Glucose Stimulation of Lipogenic Enzyme Gene Expression in Cultured White Adipose Tissue

A ROLE FOR GLUCOSE 6-PHOSPHATE*

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The expression of fatty acid synthase (FAS) and acetyl-CoA carboxylase (ACC) is low in the adipose tissue of suckling rats and increases markedly at weaning to a high carbohydrate diet. We have studied in vitro the factors regulating this phenomenon. Inguinal adipose tissue pieces from 10-day-old suckling rats were cultured for 6 or 24 h in minimal essential medium. Insulin (100 nm) added in the presence of lactate and pyruvate did not stimulate the expression of FAS and ACC. Glucose (20 mm) alone resulted in a 5–7-fold increase of FAS and ACC mRNA. Insulin potentiated the effect of glucose. 3-O-Methylglucose, a glucose analog that is transported into the cell but not metabolized, had no effect on FAS and ACC mRNA accumulation. However, 2-deoxyglucose (1 mm), a glucose analog which is phosphorylated to 2-deoxyglucose 6-phosphate, stimulated the expression of FAS and ACC to the same extent as 10 mm glucose. Glucose 6-phosphate concentrations in adipose tissue pieces cultured in various conditions changed in parallel with the FAS and ACC mRNA levels. We conclude that glucose 6-phosphate could be the metabolite involved in the stimulation of lipogenic enzyme gene expression in response to glucose.

The key enzymes of fatty acid synthesis are acetyl-CoA carboxylase (ACC)1 (EC 6.4.1.2) that catalyzes the synthesis of malonyl-CoA and fatty acid synthase (FAS) (EC 2.3.1.85), a multifunctional protein that synthesizes fatty acids by adding malonyl-CoA units to an acetyl-CoA primer. In the liver, a number of in vivo studies have shown that the expression of FAS and ACC depends on the nutritional and hormonal status of the animal (1–4). Starvation as well as diabetes are followed by a rapid decrease of FAS and ACC activity (5–9). Refeeding a starved rat with a high carbohydrate fat-free diet and treatment of diabetic rats with insulin result in a coordinate increase of the activities of these enzymes (6–9). The increase in FAS and ACC activities is due to corresponding changes in FAS and ACC mRNA concentrations that result from an increased rate of gene transcription (7–9). Using primary cultures of rat hepatocytes, an increase of lipogenic enzyme synthesis and activities was shown in response to carbohydrate and insulin (10–12).

Another major site of lipogenesis is the adipose tissue. However, there are few data available concerning the dietary and hormonal regulation of lipogenic enzyme expression in this tissue.

The suckling-weaning transition in the rat has been particularly studied. FAS and ACC activities and mRNA are low in the adipose tissue of suckling rats and increase after weaning to a high carbohydrate low fat diet (13, 14). In the rat, it is characterized by a nutritional shift from milk, a high fat low carbohydrate diet, to the laboratory chow, a high carbohydrate low fat diet (15). Concomitantly, the plasma insulin/glucagon ratio, which is low during suckling, increases at weaning (16). The development of FAS and ACC expression that occurs at weaning is impaired if rats are weaned to a carbohydrate-free diet (14). Finally, a single carbohydrate meal is able to induce the expression of these enzymes in suckling rats (14). These in vivo studies suggested that glucose and/or insulin could play a major role in the regulation of these enzymes in adipose tissue. In order to address this issue, we have developed a culture of suckling rat adipose tissue. In the present work, we investigated the regulation of FAS and ACC expression by carbohydrates and insulin.

EXPERIMENTAL PROCEDURES

Materials—Culture medium was from Gibco (Cergy Pontoise, France). Phenol, chloroform, and ethanol were from Farmitalia (Carlo Erba, Milan, Italy). Formamide (deionized before use by treatment with Bio-Rad AG 501-X8 resin) and formaldehyde were from Prolabo (Paris, France). Dextran sulfate and agarose were from Sigma. Nylon Hyperband filters, Multiprime DNA labeling system, [α-32P]dCTP (specific activity, 3,000 Ci/mm), and Hyperfilms MP were supplied by Amersham International (Amersham, Bucks, United Kingdom).

Animals—Female Wistar rats bred in our laboratory were used. They were housed in plastic cages at a constant temperature (21°C) with light from 07:00 to 19:00 h. The studies were performed on 19-day-old suckling rats and 30-day-old weaned rats. Because suckling rats begin to nibble the food of the mother at 15 days of age, the mothers were fed a high fat diet from the 14th day of lactation until weaning to avoid any consumption of carbohydrates by the pups between 16 and 21 days.

Tissue Preparation—Subcutaneous fat pads of the inguinal region were quickly removed under sterile conditions, cut with scissors in small pieces, and agitated for 30 min in minimal essential medium with Earle's salts (MEM) supplemented with 1% bovine serum albumin. Then, the pieces of adipose tissue were washed in phosphate-buffered saline. About 200 mg of tissue was incubated in a 100-mm plastic Petri dish containing 10 ml of MEM supplemented with 1% (w/v) bovine serum albumin, 10 IU/ml penicillin, and 50 µg/ml kanamycin. This medium was supplemented with various substrates and hormone in various combinations according to experimental conditions. The dishes were then incubated in an incubator aerated...
with CO₂ + air (6:95) at 37 °C for 6-24 h. After 6-24 h, the small pieces of adipose tissue were collected and frozen at −80 °C for RNA extraction.

Preparation of Total Cellular RNA—Total cellular RNA was extracted from the subcutaneous tissue using a guanidine thiocyanate method (17). RNA concentration was measured spectrophotometrically (A₂₆₀/A₃₂₀).

Northern Blot Analysis—Total RNA (20 µg) denatured in 2.2 M formaldehyde and 50% (v/v) formamide for 2 min at 95 °C was applied to a 1% (w/v) agarose gel containing 2.2 M formaldehyde gel in 20 mM MOPS, pH 7.0, 5 mM sodium acetate, and 1 mM EDTA and electrophoresed for 16-18 h at 50 V. The integrity and relative amounts of RNA were assessed by methylene blue staining.

cDNA Probes and Hybridization—The pFAS 18 CDNA for rat liver FAS mRNA is 660 base pairs in length (5) and was obtained from Dr. K. H. Kim. The full-length cDNA probe for rat liver cytosolic PEPCK mRNA, pCK10, is 2.6 kilobases in length (18) and was provided by Dr. R. W. Hanson. The Northern blots were hybridized with an oligonucleotide probe representing the sequence 1047-1070 of rat 18 S ribosomal RNA (19) labeled with [³²P]ATP in order to verify that each lane was loaded with the same amount of total RNA. Probes were labeled using a multiprime DNA labeling system (Amersham kit). Filter hybridization was as previously described (14). Autoradiograms were obtained by exposure of radioactive nylon filters to Hyperfilm MP (Amersham) at −80 °C with intensifying screens (Cronex, Du Pont, Thompson CGR, France). Quantification was performed by scanning densitometry and corrected for possible variations in the amount of RNA loaded by using the 18 S probe data.

Measurement of Glucose 6-Phosphate and ATP Concentrations—Glucose 6-phosphate concentration was determined by the cyclic procedure of Lowry and Passonneau (20). Briefly, cultured adipose tissue pieces were instantly frozen in liquid nitrogen and extracted with HClO₄ (6% final concentration). A 10-µl aliquot of the neutralized extract or standards containing known amounts of glucose 6-phosphate was used to generate NADPH quantities equimolar to the amount of glucose 6-phosphate present in the extracts. NADPH was then added in an amplifying system (coupling of reactions involving glucose 6-phosphate dehydratase and glutamate dehydrogenase) generating 6-phosphogluconate. The amount of 6-phosphogluconate formed, which is determined by classical spectrophotometric procedures, is proportional to the initial amount of glucose 6-phosphate.

ATP (the concentration of which is about 40-fold higher than glucose 6-phosphate) was determined directly on the neutralized extract (21).

RESULTS

Induction of Lipogenic Enzyme Gene Expression in Cultured Adipose Tissue—Several studies reported that adipose tissue may be kept in vitro up to 24 h with preservation of hormonal action and of the capacity to synthesize specific protein (22, 23). We thus incubated small pieces of adipose tissue of suckling rats, and we tried to induce FAS and ACC in the presence of glucose and insulin. The results are presented in Fig. 1. The FAS and ACC mRNA concentrations were barely detectable in freshly isolated white adipose tissue of 19-day-old suckling rats (Fig. 1, lane 1). After 24 h of culture, in the presence of 20 mM glucose and 100 nM insulin, a 9- and 10-fold increase of FAS and ACC mRNA concentrations, respectively, was observed (Fig. 1, lane 2). The accumulation of FAS and ACC mRNA in the presence of insulin and glucose was large since it was comparable with the level of mRNA detected in the freshly isolated white adipose tissue of 30-day-old rats weaned to a high carbohydrate diet (Fig. 1, lane 3). In order to assess the specific effect of glucose and/or insulin on lipogenic enzyme expression, the blots were hybridized with the PEPCK probe. It was previously reported that during the sucking-weaning transition, PEPCK mRNA followed a converse pattern when compared with lipogenic enzymes in adipose tissue (14). Consequently, PEPCK was chosen as a negative marker for lipogenic enzyme expression. PEPCK mRNA concentrations were high in the adipose tissue of 19-day-old suckling rats (Fig. 1, lane 1) and became undetectable after 24 h of culture in the presence of glucose and insulin (lane 2) as in the adipose tissue of 30-day-old rats weaned to a high carbohydrate diet (lane 3). Then, the time course of the accumulation of FAS and ACC mRNA in response to 20 mM glucose and 100 nM insulin was studied (Fig. 2). FAS and ACC mRNA concentrations were very low in white adipose tissue of suckling rats, increased rapidly in the presence of glucose and insulin, reached a maximal value after 6 h of culture, and declined slightly up to 24 h. PEPCK mRNA concentrations showed a totally converse time course, the concentrations declining rapidly after 2 h of culture in the presence of glucose and insulin to reach low values after 6 h. The kinetics of FAS, ACC, and PEPCK mRNA in culture are similar to that observed in suckling rats force fed with carbohydrates (14). It is thus possible to obtain in vitro a specific expression of lipogenic enzymes with a time course and quantitative characteristics similar to the in vivo situation.

Respective Role of Insulin and Glucose in the Induction of Lipogenic Enzymes—Since the maximal accumulation of FAS and ACC mRNA in cultured white adipose tissue was observed...
6 h after addition of 20 mM glucose and insulin, all the following experiments were performed during this period. The respective role of insulin and glucose in the induction of FAS and ACC expression was then investigated (Fig. 3). When adipose tissue was cultured in the absence of glucose, but in the presence of 10 mM lactate and 1 mM pyruvate as oxidative substrates, FAS and ACC mRNA remain undetectable. The addition of 5 or 20 mM glucose in the medium resulted in a marked increase in FAS and ACC mRNA concentrations even in the absence of insulin (Fig. 3). When adipose tissue was cultured in the presence of 100 nM insulin, but in the absence of glucose, no significant accumulation of FAS and ACC mRNA occurred. In these conditions, insulin decreased by 90 ± 1% (n = 3) PEPCk mRNA concentration. Thus, insulin effects on PEPCk gene transcription did not require the presence of glucose in the culture medium. Simultaneous addition of glucose and insulin in the culture medium led to a further increase in the glucose-dependent enhancement of the FAS and ACC mRNA concentrations. In summary, glucose is able to induce FAS and ACC mRNA expression in the absence of insulin. In contrast, insulin has no effect on FAS and ACC mRNA expression in the absence of glucose but has a potentiating effect on glucose-induced FAS and ACC mRNA expression.

The stimulatory effect of glucose on lipogenic enzyme expression can be mediated by glucose itself, by a metabolite derived from glucose, or by energetic compounds produced during glycolysis. In order to test these hypotheses, we have studied the effect of different hexoses on the expression of lipogenic enzymes. First, 3-O-methylglucose (20 mM), a glucose analog which is transported inside the adipocyte but not phosphorylated, had no effect on FAS and ACC expression in the absence of insulin (Fig. 4, lane 2) or in the presence of insulin (lane 3). We have then studied the effect of 2-deoxyglucose, a glucose analog which is transported inside the adipocyte, phosphorylated into 2-deoxyglucose 6-phosphate but not further metabolized. In the presence of 1 mM 2-deoxyglucose, a marked increase of FAS and ACC mRNA concentrations was observed (lane 4). The levels of FAS mRNA reached were similar to that obtained in the presence of 20 mM glucose (lane 5). Insulin potentiated the effect of 2-deoxyglucose (lane 6). Results for ACC mRNA were essentially similar to those obtained for FAS (results not shown). The possibility that hexose metabolism could be involved in the stimulation of lipogenic enzyme gene expression through the provision of energy was studied by measuring ATP concentrations 1 h after the addition of glucose and 2-deoxyglucose. Glucose (20 mM) doubled the concentration of ATP when compared with the incubation in the absence of glucose (9.9 ± 3.1 nmol/g of tissue (n = 4) versus 5.6 ± 1.3 nmol/g of tissue (n = 4)) showing that glucose is actively metabolized. In contrast, 2-deoxyglucose induced a marked decrease in ATP concentration (2.1 ± 0.4 nmol/g of tissue (n = 4) (p < 0.01). This can be explained by the fact that ATP is used for 2-deoxyglucose phosphorylation but is not produced from this compound through glycolysis.

This series of experiments suggested that the hexose 6-phosphate was responsible for the increase of FAS and ACC gene expression. Glucose 6-phosphate concentration was then measured in the adipose tissue in various conditions after 1 h of culture (Fig. 5). There was a striking parallelism between the concentrations of glucose 6-phosphate and the expression of mRNA obtained at the end of the culture.

**DISCUSSION**

Previous studies using cultured hepatocytes have shown that carbohydrates increase the activities of hepatic lipogenic enzymes such as ATP-citrate lyase (24), malic enzyme (25), fatty acid synthase (3, 12) and acetyl-CoA carboxylase (26). Recent work demonstrates that glucose increases the mRNA concentrations and the transcription of glycolytic or lipogenic related proteins such as aldolase B (27), L-type pyruvate kinase (28), and spot S14 (29) whereas it decreases that of PEPCK (30). Nevertheless the effect of carbohydrates on glycolytic and lipogenic enzymes requires the presence of insulin in the medium. For spot S14 and L-type pyruvate kinase, cis-acting sequences called "carbohydrate-responsive element" have been identified in the 5'-flanking region of the gene (31–33). Many authors have postulated that a metabolite of glucose could be involved in the stimulation of the gene expression, but direct demonstration has never been provided.
Glucose 6-Phosphate Induces the Expression of Lipogenic Enzymes

In the present study, we show that adipose tissue can be used in vitro to analyze the mechanisms involved in the induction of lipogenic enzymes at weaning. We show that, unlike previous studies performed in adult rat hepatocytes (10-12), glucose alone stimulates the expression of FAS and ACC mRNA in the adipose tissue of suckling rats, it may be hypothesized that the large hexose 6-phosphate is responsible for the accumulation of FAS and ACC mRNA.

Several lines of evidence suggest that the metabolite involved in the induction of FAS and ACC in adipose tissue is a hexose 6-phosphate; the glucose analog 2-deoxyglucose, which is transported into the cell and is phosphorylated in 2-deoxyglucose 6-phosphate, stimulates FAS and ACC expression. Since 3-O-methylglucose, which is not phosphorylated, had no effect on lipogenic enzyme expression, this would suggest that the hexose 6-phosphate is responsible for the induction of FAS and ACC. 1 mM 2-deoxyglucose is nearly as potent as 20 mM glucose to induce the lipogenic enzymes.

This could be explained if one considers the hexose phosphate as the candidate, since it accumulates in the cell to a much greater extent with 2-deoxyglucose than with glucose. Finally, a marked parallelism exists between the concentrations of glucose 6-phosphate and the levels of FAS and ACC mRNA in adipose tissue. Since extremely low concentrations of glucose 6-phosphate concentration although it was able to decrease PEPCK expression, insulin potentiates the effect of glucose and simultaneously increases the concentration of glucose 6-phosphate, probably through a stimulation of glucose transport (34). Thus, glucose 6-phosphate could be the metabolite responsible for specific activation of lipogenic enzyme gene expression in the adipose tissue. Whether a similar mechanism is also operative in the liver remains an unsolved question. However, the fact that in the liver glucose is phosphorylated to glucose 6-phosphate by glucokinase, an insulin-dependent enzyme (35, 36), might obscure the effect of glucose in this tissue.

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FIG. 5. Glucose 6-phosphate (6GP) and FAS mRNA concentrations. Adipose tissue was cultured in various conditions. Top, FAS mRNA after 6 h of culture. Bottom, glucose 6-phosphate concentration after 1 h of culture. *, **, *** differences statistically significant for p < 0.05, p < 0.01, and p < 0.001, respectively, when compared with the value obtained in the presence of lactate and pyruvate.

| Glucose | FAS mRNA (%) | Insulin |
|--------|--------------|--------|
| 0      | 0            | +      |
| 5      | +            | +      |
| 20     | +            | +      |
| 0      | 100          | 0      |

| Glucose | Glucose 6-Phosphate concentration (mg/g weight-adipose tissue) |
|--------|---------------------------------------------------------------|
| 0      | 0.2                                                          |
| 5      | 0.3                                                          |
| 20     | 0.4                                                          |
| 0      | 0.1                                                          |

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