Annu. Rev. Nutr. 1997. 17:325–52

MECHANISMS BY WHICH CARBOHYDRATES REGULATE EXPRESSION OF GENES FOR GLYCOLYTIC AND LIPOGENIC ENZYMES

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KEY WORDS: liver, adipose tissue, glucose-6-phosphate, fatty acid synthase, carbohydrate response element

ABSTRACT

Regulation of gene expression by nutrients is an important mechanism in the adaptation of mammals to their nutritional environment. This is especially true for enzymes involved in the storage of energy, such as the lipogenic and glycolytic enzymes in liver and adipose tissue. Transcription of the genes for lipogenic and glycolytic enzymes is stimulated by glucose in adipose tissue, liver, and pancreatic β-cells. Several lines of evidence suggest that glucose must be metabolized to glucose-6-phosphate to stimulate gene transcription. In adipose tissue, insulin increases the expression of lipogenic enzymes indirectly by stimulating glucose uptake. In the liver, insulin also acts indirectly by stimulating the expression of glucokinase and, hence, by increasing glucose metabolism. Glucose response elements have been characterized for the L-pyruvate kinase and S_{14} genes. They have in common the presence of a sequence 5′-CACGTG-3′, which binds a transcription factor called USF (upstream stimulatory factor). Another glucose response element, which uses a transcription factor named Sp1, has been characterized in the gene for the acetyl-coenzyme A carboxylase. The mechanisms linking glucose-6-phosphate to the glucose-responsive transcription complex are largely unknown.

0199-9885/97/0715-0325$08.00

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INTRODUCTION

Although it has long been known that nutrients regulate the expression of specific genes in prokaryotes, the demonstration of similar phenomenon in eukaryotes is recent. The regulation of specific gene expression in mammals in response to changes of nutrition has become a major aspect of modern nutrition, due to the emergence of techniques of molecular biology that have allowed cloning of most of the genes involved in the regulation of carbohydrate and fat metabolism. Major (glucose, fatty acids, amino acids) and minor (iron, vitamins) dietary constituents participate, in concert with many hormones, in the regulation of gene expression in response to nutritional changes (reviewed in 9, 29, 30, 34, 95). Major advances have been made recently in the understanding of the molecular mechanisms involved in the regulation of gene expression in response to changes in the intake of dietary carbohydrate.

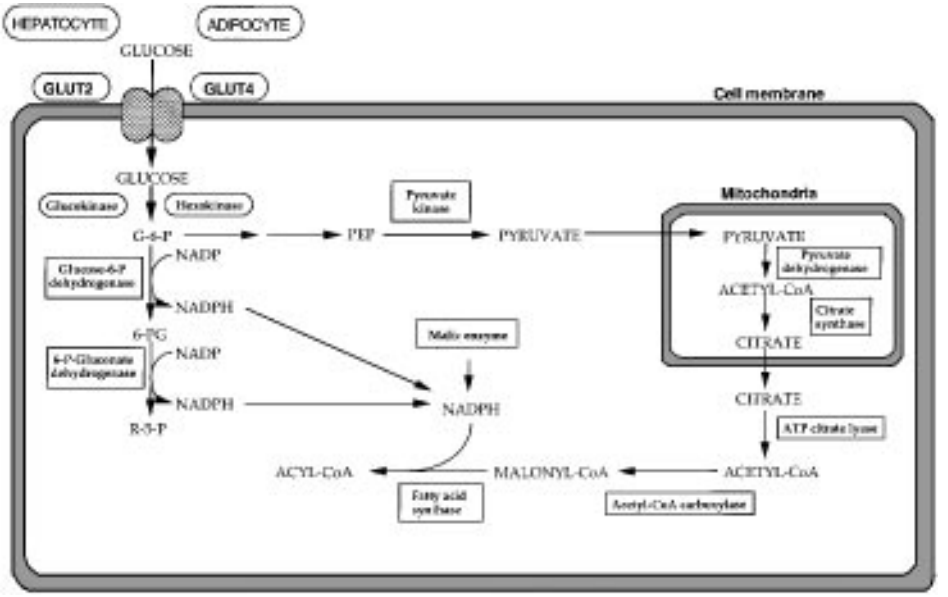

Figure 1 Pathway of lipogenesis from glucose in liver and adipose tissue. Acetyl-coenzyme A (CoA) carboxylase (EC 6.4.1.2); ATP citrate lyase (EC 4.1.3.8); citrate synthase (EC 4.1.3.7); fatty acid synthase (EC 2.3.1.85); glucokinase (EC 2.7.1.2); glucose-6-phosphate dehydrogenase (EC 1.1.1.49); hexokinase (EC 2.7.1.1); malic enzyme (EC 1.1.1.40); 6-phosphogluconate dehydrogenase (EC 1.1.1.44); pyruvate dehydrogenase complex, E1 subunit, pyruvate dehydrogenase (EC 1.2.4.1); E2 subunit, dihydrolipoamide acetyltransferase (EC 2.3.1.12); E3 subunit, dihydrolipoamide dehydrogenase (EC 1.8.1.4); pyruvate kinase (EC 2.7.1.40). G-6-P, Glucose-6-phosphate; 6-PG, 6-phosphogluconate; R-5-P, ribulose-5-phosphate; PEP, phosphoenolpyruvate.

Although carbohydrate, fat, or protein in the diet may exceed the daily caloric requirements, the main form of energy storage is triglycerides in adipose tissue (32). Triglycerides in adipose tissue originate either from the diet or from de novo synthesis. In rodents, lipogenesis occurs both in liver and in adipose tissue, whereas in birds and in humans, it is restricted to the liver (32). The pathway from glucose to acyl-coenzyme A (acyl-CoA) in liver and adipose tissue is summarized in Figure 1. There are two main differences between the two tissues. (*a*) In adipose tissue, glucose entry is facilitated by a glucose transporter (GLUT4), which in the absence of insulin is mainly located intracellularly and which translocates to the plasma membrane in the presence of insulin (76). In the liver, glucose is transported by a transporter (GLUT2) that

is present constitutively in the plasma membrane (76). (*b*) In the adipocyte, glucose is phosphorylated by hexokinase II, whereas in liver, glucose is phosphorylated by glucokinase (hexokinase IV), the synthesis of which is dependent on insulin (81). When glucose is the main substrate used for fatty acid synthesis, the enzymes of the glycolytic pathway can be considered as an extended part of the lipogenic pathway. In the liver, the major function of glycolysis may not be to provide pyruvate to be oxidized in the citric acid cycle but to allow the transformation of carbohydrate into fat (33).

The activity of the lipogenic pathway is dependent on nutritional conditions, both in liver and adipose tissue (78, 85, 86). Consumption of a diet rich in carbohydrates stimulates the lipogenic pathway, whereas starvation or consumption of a diet rich in lipids and poor in carbohydrates decreases its function. Flux through the lipogenic pathway depends on the availibility both of lipogenic substrates and of cofactors such as NADPH produced by the pentose phosphate pathway. Regulation of the activity of the lipogenic enzymes involves both short-term and long-term mechanisms. For example, the activity of acetyl-CoA carboxylase (ACC), which catalyzes the synthesis of malonyl-CoA from acetyl-CoA, is controlled by phosphorylation-dephosphorylation and polymerization-depolymerization mechanisms linked to the presence of specific metabolites (31, 50), but the quantity of this protein is also regulated by changes in its rate of synthesis (65, 73). The rates of synthesis of glycolytic and lipogenic enzymes are controlled at a transcriptional level (35). In the adult rat, the induction of hepatic and adipocyte lipogenic enzymes is rapid (4–6 h) (26, 37, 47, 48) and is dependent on the amount of carbohydrate in the diet (38).

During suckling, both lipogenesis and the activities of the lipogenic enzymes in rat liver and adipose tissue are low (milk is poor in carbohydrate and rich in fat); they increase markedly when animals are weaned onto a high-carbohydrate diet like laboratory chow (reviewed in 28). This is preceded by a quantitatively comparable increase in the level of specific mRNA (11, 79). Weaning the rat onto a high-fat diet prevents these changes, which suggests they are caused by a change in diet rather than by a change in developmental stage (11, 79). Moreover, if an inhibitor of the intestinal α-glucosidases (acarbose) is introduced into the normal weaning diet, it decreases the changes of glucose and insulin concentrations following a meal and the level of expression of fatty acid synthase (FAS) and ACC (68). Thus, the amount of carbohydrate in the diet appears to be instrumental in turning on transcription of genes coding for lipogenic enzymes in liver and adipose tissue. However, is the effect of carbohydrate direct or mediated by the increase in plasma insulin and decrease in plasma glucagon? Indeed, insulin is reported to restore lipogenic enzyme gene expression in diabetic rats (26, 47, 48, 77), and glucagon (via cAMP) inhibits expression of the genes for lipogenic enzymes (24, 54).

The aim of this review is, thus, to summarize what is known about the hormones and substrates that signal the liver and the adipose tissue that the nature of the diet has changed, and to describe the mechanisms involved in the regulation of gene expression in response to dietary carbohydrates. We focus on enzymes involved in glycolysis and lipogenesis for which a clear-cut transcriptional regulation by glucose has been shown and on the underlying cellular and molecular mechanisms.

EVIDENCE THAT GLUCOSE CONTROLS THE EXPRESSION OF GENES CODING FOR GLYCOLYTIC AND LIPOGENIC ENZYMES

Experiments in hepatocytes, adipocytes, or pancreatic β-cell lines in culture have established that glucose, in the absence of insulin, stimulates the transcription of several genes coding for enzymes involved in glycolysis [L-pyruvate kinase (L-PK)] and lipogenesis (ACC, fatty acid synthase). We briefly summarize these data.

L-Pyruvate Kinase Gene Expression in Rat Hepatocytes in Culture

In hepatocytes from fasted adult rats, insulin alone and glucose alone are unable to induce the accumulation of L-PK mRNA (16). The expression of L-PK requires both insulin and glucose (16). In the presence of insulin, the effects of glucose are concentration-dependent (5–20 mM) and show a lag period of 24 h, with the maximal level being reached after 4 days in culture. If, after 4 days in culture, insulin and glucose are removed, abundance of L-PK mRNA returns rapidly to low levels. Readdition of glucose to the culture medium causes a rapid increase in L-PK mRNA, with a maximal level reached after 24 h. These results are consistent with the idea that in order for glucose to achieve its effect on L-PK mRNA, factors must be induced in response to insulin. Later experiments showed that the lag period in insulin action in hepatocytes from fasted rats was due to the time required for induction of glucokinase (41). Once glucokinase is sufficiently active, insulin is no longer necessary to stimulate L-PK gene transcription in response to glucose.

ACC Gene Expression in Cultured Pancreatic β-Cell Line

Malonyl-CoA, which is generated by ACC, may be an important factor in glucose-induced insulin secretion by pancreatic β-cells (7, 80). ACC mRNA and protein levels are increased by long-term exposure of a pancreatic β-cell line, INS-1, to high glucose concentrations (5). INS-1 is a cell line that displays a well-differentiated pancreatic β-cell phenotype (high expression of

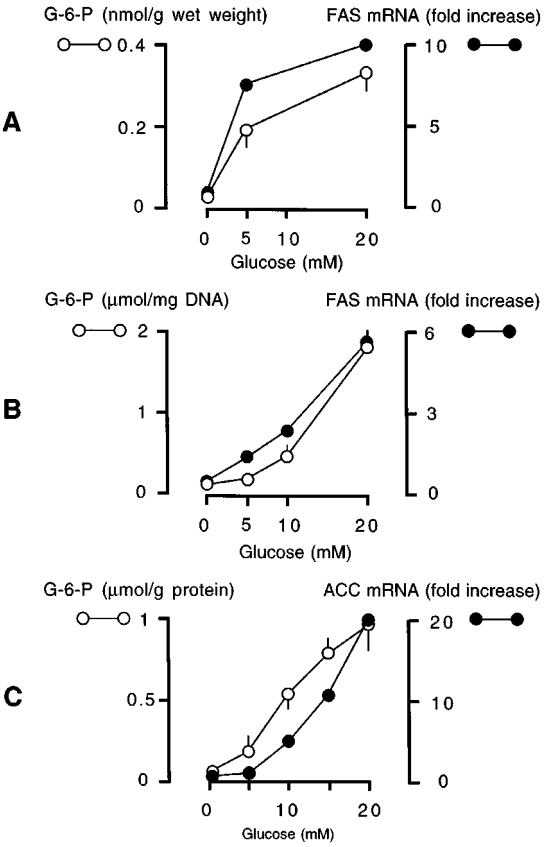

Figure 2 Correlations between glucose-6-phosphate (G-6-P) levels, fatty acid synthase (FAS), or acetyl coenzyme A carboxylase (ACC) mRNA concentrations as a function of medium glucose concentrations in adipose tissue explants (*A*) and in isolated hepatocytes in culture from suckling rats (*B*) and in INS-1 cells (*C*). (Adapted from 34, 38, 39.)

glucokinase), including the capacity to secrete insulin in response to physiological concentrations of glucose (1). Glucose causes a dose-dependent accumulation of ACC mRNA (Figure 2) that is probably linked to a transcriptional effect, since the half-life of ACC mRNA is not affected (5). The ACC gene contains two promoters, promoters I and II, which produce two types of mRNA with different 5′-untranslated sequences, depending on the promoter that is activated. In the liver, promoter I is activated under lipogenic conditions (60), whereas in most cells, including the pancreatic β-cells, ACC mRNA is generated under

the control of promoter II. In INS-1 cells, glucose strongly stimulates promoter II of ACC gene (105).

Fatty Acid Synthase Gene Expression in Adipose Tissue Explants and Hepatocytes in Culture

ADIPOSE TISSUE EXPLANTS In explants of adipose tissue from 19-day-old suckling rats, glucose and insulin rapidly increase (in only 6 h) the abundances of FAS and ACC mRNAs to similar abundances obtained in vivo in adipose tissue of 30-day-old rats weaned at 21 days onto a high-carbohydrate diet (23). Moreover, this effect is abolished by actinomycin D, which suggests that it results from the activation of transcription (24). In the absence of glucose (lactate and pyruvate being provided as energy substrates), insulin is unable to increase the level of ACC and FAS mRNA. In contrast, glucose increases FAS and ACC mRNA in the absence of insulin in a dose-dependent manner (Figure 2). The effect of glucose on FAS and ACC mRNA levels is strongly potentiated by insulin. The maximal effect of glucose is obtained at 20 mM (23). Thus, glucose controls the expression of FAS and ACC in adipose tissue, and insulin has only a potentiating effect. Importantly, the increased expression of lipogenic enzymes in response to glucose is not secondary to a general increase in energy metabolism in adipocytes, since expression of another gene, phosphoenolpyruvate carboxykinase (EC 4.1.1.32), is decreased (23, 24).

HEPATOCYTES IN CULTURE In hepatocytes from suckling rats (82), insulin and 20 mM glucose increase the FAS mRNA content. This effect is detectable after 32 h in culture and peaks after 48 h. The maximal induction requires the concomitant presence of dexamethasone, triiodothyronine, and insulin. These hormones have no effect in the absence of glucose (presence of lactate in the culture medium). However, in contrast with the studies in adipose tissue, glucose alone is unable to increase FAS mRNA concentration. Nevertheless, if hepatocytes are cultured for 48 h in the presence of hormones and then 20 mM glucose is added, expression of FAS is markedly increased after another 6 h (Figure 2). This latter phenomenon does not require the continuous presence of hormones. Finally, the glucose effect is clearly concentration-dependent. Thus, in hepatocytes and adipocytes, the presence of glucose is compulsory for the expression of FAS. However, in contrast with adipose tissue, various hormones (especially insulin) are required to induce a factor (glucokinase) necessary for the glucose effect on FAS mRNA accumulation.

A transcriptional effect of glucose was not formally demonstrated for the FAS gene in hepatocytes in this series of studies. We cannot exclude the possibility that insulin and glucose may stabilize FAS mRNA, as glucose is known to do in the hepatoma cell line HepG2 (88).

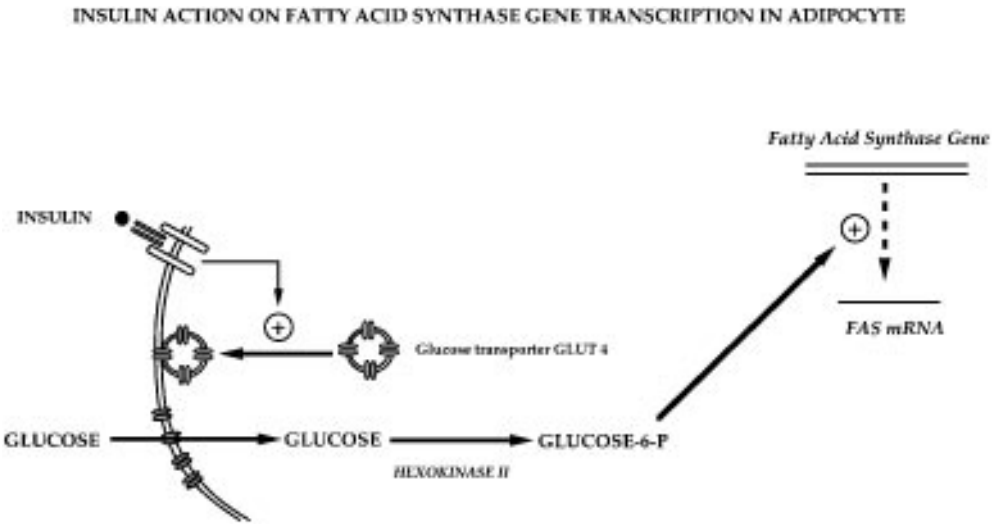

Figure 3 Model for explaining the role of insulin in the regulation of expression of the gene for fatty acid synthase in adipocytes. After binding to its receptor, insulin stimulates the translocation of glucose transporters GLUT4 to the plasma membrane. This stimulates glucose transport and phosphorylation, and glucose-6-phosphate stimulates transcription of the fatty acid synthase (FAS) gene.

CONCLUSIONS These in vitro studies demonstrate that in fully differentiated hepatic and adipose tissues, high glucose concentrations are required for inducing expression of FAS. In the absence of glucose, insulin is unable to induce expression of the FAS gene. In the presence of glucose, insulin has an indirect effect—stimulation of glucose transport or induction of glucokinase (Figures 3 and 4). This is not in agreement with other experiments performed in an adipocyte cell line in culture, which describe the presence of an insulin response element (IRE) in the promoter of the FAS gene (71). This IRE interacts with specific nuclear protein(s) from liver and adipose tissue and confers a two- to threefold increase of FAS mRNA in response to insulin (71). This modest effect of insulin on FAS mRNA could be secondary to an effect of insulin on differentiation (FAS is a late marker of adipocyte differentiation) rather than to a direct effect on the transcription of the FAS gene.

EVIDENCE THAT GLUCOSE METABOLISM IS REQUIRED TO INCREASE EXPRESSION OF GENES CODING FOR GLYCOLYTIC AND LIPOGENIC ENZYMES

Does glucose itself cause the changes in gene transcription, or is it due to a glucose metabolite?

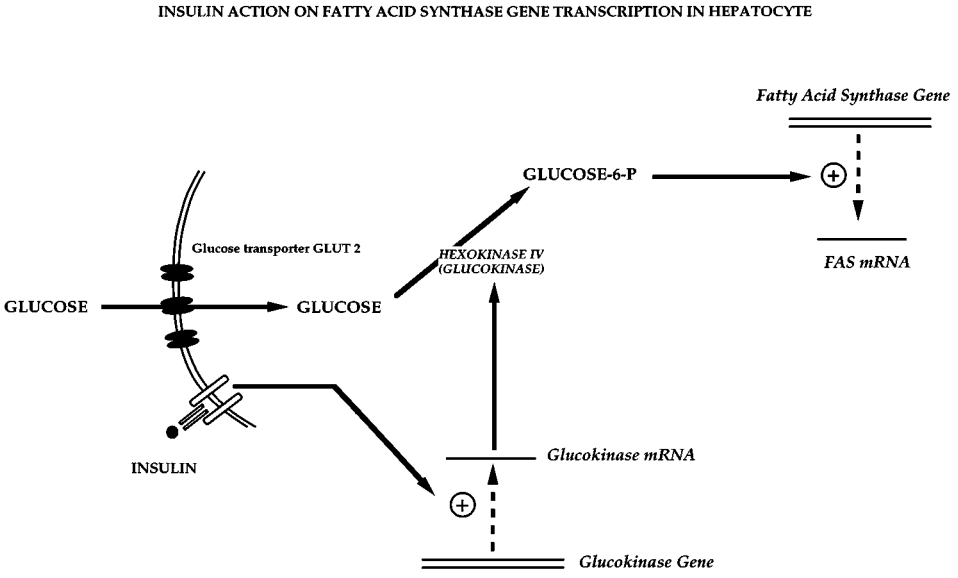

Figure 4 Model for explaining the role of insulin in the regulation of expression of the gene fatty acid synthase (FAS) in hepatocytes. After binding to its receptor, insulin stimulates transcription of glucokinase gene. The increase in glucokinase activity causes increased phosphorylation of glucose and glucose-6-phosphate stimulates transcription of the FAS gene.

Expression of FAS in Liver and Adipose Tissue

In adipose tissue explants, 3-O-methylglucose, a glucose analogue transported into the cell but not phosphorylated by hexokinases, is unable to induce the expression of FAS and ACC (23). This suggested that glucose metabolism was necessary. Further evidence that glucose metabolism was necessary came from another study (82). In suckling rat hepatocytes, the rate of glucose phosphorylation is low because glucokinase is not expressed, and the concentration of glucose-6-phosphate (taken as an index of glucose metabolism) is low even in the presence of 20 mM glucose. Under these conditions, glucose is not able to induce FAS. Culture of suckling rat hepatocytes for 48 h in the presence of insulin, triiodotyronine, and dexamethasone markedly increases expression of glucokinase (74) and glucose phosphorylation (82). Once glucokinase is induced, addition of glucose into the culture medium increases FAS and ACC mRNA levels in a dose-dependent manner (Figure 4). There is a positive correlation between the concentration of glucose-6-phosphate and FAS mRNA levels (Figure 2). The necessity of the induction of glucokinase by insulin in hepatocytes explains why the induction of FAS mRNA in response to glucose took much longer in liver than in adipose tissue (23, 82). Glucose is phosphorylated

in the adipose tissue of suckling rats by a constitutive hexokinase that is not rate limiting for glucose metabolism. In adipose tissue, there is also a striking correlation between the concentration of glucose-6-phosphate and the FAS mRNA level (Figure 2). Since the metabolism of glucose is enhanced by insulin in adipose tissue (through a stimulation of the rate of glucose transport) (Figure 3), higher glucose-6-phosphate concentrations are achieved when insulin is present in the culture medium (23). This likely explains the potentiating effect of insulin on FAS expression in adipose tissue explants.

In diabetic rats, the activity of glucokinase and the glucose phosphorylating capacity of the liver are low (3, 27, 40, 81). Concomitantly, the levels of hepatic FAS and ACC mRNA are also low (4). In adipose tissue of diabetic rats, glucose transport and phosphorylation capacities are reduced because of a decrease in the amount of the GLUT4 glucose transporter and the activity of hexokinase II; expression of FAS is also low (6). If diabetic rats are treated with vanadate, a potent insulino-mimetic agent, FAS mRNA levels and the activity of glucokinase are increased and hepatic glycolytic capacity is restored (3, 27). In contrast, GLUT4 and hexokinase II expression remain low in adipose tissue (4). Interestingly, vanadate restores expression of FAS in the liver but not in adipose tissue (4). This strongly supports the view that glucose must be metabolized to be able to stimulate expression of genes coding for glycolytic and lipogenic enzymes.

L-PK Expression in Liver Cells

Glucokinase is probably the insulin-induced factor in hepatocytes in culture that allows induction of L-PK; glucokinase provides the capacity for glucose phosphorylation. Moreover, glucose-induced expression of L-PK is independent of insulin in a hepatoma cell line that does not express glucokinase but that expresses constitutively a high hexokinase activity (55). Fructose, at 0.2 mM, activates glucokinase activity by reversing the effect of an inhibitory protein (98). Cultured hepatocytes from fed adult rats have a significant amount of glucokinase activity. Fructose (0.2 mM) by itself has no effect on glucose-6-phosphate concentration or L-PK mRNA but potentiates the effect of 20 mM glucose on both glucose-6-phosphate and L-PK mRNA levels. This effect does not require insulin (20). In the absence of insulin, 0.2 mM fructose has no effect on choramphenicol acetyltransferase (CAT) activity driven by the L-PK promoter but strongly potentiates the effect of 20 mM glucose. Finally, cotransfection of hepatocytes with the L-PK-CAT construct and with an expression vector for glucokinase also confers insulin-independent stimulation of CAT activity in response to glucose (20).

If the rate of glucose metabolism regulates expression of the genes for lipogenic and glycolytic enzymes, then overexpression of glucokinase should

lead to increased expression of those genes in liver. A line of transgenic mice overexpresses hepatic glucokinase under the control of the PEPCK promoter (21, 22). Glucokinase is increased fourfold in the livers of fasted or diabetic transgenic mice, as are the concentrations of glucose-6-phosphate and glycogen and the rate of glycolysis (21, 22). Concomitantly, the activity of hepatic L-PK is increased in fasted or diabetic transgenic mice, which is consistent with the hypothesis that overexpression of glucokinase stimulates L-PK gene transcription in the liver (21, 22). Thus, some evidence suggests that glucose must be metabolized to stimulate the expression of different genes.

ACC and L-PK Expression in a Pancreatic β-Cell Line

Glucose metabolism also is required for an effect of glucose on transcription of the ACC gene in INS-1 cells (5). Glucose induces a dose-dependent increase of glucose-6-phosphate concentration (Figure 2) and of insulin secretion. The glucose-induced increase in ACC expression and insulin secretion are inhibited by mannoheptulose and glucosamine, well-known inhibitors of glucokinase. Finally, ACC expression is not induced by 3-O-methylglucose or 6-deoxyglucose, glucose analogues that are not phosphorylated. Glucose also induces L-PK in INS-1 cells and does so in a concentration-dependent manner. The effect of glucose also is inhibited by mannoheptulose (66).

WHICH METABOLITE(S) SIGNAL THE INCREASE IN EXPRESSION OF GENES CODING FOR GLYCOLYTIC AND LIPOGENIC ENZYMES?

If glucose must be actively metabolized to affect the transcription of glucose-responsive lipogenic and glycolytic genes, what is the glucose metabolite(s) involved? The correlation between glucose-6-phosphate levels and the mRNA concentration of several glycolytic and lipogenic genes suggested that glucose-6-phosphate could be this metabolite (Figure 2). However, glucose-6-phosphate can be used in glycolysis, formation of fructose-2-6-bisphosphate, pentose-phosphate pathway, glycogen synthesis, and hexosamine synthesis. The concentration of one or more intermediates in these pathways may vary in parallel with that of glucose-6-phosphate, making them potentially suitable signal molecules.

Glucose Analogues

A number of glucose analogues have been tested in hepatocytes, adipocytes, and INS-1 cells. 2-Deoxyglucose is transported into cells by the same glucose transporters as glucose and is phosphorylated by glucokinase (K_m 55 mM vs 10 mM for glucose) and hexokinase (K_m 30 μM vs 10 μM for glucose), but 2-deoxyglucose is poorly metabolized in the glycolytic or pentose-phosphate

pathways. In most tissues, except the liver, this leads to a large accumulation of 2-deoxyglucose-6-phosphate in the cells [a minor product, possibly UDP-2-deoxyglucose, also accumulates (45)]. In adipose tissue explants from suckling rats, 2-deoxyglucose mimics the effect of glucose on FAS expression (45). However, 1 mM 2-deoxyglucose is as effective as 20 mM glucose. This suggests that 2-deoxyglucose-6-phosphate, which accumulates in adipocytes, may be the signal metabolite and that, by extension, glucose-6-phosphate is the natural signal for lipogenic enzyme expression. In INS-1 cells, 20–30 mM 2-deoxyglucose can induce both ACC and L-PK gene expression but to a lower extent than glucose does (5, 66). This could be due to the fact that transformed cells rely more on glycolysis for ATP production than do adipose cells and that high concentrations of 2-deoxyglucose severly impair the production of ATP through glycolysis.

In the liver, 2-deoxyglucose has no effect on L-PK (16, 17) or FAS mRNA accumulation (C. Prip-Buus, J. Boillot, F. Foufelle, P. Ferré and J. Girard, unpublished results). However, 2-deoxyglucose-6-phosphate is a minor component of the hepatic metabolism of 2-deoxyglucose; more than 50% of metabolites are not 2-deoxyglucose-6-phosphate (45, 84). Thus, in hepatocyes, addition of 2-deoxyglucose is not a valid test of the role of hexose-6-phosphate in regulation of gene expression.

The Glycogen Pathway

Glucose-6-phosphate is converted into glucose-1-phosphate and then into UDP-glucose in the glycogen pathway. Feeding glucose to starved animals produces a fourfold increase in liver hexose-6-phosphate, but a 50% decrease in UDP-glucose, after 1 h (97). Similarly, in the perfused liver of rats starved for 24 h, increasing the glucose concentration in the perfusion medium from 2–30 mM causes the glucose-6-phosphate concentration to rise 10-fold without any increase in UDP-glucose (104). This suggests that UDP-glucose is not a likely candidate for mediating the effects of glucose on gene expression. Glucose-1-phosphate cannot be excluded as a potential candidate because radioactive 2-deoxyglucose metabolized to 2-deoxyglucose-6-phosphate, 2-deoxyglucose-1-phosphate, and glycogen in liver and adipose tissue (10).

The Hexosamine Pathway

Intermediates in the hexosamine pathway were proposed as mediators of regulation of glucose transport and metabolism in adipocytes and muscles (67, 96) and of glucose-induced expression of the transforming growth factor (TGF)-α gene in vascular smooth muscle cells in culture (15, 70). The rate-limiting step of this pathway is glutamine:fructose-6-phosphate amidotransferase (EC 2.6.1.16) (GFAT), which catalyzes the synthesis of glucosamine-6-phosphate

from glutamine and fructose-6-phosphate. Glucosamine-6-phosphate can also be formed by phosphorylation of glucosamine catalyzed by hexokinase. Incubation of vascular smooth muscle cells with glucosamine or overexpression of GFAT in such cells stimulates expression of the TGF-α gene (15, 70). Inhibition of GFAT by pharmacological agents (6-diazo-5-oxonorleucine) or antisense olegodeoxynucleotides blunts glucose-induced TGF-α expression (87). This suggests that metabolism of glucose to glucosamine is required for the transcriptional stimulation of TGF-α in vascular smooth muscle cells in culture. Furthermore, the increase in the intracellular glucose concentration resulted in changes in the glycosylation state of some nuclear and cytosolic proteins (87). Several transcription factors are O-glycosylated (43), but it remains to be established whether O-glycosylation results in an activation of transcription.

In explants of adipocytes, glucosamine has no effect on expression of FAS or ACC (F. Foufelle & P. Ferré, unpublished results). In the INS-1 cells, glucosamine antagonizes glucose-induced expression of ACC (5). Finally, the glucose-induced expression of L-PK mRNA in the INS-1 cells is not inhibited by azaserine, an inhibitor of GFAT (66). These experiments argue against the involvement of the hexosamine biosynthetic pathways in the effect of glucose on lipogenic and glycolytic enzyme gene expression.

The Pentose-Phosphate Pathway

2-Deoxyglucose-6-phosphate may be a significant substrate for glucose-6-phosphate dehydrogenase (despite its 20-fold lower dehydrogenation rate capacity when compared with the same subsaturating concentration of glucose-6-phosphate) when it accumulates in the cell. 6-Phosphogluconate or another metabolite of the pentose-phosphate pathway could then be the signal. However, glucose-6-phosphate dehydrogenase mRNA and enzyme activity are low in liver and adipose tissue of suckling rats or starved adult rats (36, 49, 53, 83). Moreover, the rate of decarboxylation of glucose-6-phosphate is low in adipose tissue from suckling rats (39), which suggests that the pentose-phosphate pathway has a very low activity. After weaning onto a high-carbohydrate diet, or after refeeding a starved rat with a high-carbohydrate diet, the increase in glucose-6-phosphate dehydrogenase activity does not precede the accumulation of FAS or ACC mRNA; rather, it has a similar time-course (36). This suggests that 6-phosphogluconate is not the primary metabolite involved in the glucose effect on FAS or ACC gene transcription.

Mannose, an epimer of glucose also induces FAS expression in adipose tissue (F. Foufelle & P. Ferré, unpublished results) and ACC expression in INS-1 cells (5). Mannose is phosphorylated by hexokinase (or glucokinase) and then reversibly isomerized to fructose-6-phosphate, which in turn yields glucose-6-phosphate through phosphoglucose isomerase (EC 5.3.1.9). Indeed, in adipose

tissue explants with mannose as the substrate, substantial concentrations of glucose-6-phosphate can be detected (F. Foufelle & P. Ferré, unpublished results). It is thus difficult to differentiate between mannose-6-phosphate, glucose-6-phosphate, and fructose-6-phosphate as potential signaling molecules.

Xylulose-5-phosphate (19), an intermediate of the non-oxidative branch of the pentose phosphate pathway, and 3-phosphoglycerate or phosphoenolpyruvate (46), intermediates of the lower part of the glycolytic pathway, also are potential metabolites involved in the regulation of L-PK gene expression in response to glucose. Low concentrations (0.5 mM) of xylitol, a precursor of xylulose-5-phosphate, increase the expression of a L-PK/CAT construct transiently transfected to hepatoma cells, and does so to the same extent as 20 mM glucose. Because intracellular glucose-6-phosphate levels are not increased in the presence of xylitol 0.5 mM, metabolite of the pentose phosphate pathway may be involved (19). In addition, xylitol (5–10 mM) produced a fivefold increase of L-PK mRNA level in rat hepatocytes incubated in the absence of insulin (19); under the same circumstances, 25 mM glucose fails to increase L-PK mRNA (19). However, in the presence of insulin, the effects of xylitol were one third those of 25 mM glucose (19). Because xylitol is metabolized to xylulose-5-phosphate in liver cells and because it was previously reported that xylulose-5-phosphate is an activator of a specific protein phosphatase (75), xylulose-5-phosphate could be the metabolite involved in the regulation of L-PK expression (19). Thus, a xylulose-5-phosphate activated protein phosphatase could dephosphorylate a transcription factor and activate L-PK gene transcription (19). Unfortunately, intracellular xylulose-5-phosphate concentration was not measured in these experiments (19). It should be recalled that hepatic xylitol is metabolized to lactate via glycolysis and to glucose via gluconeogenesis. Experiments in adult rat hepatocytes in culture have challenged the view that xylulose-5-phosphate is the metabolite involved in regulation of gene transcription in response to glucose (F. Mourrieras, F. Foufelle & P. Ferré, unpublished data). The accumulation of FAS mRNA in response to increasing concentrations of xylitol or dihydroxyacetone (1–5 mM) was correlated with levels of glucose-6-phosphate but not with levels of xylulose-5-phosphate, phosphoenolpyruvate, 3-phosphoglycerate, or fructose-6-phosphate (F. Mourrieras, F. Foufelle & P. Ferré, unpublished data). Thus, the evidence still favors the view that glucose-6-phosphate is the best candidate for the regulation of gene transcription in response to glucose.

Does Glucose-6-Phosphate Possess All the Properties to be the Signal Molecule?

Glucose-6-phosphate is the product of non-equilibrium enzymes (hexokinase, glucokinase) and is effectively the substrate of a non-equilibrium enzyme,

6-phosphofructo-1-kinase (EC 2.7.1.11), since glucose-6-phosphate and fructose-6-phosphate are in rapid equilibrium because of the high activity of phosphoglucose isomerase. As such, its concentration varies in parallel with the external glucose concentration and in parallel with the expression of lipogenic enzymes. In the liver of adult rats starved for 24 h and refed with fructose, there is a rapid increase in liver L-PK transcription after 2–3 h, whereas refeeding with glucose induces a slower induction (6–9 h) (72). Of the various glycolytic metabolites studied, only hexose-6-phosphate concentration varied in parallel with the rate of L-PK transcription. The concentrations of fructose-1-6-bisphosphate, fructose-2-6-bisphosphate, phosphoenolpyruvate, or fructose-1-phosphate did not correlate with L-PK transcription. The early rise of hexose-6-phosphate caused by fructose, despite the fact that fructose carbons enter the glycolytic pathway at the triose phosphate level, could be due to the effect of fructose-1-phosphate (the product of fructose phosphorylation by fructokinase) on the inhibitory protein of glucokinase, thus increasing glucokinase activity (99, 100).

Thus, glucose-6-phosphate appears to be the likely signal metabolite involved in the regulation of glycolytic and lipogenic enzyme gene expression. The evidence for this is summarized as follows.

1. Glucose needs to be phosphorylated to act as a signal for gene induction.

2. Modulation of carbon flux into the hexosamine pathway does not alter the effect of glucose, and glucosamine has no effect on lipogenic enzyme gene expression.

3. The genes for lipogenic enzymes are induced under conditions in which function of the pentose-phosphate pathway is low (adipose tissue of suckling rats).

4. The glucose analogue, 2-deoxyglucose, mimics totally (adipose tissue) or partially (pancreatic β-cell line) the effect of glucose on lipogenic or glycolytic gene expression.

5. The intracellular concentration of glucose-6-phosphate varies in parallel with the intensity of gene induction.

6. In vivo, the kinetics of the increase of hexose-phosphate fit with the time-related pattern of gene induction, whereas that is not the case for other glycolytic intermediates.

Nevertheless, the possibility that glucose-1-phosphate, fructose-6-phosphate, and mannose-6-phosphate are also effective cannot be ruled out. Knowledge of

the target for the signal metabolite or genetic alterations of the various enzymes involved should allow us to differentiate between these possibilities.

MOLECULAR MECHANISMS INVOLVED IN THE CARBOHYDRATE-MEDIATED REGULATION OF THE EXPRESSION OF GENES CODING FOR GLYCOLYTIC AND LIPOGENIC ENZYMES

cis-Acting DNA Sequences Involved in Glucose Responsiveness of Genes for Lipogenic and Glycolytic Enzymes

The *cis*-acting DNA sequences involved in glucose responsiveness, the glucose response elements (GlREs) or the carbohydrate response element (ChoRE), were first identified in the L-PKgene (Figure 5) (12, 94). They were localized to the first −183 bp of the L-PK promoter, a region that was sufficient for the tissue-specific expression as well as for nutritional and hormonal regulation in transgenic mice (12). By use of transfection experiments in primary cultured hepatocytes, the element was localized further to the DNA between −183 and −96 bp (94). This region binds several nuclear factors, as shown in DNase I footprinting experiments, and has been divided into four elements: L1 (−66 to −95 bp), L2 (−97 to −114 bp), L3 (−126 to −144 bp), and L4 (−145 to

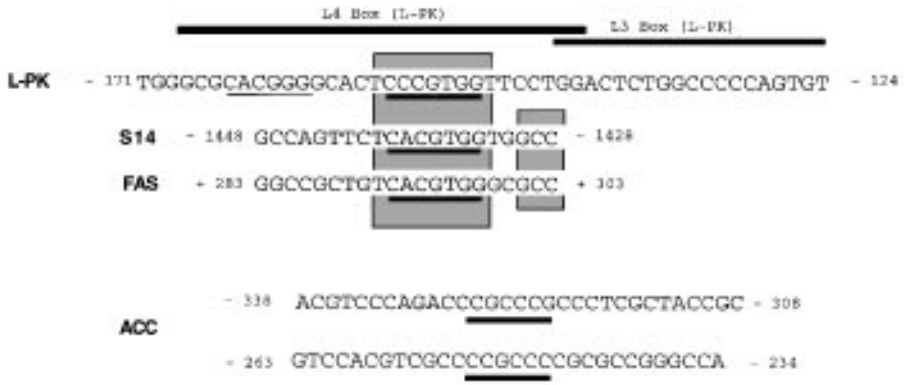

Figure 5 Minimal sequences from the L-pyruvate kinase (L-PK), S14, and acetyl-coenzyme A carboxylase (ACC) genes that are able to confer glucose responsiveness and their functionality. For the L-PK gene, the L4 [upstream stimulatory factor (USF) binding site] and L3 (HNF4 binding site) boxes are overlined. E-boxes corresponding to recognized USF binding sites are underlined. Sequence identity among the three genes is indicated by the dotted box. The three nucleotides outside the E-box that seem to be determinant for glucose responsiveness in the S14 and fatty acid synthase (FAS) genes are also boxed.

−168 bp). Glucose responsiveness is conferred by a close cooperation between the L3 and L4 elements (2, 58). A multimer of the L4 element, but not of the L3 element, confers glucose responsiveness to a glucose-unresponsive heterologous promoter (2). Nevertheless, the full glucose response is conferred only when both L3 and L4 elements are present.

A second GlRE was also characterized in the rat S_{14} gene (Figure 5) (44, 90). This gene encodes a protein of 17 kDa (51, 52, 64) that is expressed in lipogenic tissues (white and brown adipose tissue, liver, lactating mammary gland). Its expression is induced by lipogenic stimuli, including T3 and carbohydrates, and is inhibited by fasting, diabetes, polyunsaturated fatty acids, and glucagon. Expression of S_{14} correlates with that of FAS and with the diurnal rhythm of lipogenesis. The nuclear localization of the S_{14} protein (51), as well as a decreased expression of lipogenic enzymes when S_{14} expression is reduced by an antisense oligonucleotide (52), suggest that the S_{14} protein is involved in the transduction of hormonal and dietary signals involved in the long-term regulation of lipogenesis.

The region from −1601 to −1395 of the S_{14} promoter is essential for glucose responsiveness of this gene in isolated hepatocytes (90). Comparison of the sequence of this S_{14} region with that of the L4 element of the L-PK gene reveals a consensus sequence with 9 out of 10 identities. This region is able to confer glucose responsiveness to glucose-unresponsive homologous or heterologous promoters (90). The GIRE of the S_{14} promoter consists of two motifs related to the consensus binding site (CACGTG) for the c-myc family of transcription factors, separated by five base pairs (89), a similar arrangement to L-PK GIRE. In its natural context, the S_{14} GIRE requires a novel accessory factor (similar to, but distinct from, HNF-4) to support the full response to glucose.

Finally, transgenic mice carrying 2.1 kb of the 5′-flanking region (−2100 to +67) of the rat FAS gene fused to a CAT reporter gene have been generated (92). CAT activity was decreased by fasting and diabetes in liver and adipose tissue, and it was restored by refeeding fasting mice with a high carbohydrate diet or by insulin treatment of diabetic mice. Thus, the first 2.1 kb of the 5′-flanking region of FAS gene contained all sequence elements required for hormonal and nutritional regulation of FAS gene (92). A DNase I hypersensitive site has also been found in the first intron of the FAS gene (+292) with a DNA sequence (CACGTG) similar to the S_{14} and L-PK GlREs (Figure 5) (25). It was initially reported that when this sequence was inserted into a glucose-unresponsive heterologous promoter it conferred a glucose responsiveness (91). However, it was reported later that this sequence by itself was ineffective in supporting the glucose response (89). The positive result in the initial experiments was due to an erroneous duplication of this sequence during the cloning, then providing a

second CACGTG motif (89). For the FAS gene, the functionality of this element remains to be proven.

trans-Acting Factors Involved in Glucose Responsiveness of Genes of Lipogenic Enzymes

USF/MLTF (UPSTREAM STIMULATORY FACTOR/MAJOR LATE TRANSCRIPTION FACTOR) In the three GlREs characterized so far, a common DNA sequence element emerges. It corresponds to a canonical (S_{14} and FAS) or degenerated (L-PK) palindromic sequence 5′-CACGTG-3′ (E-box), which is a binding site for a specific class of proteins (Figure 5). These proteins have a stretch of amino acids rich in lysine and arginine residues, and located C terminal to this basic domain are two amphiphatic α-helices connected by a loop and followed by a conserved heptad leucine repeat or zipper motif, which contains a leucine residue every seventh residue in an α-helix. This implies that all the hydrophobic residues are on the same side of the helix, a feature that should play a role in dimerization. These structures are involved in the binding to DNA and in protein/protein interactions.

Several transcription factors belong to this class of proteins, e.g. c-myc and its partner Max, Myo-D, immunoglobulin enhancer binding proteins, and USF/MLTF. USF/MLTF (upstream stimulatory factor/major late transcription factor, designated USF in this review) was first discovered as a protein that binds to the adenovirus late promoter. Subsequently, it was proven to bind to many other genes, including the human growth hormone, mouse metallothionein I, rat γ-fibrinogen, and human insulin gene enhancer. Two USF proteins, named USF1 (M_r 43) and USF2 (M_r 44), have identical DNA binding properties. Their structures differ only in their N-terminal domains. USF binds to DNA as a dimer. The tissue distribution of USF is ubiquitous and does not seem to be altered by nutritional or hormonal status. For L-PK, S_{14}, and FAS, USF binds to the E-box of the GlRE (18, 25, 91, 102). Disruption of the E-box by point mutations abolishes the glucose responsiveness of the L-PK and S_{14} promoters (2, 90). In hepatoma cells, overexpression of native USF proteins causes transactivation of the L-PK promoter via the GlRE (56). More importantly, expression in these cells of a USF mutant represses the glucose effect. This dominant negative mutant can dimerize with and thus sequester endogenous USF but cannot bind to the GlRE (56). These studies suggest that USF proteins are major components of the transcriptional response to glucose.

A sequence from the adenovirus promoter with a core sequence of 5′-CACGTG-3′, and that binds USF, is unable to substitute for the GlREs in the L-PK and S_{14} promoters (58, 91). The flanking bases of the CACGTG core are important for glucose responsiveness: Mutation of a stretch of the three nucleotides, GCC, in the GlREs of the S_{14} and FAS genes (5′-CACGTGNNNGCC-3′) abolishes glucose responsiveness (91). Conversely, mutation of the otherwise

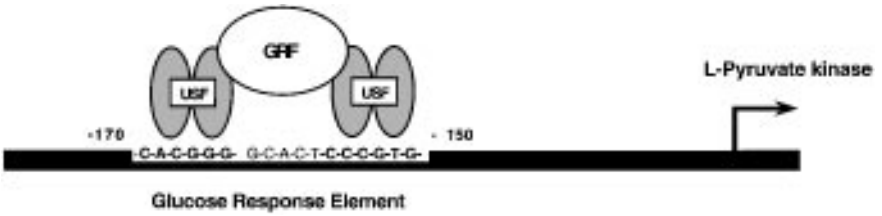

Figure 6 Model for explaining the activation of the L-pyruvate kinase (L-PK) gene in response to glucose. The GIRE is composed of two binding motifs related to the sequence CACGTG. A glucose-responsive factor, in its phosphorylated form, may cause heterodimerization of the two upstream stimulatory factor (USF) and stimulate transcription of the L-PK gene.

glucose unresponsive wild-type adenovirus sequence 5′-CACGTGACCGGG-3′ to 5′-CACGTGACCGCC-3′ confers glucose responsiveness to a heterologous promoter. This suggests that protein/protein interaction between a USF/MLTF factor and an accessory factor may contribute to specific regulation (Figure 6).

Despite the apparent involvement of USF in the glucose response of these three genes, some differences in mechanism exist among them. The L-PK GlRE contains two degenerated E-boxes. The affinities of these E-boxes for USF are much less than that of the canonical E-boxes of the S_{14} and FAS genes (25, 101). The involvement of these two E-boxes in glucose responsiveness is not clear. Disrupting one of them and transforming the other into a canonical one reduces the glucose effect, indicating that both E-boxes are necessary for the full effect (18). In a similar study, however, the opposite result was found (58). For the L-PK gene, full glucose responsiveness requires not only the E-box but also binding of a hepatic transcription factor, HNF4, on the adjacent L3 box (2, 58). The mechanism involved in this synergistic effect does not involve cooperative DNA binding (59). Since HNF4 is rather specific to the liver, a similar mechanism cannot be operative for the S_{14} and FAS genes, which are also expressed in adipose tissue. There is no evidence for interaction of the protein complex binding to the USF/MLTF site of the S_{14} and FAS genes and factors that bind to contiguous sequences. Finally, the L-PK element L4 is unable to replace the S_{14} GlRE (58). Thus, the only compelling evidence indicates that binding of USF, a common property of the three GlREs, is necessary for glucose responsiveness. Nevertheless, it is likely that the transcription complex that accounts for glucose responsiveness is not organized in the same way in each of these three genes.

SP1 The rat ACC gene contains two promoters PI and PII, which function in a tissue-specific manner and produce different mRNAs with the same coding sequences and different 5′ untranslated regions (60). PI is the promoter induced

under lipogenic conditions. PII has the structural features of a housekeeping gene (no CCAAT or TATA box) and is constitutively expressed in all tissues (61). PII is the only promoter activated by high concentrations of glucose in differentiated mouse 30A5 preadipocytes (13). The DNA sequences required for the response to glucose are located between −340 and −249 bp of PII (13) (Figure 5). Unlike the L-PK and S_{14} promoters, the PII promoter of ACC gene does not bind one of the MLTF/USF family of transcription factors, even though it has the appropriate sequences (13). The effect of glucose is mediated by the ubiquitously expressed transcription factor Sp1, which binds to the GlRE (5′-GGGCGGG-3′) in PII and activates it (13).

Transmission of the Glucose Signal

The ability of USF to bind to GlRE in nuclear extracts from tissues not expressing these genes (brain, spleen) is the same as that from tissues that so modulate expression of these genes according to the nutritional environment (liver, adipose tissue) (25, 90, 101, 102). Moreover, binding activity in nuclear extracts from lipogenic tissue was unaffected by various nutritional conditions. This suggests that the intensity of binding of USF to the GlRE is not the variable modulated by glucose. In summary, there are several hypotheses that might account for glucose responsiveness in specific tissues.

1. Glucose may modify the amount or the properties of a USF coactivator protein. For example, its nuclear localization may be affected. The sterol regulatory element binding protein 1 (SREBP-1) (103) is a transcription factor that is bound in precursor form to the endoplasmic reticulum (ER) membrane when the concentration of cholesterol is high in the ER membrane. When the concentration of cholesterol decreases, a precursor form of SREBP-1 is cleaved and migrates into the nucleus, where it binds to the SRE element of LDL receptor gene.

2. Glucose may cause USF to undergo a post-translational modification, which in turn modifies its interaction with the basic transcription machinery or a coactivator protein. When the cAMP responsive element–binding protein (CREB) is phosphorylated, it interacts with CREB-binding protein (CBP), which in turn acts as a transcriptional activator (8).

The existence of a coactivator that functions with USF is not proven. Nevertheless, post-translational modifications of USF or of a coactivator protein could involve two types of mechanisms or a combination thereof.

1. Direct allosteric modification by hexose-6-phosphate. Hexose-6-phosphates are allosteric regulators of several proteins: hexokinase is inhibited and

glycogen synthase (EC 2.4.1.11) is activated by glucose-6-phosphate; fructose-1-phosphate interacts with the glucokinase regulatory protein; etc. This mechanism is similar to the well-known regulation of the lactose operon in *Escherichia coli*. In the absence of lactose, a repressor is bound upstream of the *lac* operon and blocks transcription of genes coding for enzymes of lactose metabolism. Lactose binds to the repressor protein, causing it to dissociate from the DNA and active transcription.

2. Modification of a protein kinase/phosphatase, which in turn causes phosphorylation/dephosphorylation of a transcription factor. The activities of other transcription factors are modulated by this type of mechanism (42).

The potential role of protein phosphorylation/dephosphorylation in the regulation of lipogenic enzyme genes has been investigated (93, 105). Okadaic acid, an inhibitor of protein phosphatase 1 and 2A, abolishes the effect of glucose on hepatocytes transfected with a S_{14} construct or with the S_{14} GlRE inserted into an otherwise glucose-unresponsive promoter (93); glucose utilization was not affected. Experiments with calyculin, a more potent inhibitor of protein phosphatase 1 than okadaic acid, have implicated protein phosphatase 2A in the glucose effect. Finally, the calcium ionophore A23187, an activator of calmodulin-dependent protein kinase, also inhibits the glucose effects, suggesting that calmodulin-dependent protein kinase and protein phosphatase 2A are involved in the glucose effect. These results suggest that glucose acts on S_{14} transcription via a phosphorylation/dephosphorylation mechanism. We have also found that okadaic acid inhibited glucose-induced FAS expression in adult rat hepatocytes in culture (M. Foretz, F. Foufelle & P. Ferré, unpublished results). However, it must be pointed out that these drugs were added for 24 h in these experiments and could have modulated the glucose response through physiologically unimportant mechanisms because many protein phosphatases and their substrates would be affected by the inhibitors.

Glucose activation of ACC in INS-1 cells also involves dephosphorylation by a protein phosphatase (105). The increased binding of Sp1 caused by glucose is inhibited by okadaic acid and is activated by the catalytic subunit of type 1 protein phosphatase (14). This suggests that the increased binding of Sp1 to promoter II of ACC may be caused by a glucose-induced dephosphorylation of Sp1 by type 1 protein phosphatase (14).

CONCLUSIONS AND FUTURE DIRECTIONS

Although it is clear that glucose modulates expression of several genes belonging to the glycolytic/lipogenic pathways and that glucose metabolism is necessary for this effect, several points require further study.

1. Confirm that glucose-6-phosphate is the signal metabolite.

2. Identify the component of the transcription complex that is the target for the glucose effect remains; characterize the underlying mechanism.

3. Analyze glucose responsiveness of other genes belonging to the glycolytic and lipogenic pathways and determine if the molecular mechanisms are similar. Glucose stimulates expression of aldolase B (EC 4.1.2.13), 6-phosphofructo-2-kinase (EC. 2.7.1.105)/fructose-2,6-bisphosphatase (EC. 3.1.3.46) (57), and pyruvate carboxylase (62); the GlREs have not been identified. In addition, the adipose tissue mRNAs for E1α and E1β of the pyruvate dehydrogenase complex are increased in rats weaned to a high-carbohydrate diet but not in rats weaned to a high-fat diet (69). Moreover, glucose also causes an increase in the abundance of E1α mRNA in cultured pancreatic islets from adult rats (63). Malic enzyme, glucose-6-phosphate dehydrogenase, and ATP-citrate lyase are obvious candidates for GlREs because their expression rates are increased by high-glucose diets with time-courses similar to those for FAS and ACC.

Finally, glucose-mediated stimulation of the genes coding for enzymes related to the lipogenic pathway and the potentiating effect of fructose and insulin may explain why diets rich in sucrose (a disaccharide composed of fructose and glucose) have profound effects on lipid synthesis and adiposity. They not only provide the carbons and cofactors necessary to provide substrates for the lipogenic pathway, but in conjunction with an effect on insulin secretion, they also cause an increase in lipogenic capacity through stimulation of expression of glycolytic and lipogenic genes.

SUMMARY

Regulation of gene expression by nutrients is one of several important mechanisms by which mammals adapt to their nutritional environment. This is specially true for enzymes involved in the storage of energy, such as the lipogenic and glycolytic enzymes in the liver and adipose tissue. We reviewed the cellular and molecular mechanisms involved in the regulation of glycolytic and lipogenic enzyme gene expression by glucose.

Rates of expression of the genes for FAS and ACC are stimulated by glucose in adipose tissue in vivo and in vitro. Expression of FAS, ACC, and L-PK is stimulated in the liver and of ACC and L-PK in a pancreatic β-cell line. In each case, regulation involves stimulation of transcription. To stimulate gene transcription, glucose must be metabolized. In adipose tissue, insulin increases indirectly the expression of FAS and ACC by stimulating glucose transport. In

liver, insulin also acts indirectly by stimulating the expression of glucokinase and, hence, by increasing glucose metabolism. In liver, fructose potentiates the stimulation of gene expression by glucose because it stimulates glucokinase activity. Several lines of evidence suggest that glucose-6-phosphate is the signaling metabolite. (*a*) The effect of glucose is mimicked by 2-deoxyglucose (a glucose analogue that is phosphorylated to 2-deoxyglucose-6-phosphate by hexokinase but no further metabolized) in adipose tissue and a pancreatic β-cell line. (*b*) Intracellular concentrations of glucose-6-phosphate vary in parallel with the abundances of ACC, FAS, and L-PK mRNAs in liver, adipose tissue, and a pancreatic β-cell line. (*c*) In vivo, the kinetics of changes in hexose-phosphate levels and gene expression are consistent with glucose-6-phosphate being the signaling molecule.

GlREs have been well characterized for two genes, L-PK and S_{14}. Each of these GlREs has a sequence, 5′-CACGTG-3′, which binds a transcription factor of the basic-domain, helix-loop-helix, leucine-zipper family called USF/MLTF. The mechanisms by which these GlREs function may differ from one gene to another; for example, the ACC gene uses a Sp1 rather than USF. The mechanisms linking glucose-6-phosphate to the complex formed on the GlRE are largely unknown.

ACKNOWLEDGMENTS

The original work presented in this review was supported in part by grants from the Fondation pour la Recherche Médicale, Ministère de la Recherche et de la Technologie (grants 88-G-0119, 90-G-0292, 92-G-0499, and 95-G-0079), Bayer-Pharma (France), and Eridiana-Beghin-Say. We would like to thank many coworkers who have contributed to these studies: Christine Coupé, Betty Gouhot, Dominique Perdereau, Nathalie Lepetit, Jocelyne Maury, Joèlle Morin, Jean-Paul Pégorier, Carina Prip-Buus, Josette Boillot, and Michel Raymondjean.

Literature Cited

1. Asfari M, Janjic D, Meda P, Li G, Halban P, Wollheim C. 1992. Establishment of 2-mercaptoethanol-dependent differentiated insulin-secreting cell lines. *Endocrinology* 130:167–78
2. Bergot MO, Diaz-Guerra MJM, Puzenat N, Raymondjean M, Kahn A. 1992. *Cis*-regulation of the L-type pyruvate kinase gene promoter by glucose, insulin and cyclic AMP. *Nucleic Acids. Res.* 20:1871–78
3. Brichard S, Desbuquois B, Girard J. 1993. Vanadate treatment of diabetic rats reverses the impaired expression of genes involved in hepatic glucose metabolism. Effects on glycolytic and gluconeogenic enzymes and on glucose transporter GLUT2. *Mol. Cell. Endocrinol.* 91:91–97

4. Brichard SM, Ongemba LN, Girard J, Henquin J-C. 1994. Tissue-specific correction of lipogenic enzyme gene expression in diabetic rats given vanadate. *Diabetologia* 37:1065–72
5. Brun T, Roche E, Kim KH, Prentki M. 1993. Glucose regulates acetyl-CoA carboxylase gene expression in a pancreatic beta-cell line (INS–1). *J. Biol. Chem.* 268:18905–11
6. Burcelin R, Printz RL, Kande J, Assan R, Granner DK, Girard J. 1993. Regulation of glucose transporter and hexokinase-II expression in tissues of diabetic rats. *Am. J. Physiol.* 265:E392–401
7. Chen SY, Ogawa A, Ohneda M, Unger RH, Foster DW, McGarry JD. 1994. More direct evidence for a malonyl-CoA-carnitine palmitoyltransferase I interaction as a key event in pancreatic beta-cell signaling. *Diabetes* 43:878–83
8. Chrivia J, Kwok R, Lamb M, Hagiwara M, Montminy M. 1993. Phosphorylated CREB binds specifically to the nuclear protein CBP. *Nature* 365:855–59
9. Clarke SD, Jump DB. 1994. Dietary polyunsaturated fatty acid regulation of gene transcription. *Annu. Rev. Nutr.* 14:83–98
10. Colwell DR, Higgins JA, Denyer GS. 1996. Incorporation of 2-deoxy-D-glucose into glycogen. Implications for measurement of tissue-specific glucose uptake and utilisation. *Int. J. Biochem. Cell. Biol.* 28:115–21
11. Coupé C, Perdereau D, Ferré P, Hitier Y, Narkewicz M, Girard J. 1990. Lipogenic enzyme activities and mRNA in rat adipose tissue at weaning. *Am. J. Physiol.* 258:E126–33
12. Cuif MH, Cognet M, Boquet D, Tremp G, Kahn A, Vaulont S. 1992. Elements responsible for hormonal control and tissue specificity of L-type pyruvate kinase gene expression in transgenic mice. *Mol. Cell. Biol.* 12:4852–61
13. Daniel S, Kim KH. 1996. SP1 mediates glucose activation of the acetyl-CoA carboxylase promoter. *J. Biol. Chem.* 271:1385–92
14. Daniel S, Zhang S, De Paoli-Roach AA, Kim KH. 1996. Dephosphorylation of Sp1 by protein phosphatase 1 is involved in the glucose-mediated activation of the acetyl-CoA carboxylase gene. *J. Biol. Chem.* 271:14692–97
15. Daniels MC, Kansal P, Smith TM, Paterson AJ, Kudlow JE, McClain DA. 1993. Glucose regulation of TGF-alpha expression is mediated by products of the hexosamine biosynthetic pathway. *Mol. Endocrinology* 7:1041–48
16. Decaux JF, Antoine B, Kahn A. 1989. Regulation of the expression of the L-type pyruvate kinase gene in adult rat hepatocytes in primary culture. *J. Biol. Chem.* 264:11584–90
17. Decaux JF, Marcillat O, Pichard AL, Henry J, Kahn A. 1991. Glucose-dependent and -independent effect of insulin on gene expression. *J. Biol. Chem.* 266:3432–38
18. Diaz-Guerra MJM, Bergot M, Martinez A, Cuif M, Kahn A, Raymondjean M. 1993. Functional characterization of the L-type pyruvate kinase gene glucose response complex. *Mol. Cell. Biol.* 13: 7725–33
19. Doiron B, Cuif MH, Chen R, Kahn A. 1996. Transcriptional glucose signaling through the glucose response element is mediated by the pentose phosphate pathway. *J. Biol. Chem.* 271:5321–24
20. Doiron B, Cuif MH, Kahn A, Diaz-Guerra MJM. 1994. Respective roles of glucose, fructose, and insulin in the regulation of the liver-specific pyruvate kinase gene promoter. *J. Biol. Chem.* 269:10213–16
21. Ferre T, Pujol A, Riu E, Bosch F, Valera A. 1996. Correction of diabetic alterations by glucokinase. *Proc. Natl. Acad. Sci. USA* 93:7225–30
22. Ferre T, Riu E, Bosch F, Valera A. 1996. Evidence from transgenic mice that glucokinase is rate limiting for glucose utilization in the liver. *FASEB J.* 10:1213–18
23. Foufelle F, Gouhot B, Pégorier J, Perdereau D, Girard J, Ferré P. 1992. Glucose stimulation of lipogenic enzyme gene expression in cultured white adipose tissue. A role for glucose 6-phosphate. *J. Biol. Chem.* 267:20543–46
24. Foufelle F, Gouhot B, Perdereau D, Girard J, Ferré P. 1994. Regulation of lipogenic enzyme and phosphoenolpyruvate carboxykinase gene expression in cultured white adipose tissue: glucose and insulin effects are antagonized by cAMP. *Eur. J. Biochem.* 223:893–900
25. Foufelle F, Lepetit N, Bosc D, Delzenne N, Morin J, et al. 1995. DNase I hypersensitivity sites and nuclear protein binding on the fatty acid synthase gene: identification of an element with properties similar to known glucose-responsive elements. *Biochem. J.* 308:521–27
26. Fukuda H, Katsurada A, Iritani N. 1992. Effects of nutrients and hormones on gene expression of ATP citrate-lyase in rat liver. *Eur. J. Biochem.* 209:217–22
27. Gil J, Miralpeix M, Carreras J, Bartrons R. 1988. Insulin-like effects of vanadate

on glucokinase activity and fructose 2,6-bisphosphate levels in the liver of diabetic rats. *J. Biol. Chem.* 263:1868–71
28. Girard J, Ferré P, Pégorier JP, Duée PH. 1992. Adaptations of glucose and fatty acid metabolism during the perinatal period and the suckling-weaning transition. *Physiol. Rev.* 72:507–62
29. Goodridge AG. 1987. Dietary regulation of gene expression: enzymes involved in carbohydrate and lipid metabolism. *Annu. Rev. Nutr.* 7:157–85
30. Granner D, Pilkis S. 1990. The genes of hepatic glucose metabolism. *J. Biol. Chem.* 265:10173–76
31. Hardie DG. 1989. Regulation of fatty acid synthesis via phosphorylation of acetyl-CoA carboxylase. *Progr. Lipid Res.* 28:117–46
32. Hellerstein MK, Schwarz JM, Neese RA. 1996. Regulation of hepatic de novo lipogenesis in humans. *Annu. Rev. Nutr.* 16:523–57
33. Hers HG, Hue L. 1983. Gluconeogenesis and related aspects of glycolysis. *Annu. Rev. Biochem.* 52:617–53
34. Hillgartner FB, Salati LM, Goodridge AG. 1995. Physiological and molecular mechanisms involved in nutritional regulation of fatty acid synthesis. *Physiol. Rev.* 75:147–76
35. Iritani N. 1992. Nutritional and hormonal regulation of lipogenic enzyme gene expression in rat liver. *Eur. J. Biochem.* 205:433–42
36. Iritani N, Fukuda H, Matsumura Y. 1993. Lipogenic enzyme gene expression in rat liver during development after birth. *J. Biochem.* 113:519–25
37. Iritani N, Fukuda H, Tada K. 1996. Nutritional regulation of lipogenic enzyme gene expression in rat epididymal adipose tissue. *J. Biochem.* 120:242–48
38. Iritani N, Nishimoto N, Katsurada A, Fukuda H. 1992. Regulation of hepatic lipogenic enzyme gene expression by diet quantity in rats fed a fat-free, high carbohydrate diet. *J. Nutr.* 122:28–36
39. Issad T, Ferré P, Pastor-Anglada M, Baudon MA, Girard J. 1989. Development of insulin sensitivity in white adipose tissue during the suckling-weaning transition in the rat. Involvement of glucose transport and lipogenesis. *Biochem. J.* 264:217–22
40. Iynedjian PB, Gjinovci A, Renold AE. 1988. Stimulation by insulin of glucokinase gene transcription in liver of diabetic rats. *J. Biol. Chem.* 263:740–44
41. Iynedjian PB, Jottterand D, Nouspikel T, Asfari M, Pilot PR. 1989. Transcriptional regulation of glucokinase gene by insulin in cultured liver cells and its repression by the glucagon-cAMP system. *J. Biol. Chem.* 264:21824–29
42. Jackson S. 1992. Regulating transcription factor activity by phosphorylation. *Trends Cell Biol.* 2:104–8
43. Jackson S, Tjian R. 1988. O-glycosylation of eucaryotic transcription factors. *Cell* 55:125–33
44. Jacoby D, Zilz N, Towle H. 1989. Sequences within the 5′-flanking region of the S_{14} gene confer responsiveness to glucose in primary hepatocytes. *J. Biol. Chem.* 264:17623–26
45. Jenkins A, Furler S, Kraegen E. 1986. 2-Deoxyglucose metabolism in individual tissues of the rat in vivo. *Int. J. Biochem.* 18:311–18
46. Kang RJ, Yamada K, Tanaka T, Lu T, Noguchi T. 1996. Relationship between the concentrations of glycolytic intermediates and expression of the L-type pyruvate kinase gene in cultured hepatocytes. *J. Biochem.* 119:162–66
47. Katsurada A, Iritani N, Fukuda H, Matsumara Y, Nishimoto N, Noguchi T, Tanaka T. 1990. Effects of nutrients and hormones on transcriptional and post-transcriptional regulation of fatty acid synthase in rat liver. *Eur. J. Biochem.* 190:427–33
48. Katsurada A, Iritani N, Fukuda H, Matsumara Y, Nishimoto N, et al. 1990. Effects of nutrients and hormones on transcriptional and post-transcriptional regulation of acetyl-CoA carboxylase in rat liver. *Eur. J. Biochem.* 190:435–41
49. Katsurada A, Iritani N, Fukuda H, Matsumura Y, Noguchi T, Tanaka T. 1989. Effects of nutrients and insulin on transcriptional and post-transcriptional regulation of glucose-6-phosphate dehydrogenase synthesis in rat liver. *Biochim. Biophys. Acta* 1006:104–10
50. Kim KH. 1983. Regulation of acetyl-CoA carboxylase. *Curr. Topic Cell Regul.* 22:143–75
51. Kinlaw WB, Tron P, Friedmann A. 1992. Nuclear localization and hepatic zonation of rat "spot 14" protein: immunohistochemical investigation employing antifusion protein antibodies. *Endocrinology* 131:3120–22
52. Kinlaw WB, Church JL, Harmon J, Mariash CN. 1995. Direct evidence for a role of the "spot 14" protein in the regulation of lipid synthesis. *J. Biol. Chem.* 270:16615–18
53. Kletzien R, Prostko C, Stumpo D, McClung J, Dreher K. 1985. Molecular

cloning of DNA sequences complementary to rat liver glucose-6-phosphate dehydrogenase mRNA. *J. Biol. Chem.* 260:5621–24
54. Lakshmanan MR, Nepokroeff CM, Porter JW. 1972. Control of the synthesis of fatty-acid synthetase in rat liver by insulin, glucagon and adenosine-3′:5′-cyclic monophosphate. *Proc. Natl. Acad. Sci. USA* 69:3516–19
55. Lefrancois-Martinez AM, Diaz-Guerra MJM, Vallet V, Kahn A, Antoine B. 1994. Glucose-dependent regulation of the L-pyruvate kinase gene in a hepatoma cell line is independent of insulin and cyclic AMP. *FASEB J.* 8:89–96
56. Lefrancois-Martinez AM, Martinez A, Antoine B, Raymondjean M, Kahn A. 1995. Upstream stimulatory factor proteins are major components of the glucose response complex of the L-type pyruvate kinase gene promoter. *J. Biol. Chem.* 270:2640–43
57. Lemaigre FP, Rousseau GG. 1994. Transcriptional control of genes that regulate glycolysis and gluconeogenesis in adult liver. *Biochem. J.* 303:1–14
58. Liu ZR, Thompson KS, Towle HC. 1993. Carbohydrate regulation of the rat L-type pyruvate kinase gene requires 2 nuclear factors: LF-A1 and a member of the c-myc family. *J. Biol. Chem.* 268:12787–95
59. Liu ZR, Towle HC. 1995. Functional synergism in the carbohydrate-induced activation of L-type pyruvate kinase gene expression. *Biochem. J.* 308:105–11
60. Lopez-Casillas F, Kim KH. 1989. Heterogeneity at the 5′ end of rat acetyl-CoA carboxylase mRNA. *J. Biol. Chem.* 264:7176–84
61. Luo X, Kim KH. 1990. An enhancer element in the house-keeping promoter for acetyl-CoA carboxylase gene. *Nucleic Acid Res.* 18:3249–54
62. MacDonald MJ. 1995. Influence of glucose on pyruvate carboxylase expression in pancreatic islets. *Arch. Biochem. Biophys.* 319:128–32
63. MacDonald MJ, Kaysen J, Moran SM, Pomije CE. 1991. Pyruvate dehydrogenase and pyruvate carboxylase. Sites of pretranslational regulation by glucose of glucose-induced insulin release in pancreatic islets. *J. Biol. Chem.* 266:22392–97
64. MacDougald OA, Jump DB. 1991. Identification of functional *cis*-acting elements within the rat liver S14 promoter. *Biochem. J.* 280:761–67
65. Majerus PW, Kilburn E. 1969. Acetyl-CoA carboxylase. The roles of synthesis and degradation in regulation of enzyme level in rat liver. *J. Biol. Chem.* 244:6254–62
66. Marie S, Diaz-Guerra MJM, Miquerol L, Kahn A, Iynedjian PB. 1993. The pyruvate kinase gene as a model for studies of glucose-dependent regulation of gene expression in the endocrine pancreatic beta-cell type. *J. Biol. Chem.* 268:23881–90
67. Marshall S, Bacote V, Traxinger RR. 1991. Discovery of a metabolic pathway mediating glucose-induced desensitization of the glucose transport system. Role of hexosamine biosynthesis in the induction of insulin resistance. *J. Biol. Chem.* 266:4706–12
68. Maury J, Issad T, Perdereau D, Gouhot B, Ferré P, Girard J. 1993. Effect of acarbose on glucose homeostasis, lipogenesis and lipogenic enzyme gene expression in adipose tissue of weaned rats. *Diabetologia* 36:503–9
69. Maury J, Kerbey AL, Priestman DA, Patel M, Girard J, Ferré P. 1995. Pretranslational regulation of pyruvate dehydrogenase activity in white adipose tissue during the suckling-weaning transition in the rat. *Biochem. J.* 311:531–35
70. McClain D, Paterson A, Ross M, Wei X, Kudlow J. 1992. Glucose and glucosamine regulate growth factor gene expression in vascular smooth muscle cells. *Proc. Natl. Acad. Sci. USA* 89:8150–54
71. Moustaid N, Sakamoto K, Clarke S, Beyer RS, Sul HS. 1993. Regulation of fatty acid synthase gene transcription. Sequences that confer a positive insulin effect and differentiation-dependent expression in 3T3-L1 preadipocytes are present in the 332 bp promoter. *Biochem. J.* 292:767–72
72. Munnich A, Lyonnet S, Chauvet D, Van Schaftingen E, Kahn A. 1987. Differential effects of glucose and fructose on liver L-type pyruvate kinase gene expression in vivo. *J. Biol. Chem.* 262:17065–71
73. Nakanishi S, Numa S. 1970. Purification of rat liver acetyl coenzyme A carboxylase and immunochemical studies on its synthesis and degradation. *Eur. J. Biochem.* 16:161–73
74. Narkewicz MR, Iynedjian PB, Ferré P, Girard J. 1990. Insulin and tri-iodothyronine induce glucokinase mRNA in primary cultures of neonatal rat hepatocytes. *Biochem. J.* 271:585–89
75. Nishimura M, Uyeda K. 1995. Purification and characterization of a novel xylulose-5-phosphate-activated protein phosphatase catalyzing dephosphorylation of fructose 6-phosphate 2

kinase:fructose 2,6-bisphosphatase. *J. Biol. Chem.* 270:26341–46
76. Olson AL, Pessin JE. 1996. Structure, function, and regulation of the mammalian facilitative glucose transporter gene family. *Annu. Rev. Nutr.* 16:235–56
77. Paulauskis JD, Sul HS. 1989. Hormonal regulation of mouse fatty acid synthase gene transcription in liver. *J. Biol. Chem.* 264:574–77
78. Pearce J. 1983. Comparative aspects of fatty acid metabolism. Fatty acid synthesis in liver and adipose tissue. *Proc. Nutr. Soc.* 42:263–71
79. Perdereau D, Narkewicz M, Coupé C, Ferré P, Girard J. 1990. Hormonal control of specific gene expression in the rat liver during the suckling-weaning transition. *Adv. Enzyme Regul.* 30:91–108
80. Prentki M, Vischer S, Glennon MC, Regazzi R, Deeney JT, Corkey BE. 1992. Malonyl-CoA and long chain acyl-CoA esters as metabolic coupling factors in nutrient-induced insulin secretion. *J. Biol. Chem.* 267:5802–10
81. Printz RL, Magnuson MA, Granner DK. 1993. Mammalian glucokinase. *Annu. Rev. Nutr.* 13:463–96
82. Prip-Buus C, Perdereau D, Foufelle F, Maury J, Ferré P, Girard J. 1995. Induction of fatty-acid-synthase gene expression by glucose in primary culture of rat hepatocytes. Dependency upon glucokinase activity. *Eur. J. Biochem.* 230:309–15
83. Prostko C, Fritz R, Klietzien R. 1989. Nutritional regulation of hepatic glucose-6-phosphate dehydrogenase. *Biochem. J.* 258:295–99
84. Rencurel F, Waeber G, Antoine B, Rocchiccioli F, Maulard P, et al. 1996. Requirement of glucose metabolism for regulation of glucose transporter type 2 (GLUT2) gene expression in liver. *Biochem. J.* 314:903–9
85. Romsos DR, Leveille GA. 1974. Effect of diet on activity of enzymes involved in fatty acid and cholesterol synthesis. *Adv. Lipid. Res.* 12:97–146
86. Saggerson ED, Greenbaum AL. 1970. The regulation of triglyceride synthesis and fatty acid synthesis in rat epididymal adipose tissue. *Biochem. J.* 119:221–42
87. Sayeski PP, Kudlow JE. 1996. Glucose metabolism to glucosamine is necessary for glucose stimulation of TGF-alpha gene transcription. *J. Biol. Chem.* 271:15237–43
88. Semenkovich CF, Coleman T, Goforth R. 1993. Physiologic concentrations of glucose regulate fatty acid synthase activity in HepG2 cells by mediating fatty acid synthase messenger RNA stability. *J. Biol. Chem.* 268:6961–70
89. Shih HM, Liu Z, Towle HC. 1995. Two CACGTG motifs with proper spacing dictate the carbohydrate regulation of hepatic gene transcription. *J. Biol. Chem.* 270:21991–97
90. Shih HM, Towle HC. 1992. Definition of the carbohydrate response element of the rat S_{14} gene. Evidence for a common factor required for carbohydrate regulation of hepatic genes. *J. Biol. Chem.* 267:13222–28
91. Shih HM, Towle HC. 1994. Definition of the carbohydrate response element of the rat S_{14} gene. Context of the CACGTG motif determines the specificity of carbohydrate regulation. *J. Biol. Chem.* 269:9380–87
92. Soncini M, Yet SF, Moon Y, Chun JY, Sul HS. 1995. Hormonal and nutritional control of the fatty acid synthase promoter in transgenic mice. *J. Biol. Chem.* 270:30339–43
93. Sudo Y, Mariash CN. 1994. Two glucose-signaling pathways in S14 gene transcription in primary hepatocytes: a common role of protein phosphorylation. *Endocrinology* 134:2532–40
94. Thompson KS, Towle HC. 1991. Localization of the carbohydrate response element of the rat L-type pyruvate kinase gene. *J. Biol. Chem.* 266:8679–82
95. Towle HC. 1995. Metabolic regulation of gene transcription in mammals. *J. Biol. Chem.* 270:23235–38
96. Traxinger R, Marshall S. 1991. Coordinated regulation of glutamine:fructose-6-phosphate amidotransferase activity by insulin, glucose and glutamine. *J. Biol. Chem.* 266:10148–54
97. Van de Werve G, Jeanrenaud B. 1984. Synthase activation is not a prerequisite for glycogen synthesis in the starved rat liver. *Am. J. Physiol.* 247:E271–75
98. Van Schaftingen E, Detheux M, Da Cunha MV. 1994. Short-term control of glucokinase activity. Role of a regulatory protein. *FASEB J.* 8:414–19
99. Van Schaftingen E, Vandercammen A. 1989. Stimulation of glucose phosphorylation by fructose in isolated rat hepatocytes. *Eur. J. Biochem.* 179:173–77
100. Vandercammen A, Detheux M, Van Schaftingen E. 1992. Binding of sorbitol 6-phosphate and of fructose 1-phosphate to the regulatory protein of liver glucokinase. *Biochem. J.* 286:253–56

101. Vaulont S, Kahn A. 1994. Transcriptional control of metabolic regulation genes by carbohydrates. *FASEB J.* 8:28–35
102. Vaulont S, Puzenat N, Levrat F, Cognet M, Kahn A, Raymondjean M. 1989. Protein binding to the liver-specific pyruvate kinase gene promoter. *J. Mol. Biol.* 209:205–19
103. Wang X, Sato R, Brown M, Hua X, Golstein J. 1994. SREBP–1, a membrane-bound transcription factor released by sterol-regulated proteolysis. *Cell* 77:53–62
104. Youn J, Youn M, Bergman R. 1986. Synergism of glucose and fructose in net glycogen synthesis in perfused rat liver. *J. Biol. Chem.* 261:15960–69
105. Zhang S, Kim KH. 1995. Glucose activation of acetyl-CoA carboxylase in association with insulin secretion in a pancreatic beta-cell line. *J. Endocrinology* 147:33–41