Glucose Uptake and Glycolytic Flux in Adipose Tissue From Rats Adapted to a High-Protein, Carbohydrate-Free Diet

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Rates of glucose uptake by epididymal and retroperitoneal adipose tissue in vivo, as well as rates of hexose uptake and glycolytic flux in isolated adipocytes, were determined in rats adapted to a high-protein, carbohydrate-free (HP) diet and in control rats fed a balanced (N) diet. Adaptation to the HP diet induced a significant reduction in rates of glucose uptake, estimated with 2-deoxy-[1-^{3}H]-glucose, both by adipose tissue (epididymal and retroperitoneal) in vivo and by isolated adipocytes. Twelve hours after replacement of the HP diet with the balanced diet, rates of adipose tissue uptake in vivo in HP-adapted rats returned to levels that did not differ significantly from those in N-fed rats. The rate of flux in the glycolytic pathway, estimated with ^{3}H[5]-glucose, was also significantly reduced in adipocytes from HP-fed rats. In agreement with the above findings, the activities of hexokinase (HK), phosphofructo-1-kinase (PFK-1), and pyruvate kinase (PK) were markedly reduced in adipose tissue from HP-adapted rats. The activity of pyruvate kinase was partially reverted by diet replacement for 12 hours. The low-plasma insulin and high-glucagon levels in HP-fed rats may have played an important role in the reduction of adipose tissue glucose utilization in these animals.

RATS ADAPTED TO A high-protein, carbohydrate-free (HP) diet have been used in our laboratory as a model system to investigate adaptive mechanisms in energy-linked metabolic processes. Despite the absence of carbohydrate in the diet, rats adapted to a HP diet have a normal concentration of blood glucose, which is markedly resistant to fasting, a feature that is also characteristic of carnivorous animals[1-3] and seems to depend on the high gluconeogenic capacity with which they are endowed even before food deprivation.[3] With respect to lipid metabolism, we have shown[4,5] that in vivo lipogenesis, assessed by the rate of incorporation of tritium from ^3H_2O into tissue fatty acids (FAs), is markedly reduced in the carcass, liver, and adipose tissue from HP-adapted rats. The diet affects only the synthesis of glyceride-FAs, with no change observed in phospholipid-FA synthesis.[5] Recently,[6] we have evaluated the contribution of glucose to the synthesis of glycerol and FA moieties of adipose tissue triacylglycerols (TAG) by determining simultaneously in the same animal the rate of incorporation of ^{3}H from tritiated water, which estimates total synthesis (from all carbon sources) and of ^{14}C from glucose into the 2 fractions of TAG. It was found that glucose is a poor precursor for adipose tissue FA synthesis, contributing only 10% of total adipose tissue FA synthesis in control rats, and even less (about 7%) in HP-fed rats. In contrast, in both HP and control rats, the rate incorporation of the hexose into glyceride-glycerol was much higher than into FA and did not differ in the 2 experimental groups.[6] To further clarify the adaptive changes induced by the HP diet on adipose tissue glucose metabolism, we investigate in the present work the effect of the diet on the rate of glucose uptake by retroperitoneal and epididymal adipose tissue in vivo, as well as on the rate of hexose uptake and glycolytic flux in isolated adipocytes. The activities of key enzymes of the glycolytic pathway, hexokinase (HK), phosphofructo-1-kinase (PFK-1), and pyruvate kinase (PK) were also determined.

MATERIALS AND METHODS

Animals and Treatment

Male Wistar rats weighing initially 90 to 110 g were housed in suspended wire bottom cages with water ad libitum in a room kept at 25° ± 2°C with a 12-hour light:dark cycle. The animals were adapted for 15 days to a HP purified diet containing 70% casein, no carbohydrate, and 8% corn oil or to a balanced control diet (N) containing 17% casein, 66% carbohydrate, and 8% corn oil. The 2 diets, which were approximately isocaloric and contained equal amounts of vitamins and minerals, have been described in detail.[7] As in previous studies with the same diet,[3] after a short period of adaptation, food ingestion and the rate of body weight gain were similar in the 2 groups of rats. The animals weighed 180 to 200 g when used for the experiments, which were performed between 8:30 and 10:30 AM. Rats previously adapted to the HP diet and then fed with the balanced diet for 12 hours were also used in some experiments.

Glucose Utilization In Vivo

The technique used was based on the method of Sokoloff et al[8] as modified by Ferré et al.[9] 2-deoxy[1-^{3}H]-glucose (30 μCi; 11 Ci/mmol) was injected in 0.2 mL of 0.9% NaCl as a bolus in fed, nonanesthetized rats through a silastic catheter (Dow Corning, Midland, MI) inserted into the right jugular vein 2 days before the experiment. After flushing the catheter with saline and with the rat free in its cage, blood samples of 0.15 mL were taken 1, 3, 5, 10, 20, 30, and 60 minutes after label injection for determination of 2-deoxy[1-^{3}H]-glucose concentration (in terms of radioactivity). After the last blood sample, the rats were killed by cervical dislocation, and samples of epididymal and retroperitoneal adipose tissue were removed for determination of tissue content of 2-deoxy-[1-^{3}H] glucose-6-phosphate as described in Ferré et al.[9] Plasma glucose concentration was determined with glucose oxidase in a Beckman (Fullerton, CA) glucose analyzer.

Calculations. The quantity of glucose used per unit time was determined from the 2-deoxy-[1-^{3}H]glucose/glucose ratio versus time curves and tissue 2-deoxy-glucose phosphate (2-DG-P) using an equation derived from a 2-compartment (plasma and tissue) mathematical

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model.[8,9] Blood glucose concentration did not change significantly during the sampling period in any of the experimental groups, a requirement of the technique used.

Glucose Uptake by Adipocytes

Epididymal fat pads from 4 to 5 rats of each group were pooled together and adipocytes isolated by the method of Rodbell.[10] Cells were filtered through a 300-μm nylon mesh and washed 3 times with bicarbonate buffer containing albumin (10 mg/mL) essentially free of fatty acids and suspended in the same buffer. All steps of the procedure for fat cell isolation were performed in the absence of glucose. Because fat cells from HP-fed rats are smaller than controls, the number of cells per unit weight of adipose tissue is about 30% higher in HP rats.[11] The number of fat cells in each suspension was estimated from lipocrit values[12] and adipocyte mean volume.[13] To incubate an approximately equal number of cells in the 2 groups, the suspension was adjusted to lipocrit of 14% to 16% for HP rats and of 21% to 23% for controls. Cell suspensions (500 μL) were added to polyethylene vials containing 500 μL of a 1% albumin buffer with 0.50 μCi of 2-deoxy-[1]-^{14}C-glucose and 1 mmol/L glucose and incubated for 3 minutes at 37°C. Fifteen seconds before the end of the incubation, a 300-μL aliquot was added to a microcentrifuge tube containing 100 μL of dinonyl phtalate oil and spun immediately in a microcentrifuge for 30 seconds. After cutting through the oil phase,[13] the floating cells were added to a vial with scintillation fluid.

Glycolytic Flux in Adipocytes

The generation of ^3H_2O by cells exposed to ^{3}H [5]-glucose was used to assess the rate of the glycolytic flux. Adipocytes from epididymal fat were obtained as described for measurement of glucose uptake. The cell suspension was adjusted to 400,000 cells/mL. Portions of this stock suspension were then used for incubation or for DNA determination.[14] Samples of each suspension were added to 20-mL plastic vials and incubated in a shaking bath at 37°C for 1 hour, in 2 mL of bicarbonate buffer containing albumin (20 mg/mL), and ^{3}H-[5]-glucose (5 mmol/L, 1 μCi) equilibrated with O_2:CO_2 (19:1). At the end of incubation, 6% $HClO_4$ was added to the vials. Portions of the incubation mixtures were centrifuged at 10,000 $\times$ *g* for 10 minutes at 4°C and the supernatants adjusted to pH 8.0 with 2 mol/L potassium hydroxide (KOH). The tubes were centrifuged at 10,000 $\times$ *g* for 15 minutes at 4°C and an aliquot of supernatant was applied to a column (0.8 $\times$ 21 cm) of Dowex 1 (X8; 100 to 200 mesh; borate form) to separate ^3H_2O from ^{3}H-[5]-glucose.[15] Tritiated water derived from metabolism of the ^{3}H-[5]-glucose was not retained by the column and was recovered by thoroughly washing the column with water.

Radioactivity measurements. Measurements were made in a Beckman spectrometer with Toluene-Triton-PPO-POPOP as scintillation fluid. The degree of quenching in each sample was obtained to enable calculation of radioactivity in disintegrations per minute in all experiments.

Measurement of Enzyme Activity

HK and PFK-1. Portions of epididymal adipose tissue were homogenized in ice-cold 50 mmol/L Tris buffer, containing 5 mmol/L $MgCl_2$, 1 mmol/L EDTA, and 20 mmol/L mercaptoethanol, pH 8.2. After centrifugation at 10,000 $\times$ *g* for 15 minutes and removal of the floating fat layer, the supernatants were centrifuged for 60 minutes at 100,000 $\times$ *g*. Aliquots of new supernatant were used to determine enzyme activities.[16,17] In addition to adequate amounts of the supernatant, the assay medium for HK contained 75 mmol/L Tris-HCl, 7.5 mmol/L $MgCl_2$, 0.8 mmol/L EDTA, 1.5 mmol/L KCl, 4 mmol/L mercaptoethanol, 0.4 mmol/L nicotinamide-adenine dinucleotide phosphate $(NADP)^+$, 2.5 mmol/L ATP, 10 mmol/L creatine-phosphate, 1 mmol/L glucose, creatine phosphokinase (100 g, 1.8 units), glucose-6-phosphate dehydrogenase (10 g, 1.4 units). Controls from which glucose was omitted were run concurrently. The assay medium for PFK-1 contained, in addition to 100,000 g supernatant, 50 mmol/L Tris-HCl, 6 mmol/L $MgCl_2$, 250 mmol/L KCl, 0.17 mmol/L NADH, 1 mmol/L potassium cyanide, 3 mmol/L fructose-6-phosphate, glycerol-3-phosphate dehydrogenase (4 μg; 0.16 U), aldolase (100 μg, 0.9 U), triosephosphate isomerase (5 μg; 9.6 U).

PK. Portions of epididymal adipose tissue were homogenized in ice-cold 0.1 mol/L Tris-HCl buffer, pH 7.4, containing 50 mmol/L potassium fluoride, 7.5 mmol/L EGTA, and 30% glycerol. After centrifugation at 10,000 $\times$ *g* for 15 minutes and removal of the floating fat layer, the supernatant was centrifuged for 60 minutes at 100,000 $\times$ *g*. Aliquots of new supernatant were used to determine PK activity. The reaction mixture contained 0.1 mol/L Tris-HCl (pH, 7.4), 0.1 mol/L KCl, 4 mmol/L $MgCl_2$, 0.15 mmol/L NADH, 27 U of lactate dehydrogenase, 2 mmol/L adenosine diphosphate (ADP), 1.5 mmol/L phosphoenolpyruvate, and 50 μL of homogenate. The assays were performed in the presence or in the absence of fructose-1,6-biphosphate (F-1,6-BP).[18]

Other Chemical Analyses

The plasma concentration of insulin and glucagon was determined by radioimmunoassay using commercial kits from Amersham (Little Chalfont, UK) and Linco (St Charles, MO), respectively. The concentration of protein in homogenates was determined by the method of Lowry et al[19] using bovine serum albumin as a standard.

Statistical Methods

Differences between groups were analyzed using analysis of variance (ANOVA) with $P < .05$ as the criterion of significance.

RESULTS

Adaptation to the HP diet resulted in a decrease in the concentration of plasma insulin (25 $\pm$ 3 μU/mL, n = 9, compared with 47 $\pm$ 4, n = 10 in controls) and an increase in plasma glucagon (147 $\pm$ 15 pg/mL, n = 8, *v* 104 $\pm$ 12, n = 7 in controls).

The data in Fig 1 show that the rate of glucose uptake in vivo was significantly reduced in adipose tissue of rats adapted to the HP diet, with rates in epididymal and retroperitoneal tissues in HP rats amounting to 40% and 34% of control values, respectively. Figure 1 also shows that replacement of the HP diet by the balanced, control diet for a relatively short period (12 hours) was sufficient to normalize glucose uptake in vivo.

In agreement with the results in vivo, rates of glucose uptake, estimated with 2-deoxy-1-^{14}C-glucose in fat cells prepared in the absence of the hexose and incubated with 1 mmol/L glucose, were reduced in adipocytes from HP-fed rats to 49% of rates in adipocytes from control animals (Fig 2). Experiments with fat cells that were also prepared in the absence of the hexose and incubated with 5 mmol/L 5-^{3}H-glucose to estimate the glycolytic flux showed a significant (30%) reduction of flow of metabolites in the glycolytic pathway in adipocytes from HP-adapted rats (Fig 3).

The results of measurement of enzyme activities in adipose tissue, shown in Fig 4 (HK and PFK-1) and Table 1 (PK) were consistent with the results of the experiments on tissue glucose utilization. The activities of HK and PFK-1 were markedly reduced (to about 30% and 50% of controls, respectively) in adipose tissue from HP-adapted rats. The activity of PK was

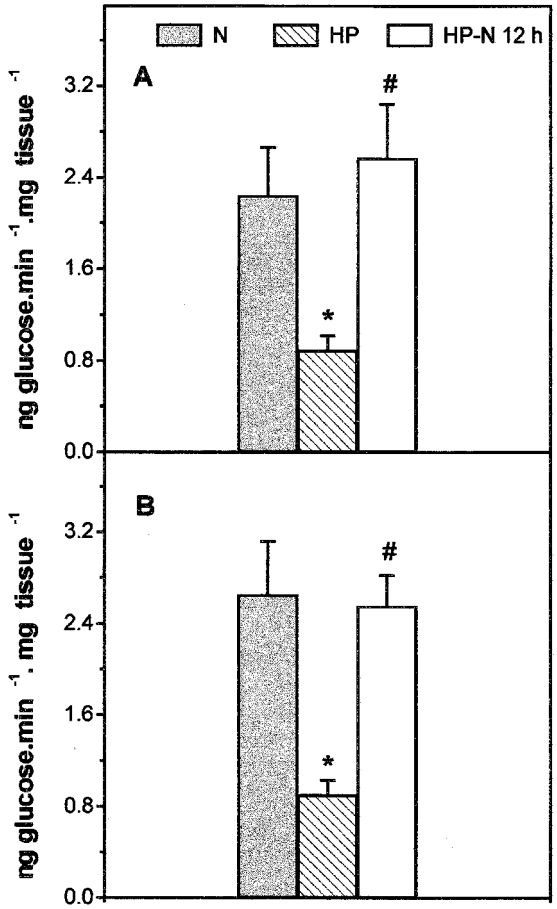

Fig 1. Rates of glucose uptake in vivo by epididymal (A) and retroperitoneal (B) adipose tissue in rats fed the HP or control (N) and in HP-adapted rats 12 hours after replacement of HP diet by control diet (HP-N, 12 hours). Each bar is the mean ± SEM of 4 to 11 determinations. *$P < .05$ *v* N; #$P < .05$ *v* HP.

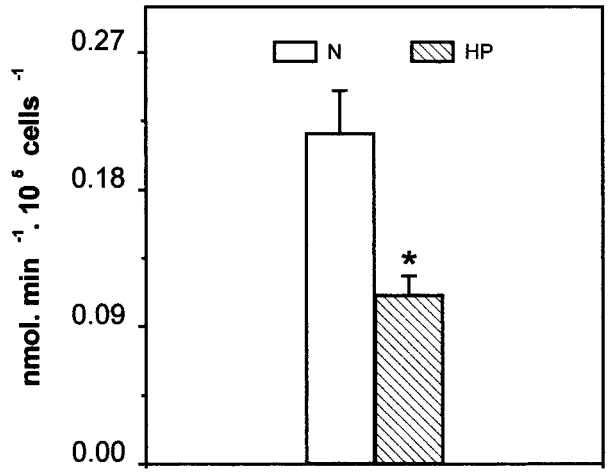

Fig 2. Rates of glucose uptake by adipocytes from rats fed the HP or N diet, estimated with 2-deoxy-[1]^{14}C-glucose. Each bar is the mean ± SEM of 7 to 8 determinations. *$P < .05$.

also reduced by about 55% in adipose tissue from HP-fed rats, both in the presence and in the absence of F-1,6-BP (Table 1). After replacement of the HP diet by the balanced diet for 12 hours, a partial, statistically significant increase in the activity of PK was detected, but only in the presence of F-1,6-BP. Activation of PK by F-1,6-BP was more pronounced in HP rats with diet reversion than in N or HP rats (Table 1), suggesting that a large fraction of the enzyme was still inhibited in these animals.

DISCUSSION

The present data clearly show that adaptation to a high-protein, carbohydrate-free diet results in a marked reduction in the rate of glucose utilization by adipose tissue, evidenced by a decrease in the tissue uptake of glucose in vivo and in the hexose uptake and glycolytic flux in isolated adipocytes, as well as by a pronounced reduction in the activity of key glycolytic enzymes: HK, PFK-1, and PK.

Confirming previous observations,[11,20] levels of plasma insulin were lower and those of glucagon were higher in rats adapted to the HP diet than in controls. These hormonal changes may have played an important role in the reduction of adipose tissue glucose utilization in HP-fed rats. The metabolism of adipose tissue seems to be controlled predominantly by metabolic and hormonal factors. Different from brown adipose tissue, there is no evidence that the utilization of glucose by white adipose tissue can be stimulated by the sympathetic nervous system, independent of plasma insulin levels. Electrical stimulation of hypothalamic ventromedial nucleus and sympathetic activation increase the rate of glucose utilization in brown adipose tissue, myocardium, and skeletal muscle (with no changes in circulating insulin levels), but not in brain or white adipose tissue.[21]

The normalization of glucose uptake in vivo and the partial restoration of PK activity after a relatively short (12 hour) period of replacement of the HP diet by the balanced diet demonstrate the capacity of adipose tissue to adapt rapidly to

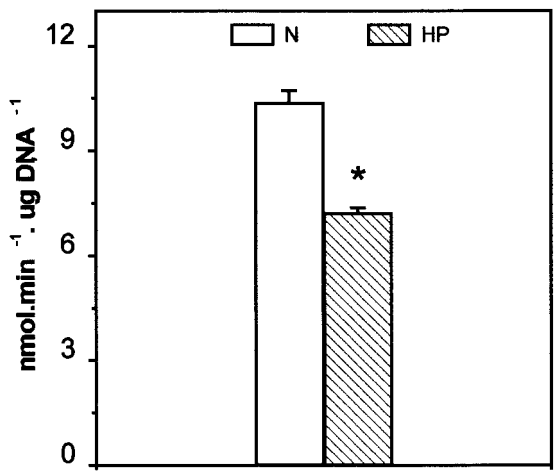

Fig 3. Glycolytic flux in adipocytes from rats fed the HP or N diet estimated with ^{3}H[5]-glucose. Each bar is the mean ± SEM of 5 determinations. *$P < .05$.

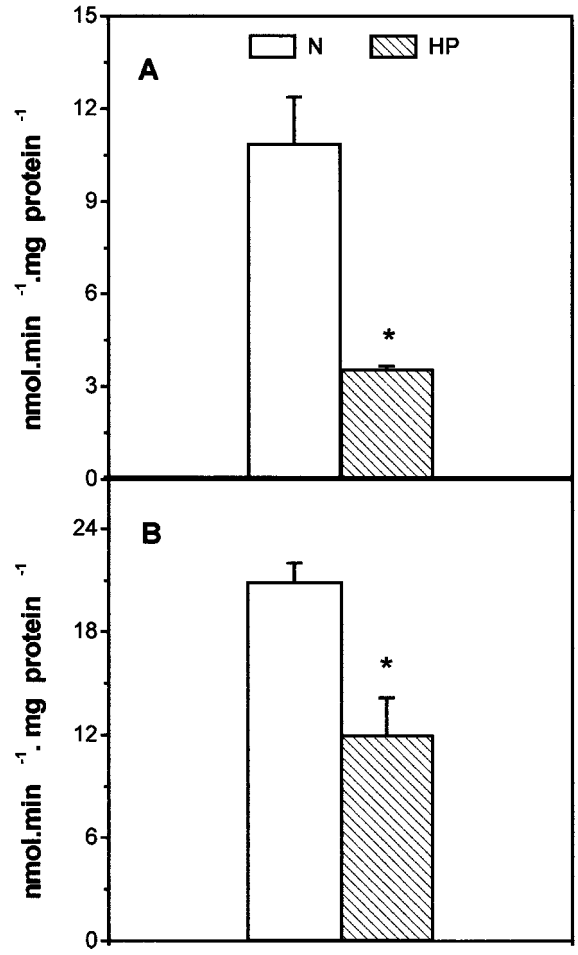

Fig 4. Activities of HK (A) and PFK-1 (B) in epididymal adipose tissue from rats fed the HP or N diet. Each bar is the mean ± SEM of 6 to 8 determinations. *$P < .05$.

markedly different dietary conditions. Plasma insulin levels of HP rats increased from initial levels of 25 ± 3 μU/mL to 54 ± 10 μU/mL after the 12-hour replacement period, while plasma glucagon decreased from 147 ± 15 pg/mL to 87 ± 10. These hormonal changes may have been important factors in the restorations observed, but a direct stimulatory effect of the increased supply of substrate (glucose) to the tissue cannot be excluded. In the course of the present study, it was observed (data not shown) that when glucose was present during the several steps of the procedure used for isolation of fat cells, differences in rates of glucose utilization between adipocytes from HP and control rats were attenuated or suppressed. This suggests that fat cells from HP rats, liberated from the restrictive metabolic and hormonal conditions prevailing in vivo, are able to improve their rate of glucose uptake without any other stimulus than the hexose itself.

Table 1. PK Activity (μmol · min^{-1} · mg protein^{-1}) in the Presence or Absence of F-1,6-BP in Epididymal Adipose Tissue From Rats Adapted to the HP Diet (before and 12 hours after reversion to the control diet) and From Control Rats

F-1,6-BP	Controls	HP	HP-N 12 Hours
−	0.60 ± 0.04 (n = 5)	0.36 ± 0.02* 0.37 (n = 6)	0.40 ± 0.01* (n = 5)
+	0.83 ± 0.08 (n = 5)	0.48 ± 0.04* (n = 6)	0.60 ± 0.02*† (n = 5)
Percent activation	38	33	50

* $P < .05$ *v* controls.
† $P < .05$ *v* HP.

It is interesting to compare the results of the present experiments with those of our recent work[6] on the effect of adaptation to the HP diet on the utilization of glucose for adipose tissue triacylglycerol synthesis in vivo. In parallel with the reduced uptake here observed, it was found that the utilization of glucose for synthesis of glyceride-fatty acids is reduced in HP-adapted rats. However, despite the reduced uptake, the contribution of glucose for glyceride-glycerol synthesis in adipose tissue from these animals did not differ significantly from that in rats fed the balanced diet.[6] This fact emphasizes the importance of maintaining adequate levels of α-glycerophosphate synthesis for the metabolic adjustments of the tissue. This view is also consistent with our previous studies[22] showing that synthesis of glycerol from nonglucose sources (glyceroneogenesis) is increased in adipose tissue from HP-fed rats, as evidenced by increased rates of incorporation of ^{14}C-pyruvate into glyceride-glycerol and increased activity of phosphoenolpyruvate carboxykinase. As previously discussed,[6] in view of the marked reduction in adipose tissue fatty acid synthesis,[4,5] most of the glycerol produced is probably used to esterify preformed fatty acids, taken up from the circulation or recycled from endogenous triacylglycerols hydrolysis.

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