Changes in Rat Adipocyte and Liver Glucose Metabolism Following Repeated Restraint Stress

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Rats exposed to repeated restraint weigh less than controls even 8 weeks after stress. Stress-induced weight loss is lean tissue, but the post-stress difference in weight between control and restrained rats is lean and fat mass. Whole-body glucose clearance is enhanced 1 day after stress, but adipocyte glucose utilization is inhibited and muscle glucose transport is unchanged. The studies described here demonstrated that glucose transport was increased in both restrained and pair-fed rats, but that glycogen synthesis was increased only in restrained rats, which may account for the improved whole-body glucose clearance. Adipocyte glucose transport was inhibited and adipose plasma membrane β-adrenergic receptor number was increased 1 day post-stress in restrained rats when weight loss was lean tissue, but were not different from control rats 5 days post-stress, when both fat and lean tissue were reduced. Thus, repeated restraint induces reversible changes in adipocyte metabolism that may represent a transition from the catabolic state of stress to a new energetic equilibrium in rats that maintain a reduced body weight for an extended period of time.

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Key words: glucose transport; glycogen; β-adrenergic receptor

We have previously demonstrated that rats exposed to restraint stress maintain a reduced body weight for extended periods of time during the post-stress period. The initial weight loss is associated with hypophagia on the days of restraint and there is no evidence of compensatory over-eating during the post-stress period to make up for the stress-induced energy deficit (1); therefore, although the rats gain weight after stress, they do not catch up to the weight of non-stressed controls (1). Repeated exposure of rats to restraint stress causes similar, but greater, changes in body weight and food intake (2, 3). Elucidating the mechanism of sustained post-stress weight loss and the temporary inhibition of food intake will assist our understanding of how stress disrupts homeostasis, and may identify mechanisms that are essential for the regulation of body weight in rats.

The weight loss in rats exposed to repeated restraint stress (3 hours restraint on each of 3 days) is exclusively lean body mass 1 day after the last restraint stress, but 5 days after the end of restraint the difference in weight between stressed and control animals is composed of both lean and fat tissue (3). This shift in body composition could only be achieved by tissue-specific changes in nutrient utilization in the days immediately following the end of stress. An oral glucose tolerance test performed 1 day after the end of restraint indicated that whole-body glucose clearance was increased. This change in clearance could not be accounted for by muscle, as glucose transport was not changed in this tissue, or by adipose tissue, because adipocyte glucose uptake was inhibited in restrained rats compared with controls (2). In the experiments described here we measured hepatocyte glucose utilization to determine whether the liver could account for increased whole-body glucose uptake with a simultaneous inhibition of adipocyte glucose uptake and loss of body fat. The results demonstrate a substantial change in hepatic glucose metabolism, but do not elucidate the primary mechanisms responsible for the metabolic changes in restrained rats.

Additional experiments described here tested the hypothesis that loss of fat during the post-stress period was due to inhibition of adipocyte *de novo* lipid synthesis from glucose. Adipocyte glucose transport and adipose tissue β-adrenergic receptor number were measured both 1 and 5 days after the end of restraint. These time points were chosen based on previous observations of significant shifts in the body composition of restrained rats during this interval of the post-stress period (3).

Materials and Methods

Twelve week old male Sprague-Dawley rats weighing 350 g were obtained from Harlan Sprague-Dawley (Houston, TX) and housed individually in wire mesh cages in a humidity and temperature controlled room (22° ± 2°C, 65%

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to 67% humidity) on a 12:12-hr light:dark cycle with lights on at 07:00 hr. All rats were fed a 40% kcal fat, 16% kcal protein diet for at least 10 days before the experiments started, as we have previously found that a high-fat diet exaggerates the effects of restraint stress on body weight (3). Body weights and food intakes were recorded daily. All food intakes, including those of pair-fed rats, were corrected for spillage. All animal protocols were approved by the Pennington Biomedical Research Center Institutional Animal Care and Use Committee.

For the repeated restraint stress protocol, rats were placed in Perspex restraining tubes (Plas Labs, Lansing, MI) for 3 hr in the morning for 3 consecutive days. The control and pair-fed rats were moved to the same room as the restrained rats and did not have access to food or water for the period of restraint. Pair-fed rats were fed *ad libitum* before stressed rats were restrained, but were given the voluntary intake of restrained rats from the first day of restraint until the end of the experiment. Experiment 1 was conducted with the rats subdivided into groups and the restraint protocol staggered over 3 days to ensure timely collection and handling of tissue and to facilitate pair-feeding to restrained animals.

Experiment 1. Hepatocyte glucose transport and glucose oxidation and incorporation into fatty acids were measured 1 day after the last restraint stress. In this experiment, glucose transport, glucose oxidation, and glucose incorporation into fatty acids were measured in hepatocytes from control, restrained, and pair-fed rats 1 day after the last repeated restraint stress. Thirty rats were fed the high-fat diet for 11 days and were then divided into three weight-matched groups: control, pair-fed, and repeated restraint stress.

One day after the last restraint stress, all rats were deprived of food for 1 to 1.5 hr, from 08.00 hr, prior to anesthesia (ketamine, 90 mg/kg body weight; Xylazine, 10 mg/kg body weight, intraperitoneally). Hepatocytes from each animal were isolated using the method of Berry (4). An incision was made through the abdominal wall and peritoneal cavity to expose the liver. An inflow cannula was inserted into the portal vein, and a perfusion (10 ml/min) was started with continuously gassed (95% O_2/5% CO_2) perfusion media (Krebs bicarbonate buffer without $CaCl_2$, 5.0 mM glucose, pH 7.45). Blood was washed out by cutting the vena cava and the perfusion was continued for 15 min. Another cannula was inserted into the inferior vena cava below the heart, and the interior vena cava near the kidney was closed with a ligature so that digestion media could be recycled. Thus, the outflow went only through the inferior vena cava below the heart. The liver digestion was started by switching the perfusion media to digestion media (Krebs bicarbonate buffer, 5.0 mM glucose, 2% bovine serum albumin [BSA], and 2 mg/ml collagenase, pH 7.45) and was stopped after 10 min. The liver was removed and cells were dispersed using a glass rod and filtered though 250-μm nylon mesh with wash media (Krebs bicarbonate buffer, 3 mM glucose, 10 mM HEPES, 0.5 mM palmitate, and 2% BSA, pH 7.45). The isolated hepatocytes were washed three times and were resuspended in an appropriate volume of wash media.

For measurement of glucose transport, an aliquot of isolated cells was washed once and suspended in transport media (Krebs bicarbonate buffer, 0.1 mM glucose, 30 mM mannitol, 10 mM HEPES, and 2% BSA, pH 7.45). Cell number was determined using a haemocytometer. Trypan Blue dye exclusion was used to determine the number of viable cells and the percentage cell viability was recorded.

Glucose uptake was measured only in basal conditions because liver glucose transport is insulin independent. One milliliter of each cell suspension was added to 2 ml of transport media containing 0.1 μCi/ml ^{14}C-mannitol and was then incubated for approximately 15 min at 37°C with shaking. Then 0.2 mM 2-deoxy D-glucose (2-DG), 1.0 mCi/mM ^{3}H 2-DG in a 50-μl volume was added and the sample was incubated for exactly 2 min. Triplicate 200-μl aliquots of the sample were transferred to vials, immediately centrifuged (5000–6000*g*) to separate cells from media, and the supernatant was aspirated off to stop the reaction. The incubation was repeated in quadruplicate for each rat. The cell fraction was counted for 2-DG incorporation and corrected for extracellular fluid volume, indicated by ^{14}C-mannitol. Results are expressed as nanomoles of glucose incorporated per 10^6 cells per 2 min.

For measurement of glucose oxidation and incorporation into fatty acids in basal and insulin-stimulated conditions, hepatocytes were suspended in incubation buffer (Krebs bicarbonate buffer, 3 mM glucose, 10 mM HEPES, 0.5 mM palmitate, and 2% BSA, pH 7.5). Triplicate 0.5-ml aliquots of each cell suspension were added to 1.0 ml of media containing 0.3 μCi/ml ^{14}C-glucose with or without 1.0 mU insulin/ml. The flasks were gassed with 95% O_2/5% CO_2, sealed with rubber stoppers carrying center wells, and incubated for exactly 2 hr at 37°C with shaking. The reaction was stopped by adding 0.5 ml of 0.5 M H_2SO_4 to media and CO_2 was collected by addition of 0.2 ml of 1.0 M benzethonium hydroxide to the center well. The center well was transferred to a scintillation vial for determination of $^{14}CO_2$ and the cells were extracted for glucose incorporation into fatty acids, as described previously (5). Results were expressed as nanomoles of glucose incorporated per 10^6 cell per 2 hr.

Experiment 2. Hepatic glycogen synthesis was measured 1 day after the end of restraint. This experiment measured glycogen synthesis in liver slices from control, restrained, and pair-fed rats 1 day after the last repeated restraint stress. Twenty-six rats were fed the high-fat diet for 11 days and were then divided into three weight-matched groups: repeated restraint stress, pair-fed, and control.

One day after the last restraint stress, all rats were food deprived for 1 to 2 hr in the morning prior to decapitation between 09:00 and 11:00 hr. The liver was removed and weighed. Six small slices of liver tissue (50–100 mg) from each rat were obtained using a Stadie Riggs tissue slicer.

The slices were weighed and preincubated for 15 min in 2 ml of Krebs bicarbonate buffer, 10 mM HEPES, 5 mM glucose, 2 mM sodium pyruvate, and 1% BSA, pH 7.45 with or without addition of 2 mU/ml of insulin. The tissue was then incubated for 60 min in 2 ml of fresh media that included 0.5 μCi U-^{14}C-glucose/μmol glucose. Reactions were stopped by transferring tissue to ice-cold saline. After washing twice with cold saline, the tissue was dissolved in 1.0 M NaOH, 66% ethanol. Glycogen (~200 μg) was added to each sample and samples were held at −20°C overnight. The samples were centrifuged at 810*g* for 20 to 30 min and the supernatant was discarded. The pellet was washed three times in cold 66% ethanol. The final pellet was dissolved in water and transferred to a scintillation vial for determination of ^{14}C -glucose. Glycogen synthesis was expressed as nanomoles of glucose per milligram of tissue per hour and as millimoles of glucose per liver per hour.

Experiment 3. β-adrenergic antagonist binding to adipose tissue membranes was measured 1 day after the last restraint stress. Eighteen adult, male Sprague-Dawley rats were divided into three weight-matched groups: repeated restraint, pair-fed, and control. The repeated restraint stress and pair feeding protocols were the same as described above except that rats were restrained on 4 instead of 3 consecutive days.

One day after the last restraint stress, the rats were food deprived for 2 hr at the start of the light period prior to decapitation. Epididymal fat was immediately homogenized in cold buffer (Krebs bicarbonate buffer, pH 7.45, 10 μg/ml aprotinin, 5 μg/ml leupeptin, and 1 mM phenylmethylsulfonyl fluoride). The homogenate was centrifuged at 1000*g* for 10 min at 4°C. The supernatant was further centrifuged at 13,500*g* for 15 min at 4°C. The final pellet was dissolved in an appropriate volume of homogenization buffer for the determination of protein concentration (BCA protein assay kit: BCA, Pierce, Rockford, IL). The final protein concentration in each sample was adjusted to 200 μg/ml.

For determination of maximum binding activity, duplicate 20-μg aliquots of freshly isolated cell membrane were incubated at 37°C for 30 min in 0.6 ml of homogenization buffer with increasing concentrations of ^{3}H dihydroalprenolol (^{3}H-DHA: 1, 5, 20, 40, 60, 80, 100, and 120 nM.) For nonspecific binding, incubation conditions were the same as maximum binding except that a saturating concentration of 5 mM/ml of propranolol was present in the incubation buffer. The reaction was stopped by adding 0.5 ml of ice-cold homogenizing buffer to the tubes and placing the tubes on ice. Membranes were collected on 0.45-μ m nitrocellulose filters (Millipore, Bedford, MA.) and were washed with 10 ml of cold Krebs buffer. The filter was dissolved in 1 ml of ethylene glycol monoethyl ether (Sigma Chemical Co., St. Louis, MO) and bound ^{3}H-DHA was determined by scintillation counting. Specific binding was calculated by subtracting nonspecific binding from maximum binding.

Experiment 4. Adipocyte glucose transport and adipose tissue β-adrenergic receptor number were measured 1 and 5 days after the last restraint. This experiment investigated post-stress effects on adipocyte glucose transport and adipose tissue β-adrenergic receptor number 1 and 5 days after the last restraint stress. The previous experiments did not reveal any differences in adipocyte glucose transport or β-adrenergic receptor binding in tissue from control and pair-fed rats, therefore, only restrained and control groups were included in this experiment.

Thirty rats were maintained on high-fat diet for 10 days and were then divided into three weight-matched groups: Control, Restraint-1, and Restraint-5. Rats in the Restraint-1 group were killed 1 day after the last day of repeated restraint and rats in the Restraint-5 group were killed 5 days after the last restraint stress. Half of the control rats were killed at a time that was equivalent to 1 day after the last restraint stress and the other half were killed at a time that was equivalent to 5 days after the last restraint stress. The start of the experimental protocol was staggered over 5 days to ensure that animals from all of the different groups were killed on the same day. Rats were food deprived for 1 to 2 hr in the morning before decapitation. Epididymal fat was dissected and weighed, and half of it was used to measure maximal β-adrenergic receptor binding, as described above except that a single concentration of 150 nM ^{3}H-DHA was used. The other half was used to measure glucose transport in isolated adipocytes.

Adipocytes were isolated by the method of Rodbell (6) and were suspended in transport buffer (1× Krebs, 0.1 mM glucose, and 2% BSA). Glucose uptake was measured in basal and insulin-stimulated conditions. Duplicate 1-ml aliquots of each cell suspension were added to 2 ml of media containing 0.1 μCi/ml ^{14}C-mannitol with or without 0.8 mU/ml of insulin, and were incubated for 30 min at 37°C with shaking. Cell number was determined by fixing an equivalent aliquot in osmium tetroxide and counting by Coulter Counter, as described previously (7). Fifty microliters of 0.2 mM 2-DG, 1.0 mCi/mM ^{3}H 2-DG was then added and the sample was incubated for exactly 2 min. Triplicate 200-μl aliquots of the sample were transferred to vials containing 100 μl of phthalic acid dinonyl ester and was immediately centrifuged to separate cells from media. The cell fraction was counted for 2-DG incorporation and was corrected for extracellular fluid volume, represented by ^{14}C-mannitol. Results are expressed as nanomoles of glucose incorporated per 10^6 cells per 2 min.

Statistical Analysis. Results for hepatocyte glucose uptake, adipocyte glucose transport, and receptor binding in Experiment 4 were analyzed by one or two-way analysis of variance (ANOVA) with *post hoc* Duncan's multiple range test. All other data for liver glucose utilization were analyzed by repeated measures ANOVA with insulin concentration as the repeated measure. The SAS system, version 6.12, was used for computations. The results for receptor binding in Experiment 3 were analyzed by a nonlinear regression model for radioligand binding data (Prism Software, GraphPad Software, San Diego, CA). The B_{max} and

K_d from each group were obtained using the average data from each rat. Data are presented as means ± SEM.

Results

Body Weight and Food Intakes of Rats in the Four Experiments. The restrained rats lost weight on the days that they were stressed, and food intake was inhibited on the 3 days of restraint in all four experiments. In Experiment 4, rats did not return to their pre-stress body weight during the 5-day post-stress period and there was no evidence of overeating to compensate for the stress-induced hypophagia (data not shown). We have previously reported that stressed rats do not reach control body weight even 40 days after the end of repeated restraint (3).

Experiment 1. Hepatocytes from both restrained and pair-fed rats had significantly higher rates of glucose transport than those from control rats ($P < 0.05$), as shown in Figure 1. Hepatocyte glucose oxidation and incorporation into fatty acids were not different among the three groups in either basal or insulin-simulated conditions (Figure 2).

Experiment 2. Restrained rats had significantly higher rates of liver glycogen synthesis in basal conditions compared with control and pair-fed groups, when data were expressed either as nanomoles of glucose incorporated per milligram of tissue per hour or as millimoles of glucose incorporated per liver per hour (see Fig. 3). This significant difference was not apparent in insulin-stimulated conditions because glycogen synthesis in tissue from restrained rats did not increase beyond the levels found in basal conditions.

Experiment 3. Figure 4 clearly shows that at the same ^{3}H-DHA concentration, specific binding of ^{3}H-DHA to adipose plasma membrane was saturated for tissue from control and pair-fed rats, but not from restrained rats. The B_{max} (control, 147 fmol/μg; pair-fed, 167 fmol/μg) and K_d (control, 75 pM; pair-fed, 62 pM) for control and pair-fed rats were similar. Since binding was not saturated for restrained rats, B_{max} and K_d could not be calculated. However, as concentration of ligand needed for saturation is determined by receptor number, it may be assumed that receptor number was increased in restrained rats. Due to large inter-animal variation, we could not compare K_d among the three groups of rats.

Experiment 4. Restraint stress ($P < 0.05$) and insulin ($P < 0.05$) had significant effects on adipocyte glucose transport measured 1 and 5 days after restraint, as shown in Figure 5A, but there was no interaction between stress and insulin ($P > 0.1$). Glucose transport in adipocytes from Restraint-1 rats was significantly reduced compared with that in control rats in both basal and insulin-stimulated conditions. Transport in cells from Restraint-5 rats was not different from either the control or Restraint-1 groups. Adipose tissue β-adrenergic receptor binding was significantly higher in tissue from Restraint-1 rats than either control or Restraint-5 rats. ($P < 0.05$), but there was no difference between control and Restraint-5 animals (see Fig. 5B).

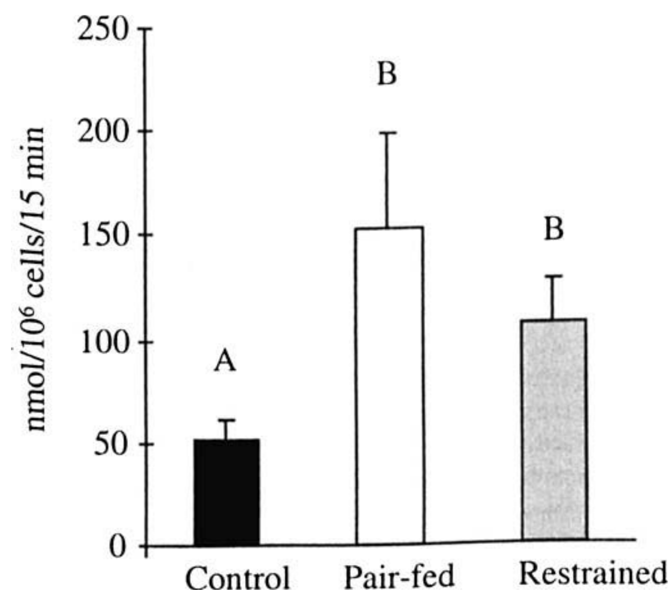
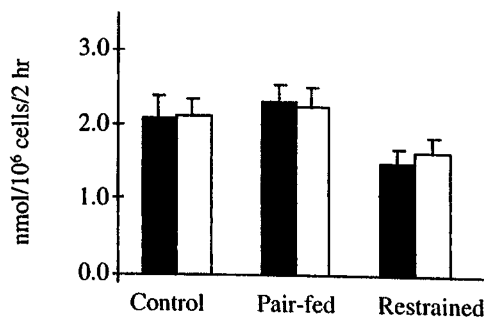


Figure 1. Glucose transport in isolated hepatocytes from control, pair-fed, and restrained rats in Experiment 1. Data are means ± SEM for groups of 10 rats. Values that do not share a common letter are significantly different at P < 0.05. Cells from pair-fed and restrained rats had significantly higher rates of glucose transport than those from control rats.

Basal
1.0 mU/ml Insulin

A: Glucose Oxidation



B: Glucose Incorporation into Fatty Acids

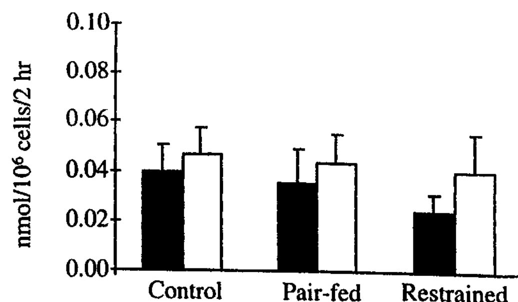


Figure 2. Glucose oxidation and incorporation into fatty acids in hepatocytes from control, pair-fed, and restrained rats in Experiment 1. Data are means ± SEM for groups of 9 rats. There were no statistically significant differences between groups for either measurement.

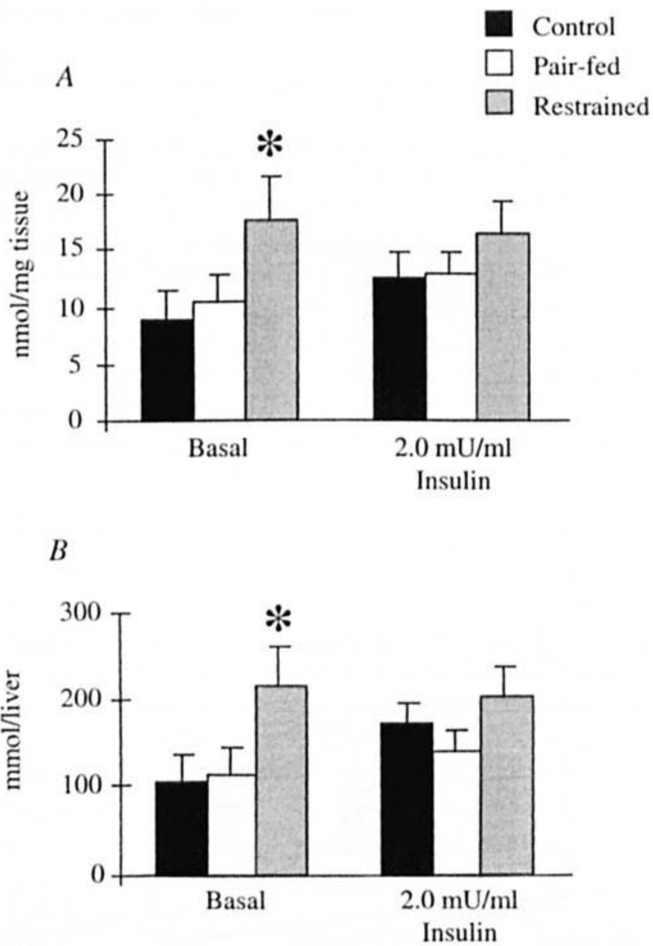


Figure 3. Glycogen synthesis in liver slices from control, pair-fed, and restrained rats in Experiment 2. Data are mean ± SEM for groups of 8 rats. (A) Glycogen synthesis expressed per milligram of tissue. (B) Glycogen synthesis expressed per liver. Glycogen synthesis was significantly higher ($P < 0.05$) in restrained than control or pair-fed rats, as denoted by an asterisk.

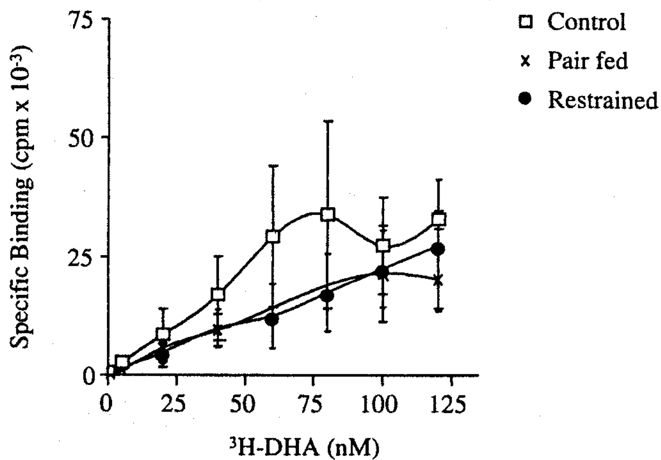
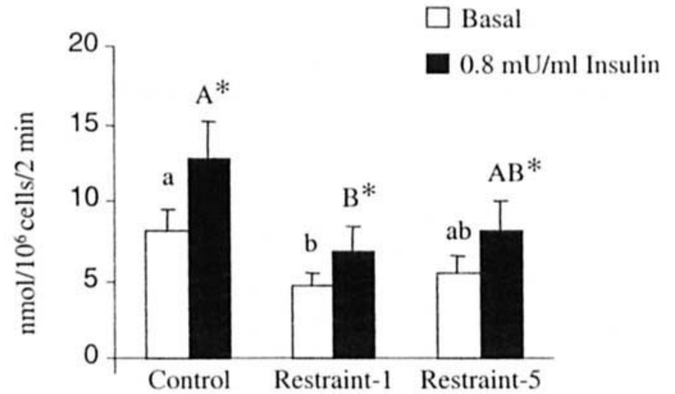


Figure 4. Specific binding of ^{3}H-DHA to adipose plasma membrane of control, pair-fed, and restrained rats from Experiment 3. Data are means ± SEM for 5 or 6 rats per group.



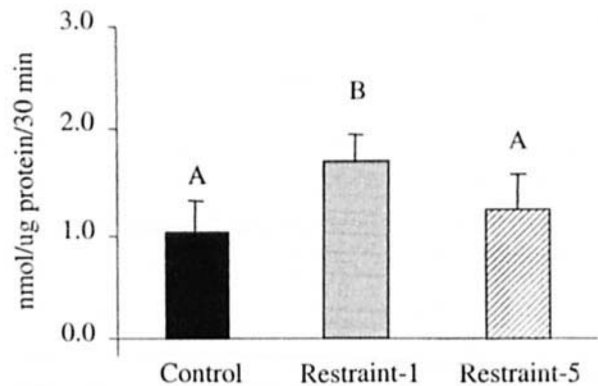


Figure 5. (A) Adipocyte glucose transport in control, Restraint-1, and Restraint-5 groups of rats from Experiment 4. Data are means ± SEM for groups of 10 rats. Insulin significantly increased glucose transport in all three groups of rats ($P < 0.05$), as indicated by an asterisk. Both basal and insulin-stimulated glucose transport were significantly lower in Restraint-1 rats than controls ($P < 0.05$), indicated by lower case superscripts for basal transport and upper case superscripts for insulin-stimulated transport. Restraint-5 rats were not different from either control or Restraint-1 groups. (B) β-Adrenergic receptor binding of ^{3}H-DHA in control, Restraint-1, and Restraint-5 groups of rats from Experiment 4. Data are means ± SEM for groups of 10 rats. β-Adrenergic ligand binding was significantly higher for Restraint-1 than control or Restraint-5 animals ($P < 0.05$). There was no difference between control and Restraint-5 rats.

Discussion

Previous experiments have shown that repeated restraint stress causes a sustained down-regulation of body weight in rats, such that they weigh significantly less than their controls even 40 days after the end of restraint (3). The weight loss during stress is accounted for by catabolism of lean tissue, but within 5 days of the end of stress the composition of weight loss is a combination of lean and fat mass. Whole-body glucose clearance is also significantly improved 1 day after the end of stress despite a substantial inhibition of adipocyte glucose transport and no measurable change in muscle glucose uptake (2). Because the shift in body composition can only be achieved by tissue-specific changes in nutrient utilization, these studies tested the effects of repeated restraint stress on liver and fat metabolism

during the early stages of the post-stress period. All of the measurements were made *in vitro* on isolated cells or tissue slices, and differences between groups represented responses mediated by cellular factors such as levels of protein expression. They did not account for differences between groups that were mediated by acute *in vivo* neural or hormonal input to the cells.

Hepatocyte glucose transport was significantly increased 1 day after the end of restraint in restrained and pair-fed rats compared with controls. This may account for our previous observation of increased whole-body glucose clearance in restrained rats (2). Net hepatic glucose uptake is determined by the balance between glucose uptake and output and it has been shown that the stress of exercise, trauma, or infection stimulates hepatic glucose production (8–10). We did not measure glucose production; therefore, the increased glucose transport into hepatocytes of restrained rats could reflect either an increase in net glucose uptake, if hepatic glucose production was normal, or no net change in uptake if glucose production was increased. We hypothesize that the first situation was true for restrained rats and the second was true for pair-fed rats. There are four alternative metabolic pathways for glucose in the liver: (i) local oxidation to provide energy; (ii) glycogen synthesis to store glucose; (iii) lipid synthesis for energy storage; and (iv) transport out of the liver as an energy substrate for other tissues. Liver glycogen, but not lipid, was increased during the post-stress period in restrained rats compared with pair-fed rats (2) and results from these experiments show that glycogen synthesis was increased (see Fig. 3), indicating an increase in net glucose uptake that is insulin independent. In pair-fed rats, glucose oxidation and glycogen and lipid synthesis were not changed, suggesting that the increased hepatic glucose uptake was associated with increased glucose efflux and no net change in glycogen stores.

Although the liver probably accounts for increased whole-body glucose clearance in both restrained and pair-fed animals, the mechanisms responsible for this increase may be different as the glucose has a different metabolic fate in the two groups. It has been reported that stress hormones such as norepinephrine, epinephrine, and cortisol inhibit insulin release and cause insulin resistance in peripheral tissue (11). If these hormones are chronically elevated, insulin-independent hepatic glucose uptake could increase to keep blood glucose within the normal range. Single time-point measures of brain catecholamines and serum corticosterone immediately following a single 3-hr restraint (1) and serum corticosterone 1 day after repeated restraint (3) were not different between control and restrained animals. The observations imply that changes in hepatic metabolism are not due sustained activation of the Corticotropin-releasing factor or sympathetic systems, but that repeated restraint initiates a cascade of events that effects tissue metabolism even 24 hr after the stress has ended. Single measures of hormone concentration do not exclude the possibility of a change in the circadian pattern of hormone release that alters the balance between anabolic and catabolic hormones and, ultimately, peripheral tissue metabolism. In contrast to restrained rats, pair-fed rats were food-restricted throughout the experimental period and neural or endocrine responses to this on-going stress may have directly promoted hepatic glucose transport.

In 1963, Russek (12) hypothesized that the central "feeding center" could respond to changes in hepatic glucose flux by monitoring the arterio-portal glucose gradient. Niijima (13, 14) subsequently proposed that glucose-sensitive vagal afferent fibers from the liver to the hypothalamus played a role in controlling food intake and others (15–18) have reported that liver glucose metabolism influences feeding behavior. Infusion of glucose into the portal, but not the jugular, vein inhibits feeding and increases liver glycogen content (19) unless the rats are fasted and already have a reduced liver glycogen content (20). Liver glycogen was increased in restrained rats in the post-stress period and the rats did not overeat to compensate for their reduced food intake during stress, consistent with the hypothesis that liver glycogen inhibits feeding. The difference in hepatic glycogen between restrained and pair-fed rats may represent a post-stress response in the restrained rats, but in addition, it was likely that the hungry, pair-fed rats ate their food in large meals early in the day, whereas restrained rats were eating *ad libitum*. This difference in meal patterns would have had an independent effect on liver glycogen content, which in turn may have influenced the appetite of the animals.

Weight loss in rats exposed to repeated restraint is entirely lean body mass during the stress but 5 days post-stress the difference in weight between control and restrained rats is composed of both lean and fat tissue (2), implying a selective inhibition of fat accretion once stress has ended. Experiments 3 and 4 demonstrated that adipose tissue β-adrenergic receptor number was increased in restrained rats the day after stress ended, but was not different from controls 5 days after the end of stress. We measured adipose tissue plasma membrane binding of ^{3}H-DHA, a β-adrenergic receptor antagonist (21, 22) that binds to all three β-adrenergic receptor subtypes, β_1, β_2, and β_3, all of which are present in rat adipose tissue (23, 24). Activation of these receptors would increase lipolysis and decrease glucose transport in adipose tissue (24, 25). Different subtypes of β-adrenergic receptors make variable contributions to these effects depending on species, age, and receptor activity (26–28), but in adipose tissue, β_3-adrenergic receptors are primarily responsible for catecholamine-stimulated lipolysis and acute inhibition of glucose transport (29). In our experiments it would have been preferable to identify binding activity of each receptor subtype individually; however, specific β_3-adrenergic agonists or antagonists were not commercially available.

The increased β-adrenergic receptor number in adipose tissue from restrained rats would enhance the stimulation of lipolysis and inhibition of glucose transport caused by β-ad-

renergic receptor activation (24). Thus, the post-stress inhibition of glucose transport (2) and loss of body fat from restrained rats (3) could be secondary to an up-regulation of β-adrenergic receptors. Under conditions of sustained sympathetic activation, adrenergic receptors are usually down-regulated or desensitized (25, 30). Thus, the increased number of receptors is consistent with previous observations that repeated restraint stress is not associated with chronic activation of the catecholaminergic system (1, 2). The mechanism responsible for an increase in β-adrenergic receptor number in restrained rats needs further investigation.

β-Adrenergic receptor number can be regulated by factors such as corticosterone, TNF-α, and protein kinase C (31–33). Corticosterone has been reported to prevent the down-regulation of β_2-receptors by a β-agonist, so that the combination of corticosterone and β-agonist resulted in no net change in receptor number or gene expression (34). This could also be the case in pair-fed rats, which did not show any differences in β-adrenergic receptor number, although they were under the continuous stress of food restriction and corticosterone was elevated throughout the period of food restriction (2).

In summary, studies described provide additional information on post-stress metabolic changes in rats that have been exposed to repeated restraint. Although these studies do not identify the primary mechanisms responsible for the maintenance of a reduced body weight, they demonstrate a temporary modulation of adipose tissue metabolism during the post-stress period that may account for the changes in body composition that occur within days of the end of restraint. In addition, the results suggest that changes in liver glucose metabolism may contribute to the failure of the rats to overeat when they have an opportunity to compensate for stress-induced negative energy balance. The present studies help to define, on a temporal basis, the contribution of peripheral tissue metabolism to end-point energetic responses during the post-stress period. Future studies will investigate the mechanisms responsible for these chronic responses to repeated acute activation of the Corticotropin-releasing factor system.

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