associated with the glycogen protein particle; PKA, protein kinase A; PP-1I, protein phosphatase-1 bound to inhibitor proteins; PP-1C, protein phosphatase-1 catalytic subunit; -(P), phosphorylated form. (↓) indicates a decrease in activity or content and (↑) indicates an increase in activity.

the HC rat, suggesting tissue-specific responses under conditions of chronic hyperinsulinemia/obesity.

Only a few reports are available on the regulation of glycogen synthase in adipose tissue under conditions of chronic hyperinsulinemia/obesity. In spontaneously insulin-resistant rhesus monkeys it was shown that the basal level of total glycogen synthase activity was elevated in isolated adipocytes from abdominal adipose tissue although the basal levels of the active form was not altered between the insulin-resistant and control groups.[9] In adipocytes isolated from obese and NIDDM patients there was no change in the basal or insulin-stimulated levels of the active form of glycogen synthase although the total activity was reduced in NIDDM patients compared with normal or obese patients.[8] Chronic exposure to insulin of cultured adipocytes from normal rats increased glycogen synthase activity, both the active and total forms.[22] Our results on HC rats indicate increased basal activities of both the active and total forms of glycogen synthase and hence differ from the results on human subjects and the rhesus monkeys. They, however, are in agreement with the study on cultured epididymal adipocytes isolated from the rat epididymal adipose tissue.[22] This difference could be attributable to species variation, extent of the hyperinsulinemic condition and the type of the adipose tissue used (epididymal adipose tissue versus abdominal adipose tissue).

There are no reports on the activity of MAPK, pp90^{rsk}, or PP-1 in adipose tissue under chronic hyperinsulinemic/obese conditions, although these enzymes have a significant physiologic role to play in the stimulation of glycogen synthase. Our study shows that the basal activities of the MAPK, pp90^{rsk} and PP-1 are increased in the hyperinsulinemic HC rat leading to increased activation of glycogen synthase, the rate-limiting enzyme in glycogen synthesis (*Figure 7*). The increased basal activity of PP-1 in the homogenate and the glycogen protein particle in the HC rats could be because less of the C subunit is bound to the heat-stable protein inhibitors as is indicated by reduction in the released level of PP-1 activity after trypsin treatment in HC rats (*Figure 2*). This is further strengthened by the reduced basal activity of PKA, which regulates glycogen synthesis by its direct effect on glycogen synthase and indirectly by its effect on PP-1 (*Figure 7*).

The higher basal activities of MAPK, PP-1 and glucose uptake may be a response to chronic hyperinsulinemia in these rats. The increased protein content of the glucose transporter and the catalytic subunit of PP-1 may contribute to the higher basal glucose uptake and higher basal activity of PP-1 in adipocytes from HC rats. The reduced ability of insulin to stimulate these processes in isolated adipocytes from HC animals may be a compensatory mechanism for the effects induced by chronic hyperinsulinemia.

This study reflects the effects of a nutritional modification in the form of a high carbohydrate milk formula during the suckling period of female rats on the epididymal adipose tissue metabolism of their progeny. These progeny acquire the traits of chronic hyperinsulinemia and adult onset obsesity from their mothers without themselves undergoing any dietary treatment during their suckling period. It is possible that chronic hyperinsulinemia causes a positive energy balance for fat in the adipose tissue leading to the triglyceride deposition in these rats. The upregulation of the basal activities of the specific kinases and the phosphatase in the adipose tissue may support the anabolic metabolism in this tissue.

Acknowledgments

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