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Evidence for Suppression of Hepatic Glucose-6-Phosphatase with Carbohydrate Feeding

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SUMMARY

The mechanism by which exogenous glucose stimulates the incorporation of hepatic glucose-6-phosphate into glycogen in fasted rats has not been clearly delineated. We gave glucose intragastrically over a 3.5-h period during which liver glycogen was deposited at linear rates. Simultaneous primed continuous infusion of [2-^{3}H] or [3-^{3}H]glucose established that under these conditions absolute carbon flow through hepatic glucose-6-phosphatase was greatly suppressed.

After 1 h, hepatic [UDP-glucose] and [glucose-6-phosphate] had fallen by 50–60% and the former remained low throughout the experiment. By contrast, [glucose-6-phosphate] rebounded to its initial value by 2 h and remained at this level during the subsequent hour.

We interpret the data as follows. Exogenous glucose, in addition to acting as a precursor of liver glucose-6-phosphate, causes diversion of the latter away from free glucose formation and into glycogen synthesis. The fall in [UDP-glucose] is in accord with a glucose-induced activation of glycogen synthase, as proposed by Hers (Annu. Rev. Biochem. 1976; 45:167–89.). However, the fall-rise sequence of glucose-6-phosphate concentration constitutes the first direct evidence in vivo for simultaneous inhibition at the level of glucose-6-phosphatase. DIABETES *33*:192–195, February 1984.

There is good evidence that in the rat, and probably also in man, the bulk of glucose ingested after a fast is first metabolized to a 3-carbon compound (probably lactate) before incorporation into liver glycogen.[1,2] Implicit to this construct is the requirement that carbon flow through the gluconeogenic pathway in liver continue for at least several hours after the administration of glucose in the fasted state. Since under these circumstances glycogen deposition is brisk while net hepatic glucose output is suppressed, it is probable that glucose-6-phosphate (glucose-6P) carbon is diverted away from free glucose and into glycogen formation. How this crucial metabolic switch is effected constitutes a controversial issue and represents a major void in our understanding of liver intermediary metabolism.

One possible mechanism, advocated by Hers and colleagues,[3–7] would be that glucose brings about the activation of glycogen synthase secondary to inhibition of glycogen phosphorylase. This would be expected to accelerate carbon flow into glycogen with a concomitant fall in tissue levels of UDP-glucose and glucose-6P. Since the latter is normally present in liver at concentrations far below its apparent Km for glucose-6-phosphatase (glucose-6Pase) a further reduction in its concentration was considered to be sufficient to cause diminished production of free glucose (i.e., simultaneous, direct inhibition of glucose-6Pase need not be invoked). This formulation, often referred to as the "pull" hypothesis, emerged in large part from short-term experiments (generally 1–10 min) in which glucose loads were administered to fed rodents.[3–5] An opposing view, namely, that the primary effect of glucose is to cause suppression of the glucose-6Pase reaction has been expressed by El-Refai and Bergman,[8] in keeping with the earlier suggestion of Stadtman.[9] However, this concept, the so-called "push" hypothesis, was derived mainly from theoretical considerations.[8]

In an attempt to clarify these issues we administered glucose to fasted rats and measured certain key parameters over the following 3 h. In this time period, liver glycogen rises from negligible to high levels.[1] The rationale was as follows. If it could be established that infusion of glucose reduced absolute carbon flux through the glucose-6Pase step (in addition to attenuating net hepatic glucose output) at a time when liver glycogen synthesis was active, directional changes in the tissue level of metabolites such as UDP-glucose, glucose-6P, fructose-6P, and fructose-1,6-bisphosphate should provide insight into operative mechanisms. The data outlined below support our earlier speculation[1] that both

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"pull" and "push" systems play important roles and provide the first in vivo support for direct inhibition of glucose-6Pase in the postprandial state.

EXPERIMENTAL PROCEDURES

Animals. Male Sprague-Dawley rats weighing 100–160 g were used. They were maintained on a high-sucrose, low-fat diet as described previously[10] and were housed in a room with lighting from 3:00 p.m. to 3:00 a.m. At 1:00 p.m. on day 1 animals were lightly anesthetized with ether and surgically fitted with intragastric, femoral artery and femoral vein catheters. They were then placed in restraining cages with water but no food available and left in a dark, quiet room until experiments began at 9:00 a.m. on day 2 (i.e., they were fasted for about 20 h).

Glucose turnover studies. Animals received water or glucose (84 or 167 mg/100 g body wt/h) intragastrically for a period of 210 min.[1] At 90 min a primed continuous infusion of [2-^{3}H] or [3-^{3}H]glucose was given intravenously and arterial blood samples were taken for analysis at 120, 150, 180, and 210 min. As shown previously,[1] a steady state both for plasma glucose concentration and specific activity was generally achieved by 120 min; the 120–210-min interval was used for calculation of endogenous glucose production rates. The formula employed was (cpm/min of [^{3}H]glucose infused ÷ cpm/μmol in circulating glucose) minus the μmol/min of exogenous glucose given intragastrically. Livers were removed at the termination of experiments for analysis of glycogen.[11]

Studies on liver metabolite levels. Animals received water or glucose (167 mg/100 g body wt/h) intragastrically. At the indicated times they were anesthetized with a minimum of stress (to avoid spurious perturbations of hepatic metabolite levels) by the intravenous administration of pentobarbital (8 mg/100 g body wt) and removed from their restraining cages. The abdomen was opened and the liver was rapidly freeze-clamped between aluminum plates at the temperature of liquid N_2. The time elapsed between injection of anesthetic and freezing of the liver was generally no more than 30 s. Livers were pulverized in liquid N_2 and extracted with perchloric acid. Neutral extracts were analyzed for UDP-glucose, glucose-6P, fructose-6P, and fructose-1,6-bisphosphate by conventional enzymatic techniques.

Materials. [2-^{3}H] and [3-^{3}H]glucose were from New England Nuclear (Boston, Massachusetts) and were estimated to be >99% pure when analyzed by the method of Mills et al.[12] Other materials were of the highest purity commercially available.

RESULTS

In the present experiments endogenous glucose production was assessed in fasted rats receiving a primed continuous infusion of [2-^{3}H] or [3-^{3}H]glucose intravenously together with water (basal), a low glucose load (84 mg/100 g body wt/h) or a high glucose load (167 mg/100 g body wt/h) intragastrically. The validity of the procedure requires that certain conditions be fulfilled. First, during the period used for calculation an isotopic steady state for circulating glucose should have been achieved. This was closely approximated in all cases (data not shown; see also ref. 1). Second, during the metabolism of glucose there must be irreversible loss of label. This requirement was also met since ^{3}H from both the 2 and 3 positions of glucose is lost to body water during the process of glycolysis, thus avoiding errors due to recycling of lactate. Third, to obtain the rate of endogenous glucose production during the administration of intragastric glucose the rate of entry of exogenous glucose into the circulating glucose pool must be known (since this value is subtracted from the calculated rate of total glucose turnover). We had previously assumed[1] that under our experimental conditions the rate of glucose entry closely approximates the intragastric infusion rate (because of the known rapidity of glucose absorption from the gut). The validity of this assumption was established in the present studies by the fact that calculated rates of glucose production in rats receiving a high glucose load intragastrically were essentially the same whether the tracer glucose ([3-^{3}H]glucose in this case) was given intravenously or intragastrically (0.6 ± 0.2 and 0.8 ± 0.4 μmol/min/100 g body wt, respectively; N = 6–8). In addition, analysis of intestinal content at the termination of experiments revealed <1% retention of the administered glucose.

As expected, both the low and high glucose loads resulted in marked glycogen deposition in the liver despite the fact that circulating glucose levels rose only to the region of 7–8 mM (Table 1). Although the glycogen values shown refer only to the 210-min time point it is known that under these

TABLE 1
Effect of exogenous glucose on apparent rates of endogenous glucose production in fasted rats

Tracer infused	Intragastric infusion	Mean plasma glucose concentration (120–210 min) (mM)	Calculated endogenous glucose production (120–210 min) (μmol/min/100 g body wt)	Liver glycogen at 210 min (mg/g wet wt)
[2-^{3}H]Glucose	Water	5.4 ± 0.1 (7)	8.4 ± 0.5 (7)	ND
	Low glucose	7.4 ± 0.1 (6)	4.8 ± 0.4 (6)	19.4 ± 2.8 (6)
	High glucose	8.5 ± 0.2 (18)	2.9 ± 0.4 (18)	28.4 ± 1.1 (18)
[3-^{3}H]Glucose	Water	4.7 ± 0.1 (9)	6.3 ± 0.3 (9)	ND
	Low glucose	7.4 ± 0.1 (9)	2.6 ± 0.5 (9)	19.2 ± 2.4 (3)
	High glucose	8.3 ± 0.4 (14)	0.2 ± 0.1 (14)	27.6 ± 1.3 (7)

Fasted rats received water, low glucose (84 mg/100 g body wt/h), or high glucose (167 mg/100 g body wt/h) intragastrically from zero time. After 90 min, a primed continuous infusion of [2-^{3}H] or [3-^{3}H]glucose was given intravenously and apparent endogenous glucose production rates were calculated as described in EXPERIMENTAL PROCEDURES. Initial liver glycogen content was generally <1 mg/g. Values are means ± SEM for the number of animals shown in parentheses. ND: not determined.

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experimental conditions hepatic glycogen synthesis occurs at a linear rate over the time period studied.[1] Also confirmed in Table 1 is the fact that endogenous glucose production,* as measured using [3-^{3}H]glucose, is progressively suppressed with increasing glucose loads.[1] It should be noted that these values refer only to net glucose production, i.e., they provide no information on absolute carbon flow through the hepatic glucose-6Pase reaction. In other words, it is theoretically possible that under conditions where net hepatic glucose output is zero futile cycling of glucose might be extensive because of the simultaneous operation in liver of glucokinase and glucose-6Pase.[13] Such cycling would result in little loss of ^{3}H from [3-^{3}H]glucose because the activities of hexose phosphate isomerase and glucose-6Pase greatly exceed those of phosphofructokinase and the subsequent enzymes of glycolysis (glucose must be metabolized to the triose phosphate level before ^{3}H is released from carbon 3). For this reason a parallel series of experiments was carried out using [2-^{3}H] glucose as tracer. In this case the operation of a glucose → glucose-6P → glucose cycle in liver will result in loss of ^{3}H because of the rapid equilibration of glucose-6P and fructose-6P.[13]† However, release of ^{3}H due to whole body glucose metabolism should be similar with both isotopes of glucose.

As seen from the table, calculated rates of glucose production were higher when [2-^{3}H]glucose replaced [3-^{3}H]glucose as the infused tracer, suggesting that significant intrahepatic cycling of glucose was occurring. More significant in terms of the goals of this study was the observation that even in the experiments with [2-^{3}H]glucose, the low and high glucose loads suppressed apparent endogenous glucose production by some 43% and 65%, respectively. Clearly, absolute carbon flux through hepatic glucose-6Pase had been severely restricted.

Figure 1 shows the profile of liver glucose-6P and UDP-glucose levels when fasted rats received water or the high glucose load intragastrically. For reasons not clear the concentration of UDP-glucose fell by 28% during the first hour of water infusion,‡ but thereafter returned to basal values (Figure 1B). With glucose administration, however, [UDP-glucose] fell by 60% at 1 h and remained at this reduced level throughout the experiment. A different response to glucose was seen for [glucose-6P] (Figure 1A). This was depressed by 46% at 1 h but then rebounded such that basal levels had been restored during the 2–3-h interval. Concentrations of fructose-6P and fructose-1,6-bisphosphate (about 15–20 nmol per g liver) were not significantly affected by the glucose infusion (not shown).

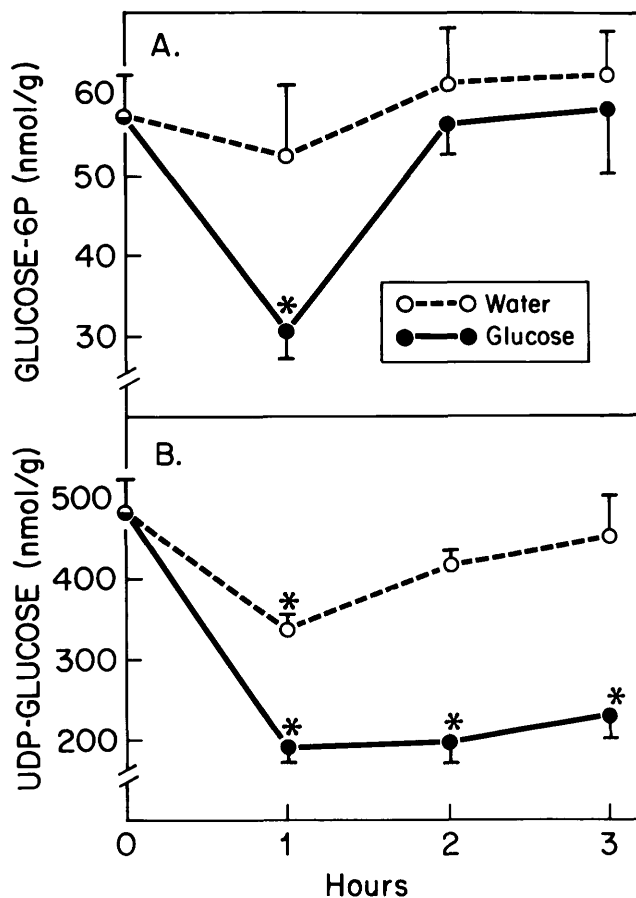

FIGURE 1. Response of liver UDP-glucose and glucose-6P levels to glucose infusion in fasted rats. Fasted rats received water or glucose (167 mg/100 g body wt/h) intragastrically. At the indicated times livers were taken for analysis of metabolites as described in EXPERIMENTAL PROCEDURES. Values are means ± SEM for 5–10 animals at each time point. Asterisks indicate significant difference from zero time value ($P < 0.001$).

DISCUSSION

In a previous study[1] the following events were documented in fasting rats given exogenous glucose loads similar to those used in the present work. First, liver glycogen was deposited at a linear rate for at least 3 h after glucose administration. Second, the bulk of this glycogen was formed by a pathway necessitating continued carbon flow through the gluconeogenic machinery of the liver. Third, net hepatic glucose production was suppressed. It seemed likely that, in addition to acting as a precursor of liver glucose-6P (both direct and indirect), glucose also effected the diversion of this intermediate away from free glucose and into glycogen formation. This conclusion hinged on the assumption that glucose loading not only damped net hepatic glucose output but also diminished absolute carbon flow through the glucose-6Pase reaction. The present work clearly establishes that such was the case.

How does glucose administration bring about this switch in hepatic glucose-6P metabolism? According to Hers and colleagues[6,7] the answer lies in the ability of glucose to bind to glycogen phosphorylase a, which in turn initiates the following sequence of events: conversion of phosphorylase a into the less active phosphorylase b → deinhibition of glycogen synthase phosphatase → conversion of glycogen synthase b into the more active glycogen synthase a → accelerated glycogen synthesis with concomitant reduction in the tissue levels of UDP-glucose and glucose-6P.

*We assume that the major site of endogenous glucose production is the liver.
†This is borne out by the following experiment. Fasted rats were infused with [2-^{3}H] or [3-^{3}H]glucose intragastrically for 2 or 3 h at a rate of 167 mg/100 g body wt/h (sufficient to bring the specific activity of circulating glucose to within 80–100% of that of the administered glucose by the 1-h time point). However, the relative specific activity of liver glycogen-glucose at 2 and 3 h was only 12% and 14.7%, respectively, in animals receiving [3-^{3}H]glucose, and fell to 5.1% and 7.9%, respectively, when [2-^{3}H]glucose was infused (averages of 2–4 animals at each time point).
‡Unexpected changes in the circulating levels of certain hormones have also been reported after intragastric water infusion in man.[14]

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The fall in [glucose-6P] is seen as the sole factor responsible for the diminished output of free glucose from the liver under these conditions.[6,7] Yet on the basis of computer modeling El-Refai and Bergman[8] arrived at an opposite prediction, namely, that glucose loading of fasted animals should cause an increase in hepatic [glucose-6P], which in turn would serve to push glucose carbon into glycogen. A glucose-induced inhibition of glucose-6Pase was considered the most plausible mechanism; simultaneous activation of glycogen synthase, with or without phosphorylase suppression, was felt to be unnecessary.

Our findings suggest that the situation is more complicated and that in fact both "pull" and "push" mechanisms are operative depending on the time interval examined after glucose administration. The early fall in hepatic UDP-glucose and glucose-6P levels would support the Hers mechanism as the initial event. However, the subsequent rebound in [glucose-6P] to basal levels at a time when glycogen synthesis was active while carbon flow through glucose-6Pase was greatly diminished (the 2–3-h interval) indicates that an additional mechanism came into play. We submit that this entails direct inhibition of glucose-6Pase. Furthermore, the conclusion that the failure of [glucose-6P] to rise above its basal level in the face of inhibition of glucose-6Pase resulted from disproportionate activation of glycogen synthase relative to the capacity of UDP-glucose pyrophosphorylase, as indicated by the sustained lowering of [UDP-glucose], seems warranted.

Alvares and Nordlie, in an attempt to reconcile an apparent discrepancy between the combined activities of glucokinase plus hexokinase and observed rates of glucose uptake in the perfused rat liver, also suggested a regulatory role for glucose-6Pase.[15] In their view, glucose-6Pase, operating in reverse with various phosphoryl donors, constitutes a supplementary mechanism for glucose phosphorylation. We do not dispute the phosphotransferase capacity of glucose-6Pase demonstrable in vitro. However, in light of more recent studies[16] the efficient operation of this system in vivo seems questionable, particularly at glucose concentrations in the 7–8-mM range as seen here. Accordingly, we favor the simpler view that most of the glucose-6P formed in liver is derived from the gluconeogenic pathway both in the fasted and immediate postprandial states and that in the latter a direct suppressor of the conventional glucose-6Pase system is generated.

To speculate on the nature or mechanism of action of the putative glucose-induced inhibitor of hepatic glucose-6Pase would be premature at this stage. From the work of Arion and colleagues[16] it appears that the phosphohydrolase itself is a rather nonspecific enzyme that resides within the lumen of the endoplasmic reticulum. Specificity for glucose-6P is conferred by the presence on the membrane of a translocase that transports substrate from the extra- to intramicrosomal compartment. That the translocase rather than the phosphohydrolase itself is subject to metabolic regulation seems to us intuitively attractive. Studies to demonstrate this are in progress.

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