Induction of fatty acid synthase and S14 gene expression by glucose, xylitol and dihydroxyacetone in cultured rat hepatocytes is closely correlated with glucose 6-phosphate concentrations

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INTRODUCTION

It is now well established that the transcription of several genes belonging to the glycolytic and lipogenic pathway is stimulated in the presence of a high glucose concentration in adipocytes and hepatocytes. We have previously proposed that glucose 6-phosphate could be the signal metabolite that transduces the glucose effect. This proposal has recently been challenged and both an intermediate of the pentose phosphate pathway, xylulose 5-phosphate, and metabolites of the later part of glycolysis (3-phosphoglycerate and phosphoenolpyruvate) have been proposed. To discriminate between these possibilities, we have measured concomitantly, in primary cultures of adult rat hepatocytes, the expression of the fatty acid synthase (FAS) and S14 genes and the concentration of glucose metabolites. We have used various substrates entering at different steps of the glycolytic pathway (glucose, dihydroxyacetone) and the pentose phosphate pathway (xylitol). When compared with 5 mM glucose, 25 mM glucose induces a marked increase in both S14 and FAS gene expression, detectable as early as 2 h and peaking at 6 h. Increasing concentrations (1–5 mM) of xylitol and dihydroxyacetone in the presence of 5 mM glucose are also able to induce S14 and FAS gene expression progressively. Among the various glucose metabolites measured, glucose 6-phosphate, in contrast with xylulose 5-phosphate and metabolites of the lower part of glycolysis, is the only one that shows a clear-cut parallelism between its concentration and the degree of S14 and FAS gene expression. We conclude that glucose 6-phosphate is the most likely signal metabolite for the glucose-induced transcription of this group of genes.

MATERIALS AND METHODS

Animals

Animal studies were conducted in accordance with the French Guidelines for the Care and Use of Experimental Animals. Female Wistar rats (200–300 g body weight) from Ifa-Credo, (L’Arbresle, France) were used. They were housed in plastic cages at a constant temperature (22 °C) with light from 07:00 h to 19:00 h for at least 1 week before the experiments.

Abbreviations used: ACC, acetyl-CoA carboxylase; FAS, fatty acid synthase; L-PK, L-pyruvate kinase; T3, 3,3,5-tri-iodothyronine.

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Isolation and primary culture of hepatocytes

Hepatocytes were isolated by the collagenase method [14]. Cell viability was assessed by the Trypan Blue exclusion test and was always higher than 85%. Hepatocytes were seeded at a density of 8 x 10^6 cells per dish in 100 mm Petri dishes in minimal essential medium with Earle salts (MEM 199; Gibco BRL, Paisley, Scotland, U.K.) supplemented with 100 i.u./ml penicillin, 100 µg/ml streptomycin, 0.1% (w/v) BSA, 2% (w/v) Ultroser G (IBF, Villeneuve la Garenne, France), 100 mM dexamethasone (Sigma, St. Louis, MO, U.S.A.), 1 nM insulin (Actrapid; Novo Nordisk, Copenhagen, Denmark) and 100 mM 3,5,3',5'-tri-iodothyronine (T3) (Sigma, St. Louis, MO, U.S.A.). After cell attachment (4 h), the medium was replaced by a medium similar to the seeding medium but lacking Ultroser and albumin and containing 100 mM insulin. Hepatocytes were then cultured in the presence of various substrates, glucose (5 or 25 mM), xylitol (1, 2 or 5 mM, plus 5 mM glucose), dihydroxyacetone (1, 2 or 5 mM, plus 5 mM glucose).

Metabolite concentration assay

The concentrations of metabolites in cultured hepatocytes, including glucose 6-phosphate, fructose 6-phosphate, 3-phosphoglycerate, phosphoenolpyruvate and pyruvate, were assayed enzymically as described previously [15]. Briefly, after removing the culture medium, cells were scraped into 0.5 ml of 6% (v/v) ice-cold HClO4. After centrifugation, the acid supernatant was neutralized with KOH and an aliquot was used for enzymic spectrophotometric determination of metabolite concentrations. Xylulose 5-phosphate was assayed spectrophotometrically in a reaction using transketolase (EC 2.2.1.1) in the presence of ribose 5-phosphate, thiamine pyrophosphate and Mg2+, coupled to triose phosphate isomerase (EC 5.3.1.1) and α-glycerophosphate dehydrogenase (EC.1.1.1.8) in the presence of NADH.

Isolation of total RNA and Northern-blot hybridization

Total cellular RNA species were extracted from hepatocytes by the guanidine thiocyanate method [16]. Total RNA (20 µg) was denatured by heating at 95°C for 3 min. RNA was size-fractionated by electrophoresis on 1% (w/v) agarose gel containing 2.2 mM formaldehyde in 20 mM MOPS (pH 7.0)/50 mM sodium acetate/1 mM EDTA for 16–18 h at 50 V. RNA was then transferred and UV cross-linked to nylon membrane (Hybond-N; Amersham, Arlington Heights, IL, U.S.A.). Filter hybridizations were performed as described previously [17]. Autoradiograms of Northern blots were scanned and quantified with an image processor program.

FAS cDNA was as previously described [17]. β-actin cDNA was a gift from Dr. C. Forest (CNRS, Meudon, France). Mouse S14 cDNA was a gift from Dr. R. Planells (INSERM, Marseille, France) [18].

Statistical analysis

Results are expressed as means ± S.E.M. When quantified, FAS mRNA concentrations were normalized with respect to the β-actin hybridization signal.

RESULTS AND DISCUSSION

Glucose induces FAS expression in adult rat hepatocytes

Because the experiments we had performed previously to show a glucose effect on FAS expression were done on suckling rat hepatocytes, in a first series of experiments we tested the conditions necessary to obtain a glucose effect on FAS expression in adult rat hepatocytes.

We therefore cultured adult rat hepatocytes for 24 h in the presence of insulin, T3, and dexamethasone to achieve the full expression of glucokinase as previously described [19], and in the presence of 5 or 25 mM glucose. The expression of the FAS gene was 10-fold higher when the cells were cultured in the presence of 25 mM glucose than in the presence of 5 mM glucose, whereas β-actin expression was not affected (Figure 1). Similar results were obtained for the S14 gene expression (results not shown).

To determine the kinetics of induction of the FAS and S14 genes by glucose, hepatocytes were cultured for 18 h after attachment in the presence of 5 mM glucose (a non-inducing concentration) and in the presence of insulin, dexamethasone and T3. The medium was then replaced by a similar one except that the concentration of glucose was 25 mM. The cells were harvested at 0, 2, 4, and 6 hours after the medium change. The FAS and S14 gene expression increased 2-fold after 2 h of culture in the presence of 25 mM glucose when compared with that in 5 mM glucose, and 8–10-fold after 4 h and 6 h of culture (Figure 2).

This experiment gave us the rationale for those that followed. Because an increase in FAS and S14 expression is already detectable after 2 h in the presence of 25 mM glucose and maximal after 4 h, we decided to harvest the cells after 4 h for the assay of metabolite concentrations and after 6 h for the measurement of FAS and S14 mRNA concentrations in the same culture.

Comparison of the effect of glucose and xylitol on FAS and S14 gene expression and on metabolite concentrations

The proposal that xylulose 5-phosphate was the signal metabolite was based on the following ideas and findings [12]: (1) in some cells, 2-deoxyglucose 6-phosphate can be metabolized further and especially into pentose phosphate pathway intermediates, (2) xylitol, a precursor of xylulose 5-phosphate, is able to stimulate the transcription of a reporter gene driven by the l-PK promoter in a hepatocyte cell line (AT3F) at a low concentration (0.5 mM) without detectable changes in glucose 6-phosphate concentration; (3) in primary cultured hepatocytes, xylitol (5–10 mM) is able to induce a 6-fold increase in l-PK mRNA concentration.

Figure 1  Effects of 5 or 25 mM glucose on FAS mRNA concentration in cultured hepatocytes

Hepatocytes were isolated from female rats and plated for 4 h on dishes as described in the Materials and methods section. Then the hepatocytes were cultured in the presence of 100 nM insulin, 100 nM T3, 100 nM dexamethasone and 5 or 25 mM glucose. After 18 h, cells were harvested for RNA extraction. A representative Northern blot from three independent experiments is shown. Each lane corresponds to an independent RNA extraction.
Glucose regulation of fatty acid synthase and S14 gene expression

Figure 2  Kinetics of induction of FAS and S14 gene expression in the presence of 25 mM glucose in cultured hepatocytes

After 18 h of culture in the presence of 100 nM insulin, 100 nM T3, 100 nM dexamethasone and 5 mM glucose (G 5), cells were cultured in the presence of the hormones and 25 mM glucose (G 25) for 0, 2, 4 or 6 h and harvested for RNA extraction (for further details see the Materials and methods section).

...even in the absence of insulin (it must be pointed out, however, that in these latter experiments the xylulose 5-phosphate concentration was not measured, nor was that of glucose 6-phosphate); and (4) xylulose 5-phosphate has been shown to activate phosphatase 2A-mediated dephosphorylation [20], and this phosphatase is also involved in the dephosphorylation of transcription factors [21].

...We then compared in the same culture the effect of 5 and 25 mM glucose and 1, 2 or 5 mM xylitol (in the presence of 5 mM glucose, which represents the basal non-inducing condition) on FAS and S14 gene expression and on glucose 6-phosphate and xylulose 5-phosphate concentrations (Figure 3). A 6 h culture in the presence of 25 mM glucose induced a 9-fold increase in FAS and S14 gene expression (quantification is shown only for FAS mRNA in Figure 3); there was no detectable change in xylulose 5-phosphate concentration but a 9-fold increase in glucose 6-phosphate concentration. Xylitol (1 mM) enhanced FAS and S14 gene expression only marginally despite a 10-fold increase in xylulose 5-phosphate concentration. At 2 and 5 mM xylitol there was a further and dose-dependent increase in xylulose 5-phosphate concentration and respectively 3-fold and 10-fold increases in FAS gene expression. Interestingly, 1, 2 and 5 mM xylitol induced respectively 2-fold, 4-fold and 10-fold increases in glucose 6-phosphate concentration. This latter finding is not unexpected because metabolites from the pentose phosphate pathway can be recycled back to glycolysis. In summary, whereas there is a tight parallelism between the induction of FAS gene expression and glucose 6-phosphate concentration, the same relationship does not hold for xylulose 5-phosphate. To be sure that we were not missing a peak of xylulose 5-phosphate at later times in the presence of 25 mM glucose, we followed its concentration from 1 to 6 h after the addition of 25 mM glucose under conditions similar to that described for Figure 2. Glucose (25 mM) did not induce an increase in xylulose 5-phosphate concentration at any sampling time. In contrast there was a rapid and sustained increase in glucose 6-phosphate concentration (results not shown).

Clearly, in our hands, xylulose 5-phosphate does not qualify as a signal metabolite for FAS and S14 induction. In contrast with glucose 6-phosphate, it does not vary either in proportion to the medium glucose concentration or in proportion to the FAS gene expression.

One of the arguments to suggest that a metabolite of the pentose phosphate pathway was the signal metabolite stemmed from the fact that 2-deoxyglucose 6-phosphate, when accumulating into adipocytes or INS1 cells, could become a significant

Figure 3  Effects of 1, 2 or 5 mM xylitol and 5 or 25 mM glucose on FAS and S14 mRNA and metabolite concentrations in cultured hepatocytes

After 18 h culture in the presence of 100 nM insulin, 100 nM T3, 100 nM dexamethasone and 5 mM glucose (G 5), cells were cultured in the presence of 5 or 25 mM glucose (G 5, G 25), and 1, 2 or 5 mM xylitol (Xyl) plus 5 mM glucose. Cells were harvested after 1 h for metabolite determination or after 6 h for FAS and S14 mRNA extraction. Top panel: representative Northern blot of three independent experiments. Bottom panels: quantification of FAS mRNA corrected for β-actin expression. Glucose 6-phosphate and xylulose 5-phosphate were determined in triplicate for each condition in each culture. The results are expressed as a percentage of the values obtained in the presence of 5 mM xylitol/5 mM glucose, and as means ± SEM for three independent cultures. Concentrations (nmol per 10⁶ hepatocytes) of metabolites in the presence of 5 mM xylitol/5 mM glucose were: glucose 6-phosphate, 2.05 ± 0.31; xylulose 5-phosphate, 1.33 ± 0.25.
owing to the presence of enzymes of the gluconeogenic pathway. 

generate intermediates of the earlier part of glycolysis in the liver 

reactions of glycolysis, although dihydroxyacetone can also 

colytic pathway at the level of dihydroxyacetone phosphate after 

presence of 5 mM glucose). Dihydroxyacetone enters the gly-

25 mM glucose or 1, 2 or 5 mM dihydroxyacetone (in the 

of glycolysis, hepatocytes were incubated in the presence of 

To test the potential importance of metabolites of the later part 

Potential involvment of metabolites of the later part of glycolysis 

Figure 4 Effects of 1, 2 or 5 mM dihydroxyacetone and 5 or 25 mM 

The experimental procedure was the same as described in the legend to Figure 3 except that 

cells were incubated in the presence of dihydroxyacetone instead of xylitol. A representative 

Northern blt from three independent experiments is shown. Abbreviations: G 5, 5 mM glucose; 

G 25, 25 mM glucose; DHA 1, 1 mM dihydroxyacetone; DHA 2, 2 mM dihydroxyacetone; DHA 

5, 5 mM dihydroxyacetone.

substrate for glucose-6-phosphate dehydrogenase, despite the 

fact that its dehydrogenation rate was 1/20 that of glucose 

6-phosphate. However, it was shown previously that glucose-6- 

phosphate dehydrogenase mRNA and activity are low in the 

liver and adipose tissue of suckling rat or starved adult rats 

[22–25] in which glucose is very efficient at inducing FAS and l- 

PK gene expression. Moreover the decarboxylation of glucose 6- 

phosphate is extremely low in suckling rat adipose tissue, in 

which 2-deoxyglucose induces FAS gene expression [26]. Finally, 

after weaning on a high-carbohydrate diet, or after refeeding a 

starved rat with a high-carbohydrate diet, the increase in glucose- 

6-phosphate dehydrogenase mRNA does not precede the ac-

cumulation of FAS mRNA but has a similar time course [25]. 

This strongly suggests that a metabolite of the pentose phosphate 

pathway is not involved in the induction of the FAS gene by 

glucose. In fact the effects of xylitol on FAS gene expression can 

be explained entirely by the increase in glucose 6-phosphate. This 

could certainly also explain the effect of xylitol on l-PK gene 

expression in cultured hepatocytes [12]. 

In their study on AT3-F cells, Doiron et al. [12] stated that the 

3-fold increase in l-PK transcription rate occurred without 

detectable change in glucose 6-phosphate concentration. How-

ever, metabolite measurements were performed after 20 min of 

culture in the presence of xylitol, whereas chloramphenicol 

acetyltransferase reporter activities were performed after 36 h. 

It is therefore difficult to be sure that glucose 6-phosphate con-

centration was not increased at later time points.

Potential involvment of metabolites of the later part of glycolysis 

in the induction of FAS and S14 gene expression by glucose 

To test the potential importance of metabolites of the later part 

glycolysis, hepatocytes were incubated in the presence of 

25 mM glucose or 1, 2 or 5 mM dihydroxyacetone (in the 

presence of 5 mM glucose). Dihydroxyacetone enters the gly-

colytic pathway at the level of dihydroxyacetone phosphate after 

its phosphorylation by a triokinase, thus bypassing the first 

reactions of glycolysis, although dihydroxyacetone can also 

generate intermediates of the earlier part of glycolysis in the liver 

owing to the presence of enzymes of the gluconeogenic pathway.

Dihydroxyacetone at 1, 2 and 5 mM and in the presence of 

5 mM glucose induced respectively 2, 4 and 8-fold increases in 

FAS gene expression and a parallel increase in S14 gene 

expression (quantification was performed only for FAS mRNA) 

(Figure 4 and Table 1). When considering metabolites from the 
later part of glycolysis, 3-phosphoglycerate, pyruvate and phos-

phoenolpyruvate, their concentrations varied at most 2-fold 

under the various conditions used but bore no relationship to 

FAS or S14 gene expression. In contrast, there was again a good 

parallelism between the effect of dihydroxyacetone on FAS gene 

expression and on glucose 6-phosphate concentration because 1, 

2 and 5 mM dihydroxyacetone increased its concentration re-

spectively 3-fold, 4-fold and 6-fold (Table 1).

In the study by Kang et al. [13] on the relationship between 
glycolytic metabolite concentrations and l-PK gene induction, 

the protocol used might have obscured any potential relationship 

between a given metabolite and l-PK gene induction, 

Indeed, hepatocytes were cultured for 16 h in the absence of insulin, 
a condition that should strongly limit the expression and 
activity of glucokinase. Thereafter a high glucose concentration in the 

medium was added together with insulin, and the metabolites 
were measured during the first 6 h. This was probably an 

insufficient length of time for insulin to reinduce glucokinase 

expression and glucose phosphorylation. In contrast, l-PK 
mRNA were measured after 30 h of culture, a sufficient duration 
to promote glucose phosphorylation. It is thus difficult to safely 
conclude a role for a given metabolite in l-PK gene induction by 

glucose in that study.

Glucokinase and gene induction by glucose 

In hepatocytes, as stated previously, phosphorylation of glucose 
into glucose 6-phosphate is achieved through the action of 
glucokinase; this represents an obligatory step for glucose action 
on gene expression. It has recently been demonstrated that 
glucokinase and its regulatory protein, which could act as an 
anchor for this enzyme [27], are localized in the nucleus in the 
presence of 5 mM glucose, but migrate into the cytoplasm when 
the glucose concentration is high [28,29]. It could then be

Table 1 Effects of glucose and dihydroxyacetone on FAS mRNA and 
glycolytic metabolite concentrations in cultured hepatocytes 

<table>
<thead>
<tr>
<th>Condition</th>
<th>Fas mRNA</th>
<th>G6P</th>
<th>F6P</th>
<th>3PG</th>
<th>PEP</th>
<th>Pyruvate</th>
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<tr>
<td>G 5</td>
<td>11 ± 3</td>
<td>13 ± 5</td>
<td>43 ± 10</td>
<td>59 ± 10</td>
<td>78 ± 19</td>
<td>47 ± 3</td>
</tr>
<tr>
<td>G 25</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>G 5 + DHA 1</td>
<td>27 ± 9</td>
<td>55 ± 18</td>
<td>90 ± 23</td>
<td>82 ± 9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>G 5 + DHA 2</td>
<td>50 ± 8</td>
<td>56 ± 9</td>
<td>111 ± 31</td>
<td>86 ± 18</td>
<td></td>
<td></td>
</tr>
<tr>
<td>G 5 + DHA 5</td>
<td>91 ± 26</td>
<td>76 ± 13</td>
<td>110 ± 12</td>
<td>75 ± 29</td>
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</tbody>
</table>

After 18 h of culture in the presence of 100 mM insulin, 100 mM T₈, 100 mM dexamethasone and 
5 mM glucose, cells were cultured in the presence of 5 or 25 mM glucose, or 1, 2, and 
5 mM dihydroxyacetone plus 5 mM glucose. Cells were harvested after 1 h for metabolite 
determination or after 6 h for FAS mRNA determination (for further details see the Materials 
and methods section). Glucose 6-phosphate (G6P), fructose-6-phosphate (F6P), 3-phos-
phoglycerate (3PG), phosphoenolpyruvate (PEP) and pyruvate concentrations were determined 
in triplicate for each condition in each culture. Abbreviations: G 5, 5 mM glucose; G 25, 25 mM 
glucose; DHA 1, 1 mM dihydroxyacetone; DHA 2, 2 mM dihydroxyacetone; DHA 5, 5 mM 
dihydroxyacetone. The results are expressed as percentages of the values obtained in the 
presence of 25 mM glucose and as means ± S.E.M. for three independent cultures. Concentrations (nmol per 10⁶ hepatocytes) of metabolites in the presence of 25 mM glucose were: G6P, 1.38 ± 0.18; F6P, 1.25 ± 0.39; 3PG, 1.43 ± 0.20; PEP, 0.60 ± 0.06; pyruvate, 7.62 ± 0.7.

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>G 5</th>
<th>G 25</th>
<th>G 5 + DHA 1</th>
<th>G 5 + DHA 2</th>
<th>G 5 + DHA 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>F6P</td>
<td>0.19</td>
<td>0.20</td>
<td>0.24</td>
<td>0.25</td>
<td>0.26</td>
</tr>
<tr>
<td>3PG</td>
<td>1.43</td>
<td>1.25</td>
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<tr>
<td>PEP</td>
<td>0.60</td>
<td>0.36</td>
<td>0.45</td>
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<td>0.60</td>
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<tr>
<td>Pyruvate</td>
<td>7.62</td>
<td>10.60</td>
<td>12.35</td>
<td>14.40</td>
<td>16.29</td>
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</table>
questioned whether this nuclear localization, leading to a potential production of glucose 6-phosphate inside the nucleus by glucokinase on its way to the cytoplasm, would be more efficient than cytoplasmic glucose 6-phosphate production for glucose-responsive gene induction.

Clearly, if one compares in the present study the relationship between the concentration of glucose 6-phosphate and the extent of induction of S14 and FAS genes by glucose, it is similar to that obtained with substrates that can generate glucose 6-phosphate without the intervention of glucokinase, such as xylitol and dihydroxyacetone. This suggests that the nuclear production of glucose 6-phosphate is not a major prerequisite for gene induction by glucose.

Concluding remarks

The bulk of evidence from the present and previous studies suggests that glucose 6-phosphate is the most likely signal metabolite involved in the regulation of glycolytic and lipogenic enzyme expression: (1) glucose itself does not qualify because if it is to act as a signal for gene induction, its phosphorylation into 2-deoxyglucose 6-phosphate, mimicks expression, and 2-deoxyglucose, whose metabolism stops after the pathway, their concentration does not vary in parallel with gene expression; (2) with regard to other metabolites from the glycolytic pathway, their own and metabolites resulting from their metabolism in the citric acid cycle are not involved; (3) with regard to metabolites involved in the regulation of glycolytic and lipogenic enzymes, it is to act as a signal for gene induction, its phosphorylation into glucose 6-phosphate is not a likely candidate and it must be emphasized that the expression of these genes can be induced in conditions in which the activity of the pentose phosphate pathway is very low; (4) with regard to other metabolites from the glycolytic pathway, their concentration does not vary in parallel with gene expression, and 2-deoxyglucose, whose metabolism stops after its phosphorylation into 2-deoxyglucose 6-phosphate, mimicks totally (adipose tissue) or partly (pancreatic -cell line) the effect of glucose on lipogenic or glycogenic gene expression; and (5) in contrast with other metabolites, the intracellular concentration of glucose 6-phosphate varies in parallel with the intensity of gene induction and its kinetic pattern fits in vivo and in vitro with the pattern of gene induction.

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REFERENCES


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