Rapid Publication

Time Course and Significance of Changes in Hepatic Fructose-2,6-Bisphosphate Levels During Refeeding of Fasted Rats

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Abstract. The time course of changes in hepatic fructose-2,6-bisphosphate (F-2,6-P_2) and glycogen content was examined in fasted rats infused with glucose intragastrically or allowed to eat a chow diet ad lib. Initial values for the two parameters were ~0.4 nmol/g and 2 mg/g of tissue, respectively. Contrary to what might have been expected on the basis of reported studies with hepatocytes exposed to glucose (i.e., a rapid elevation of F-2,6-P_2), the rise in F-2,6-P_2 levels in vivo was a late event. It began only 4–5 h after glucose administration or refeeding, at which time glycogen content had reached ~35 mg/g of tissue. Thereafter, [F-2,6-P_2] climbed rapidly, attaining fed values in the region of 10 nmol/g as glycogen stores became maximal (~60 mg/g of tissue).

Although the biochemical basis for these changes is still unclear, the delayed increase in [F-2,6-P_2] is entirely consistent with the fact that much of the glycogen deposited in liver in the early postprandial phase is gluconeogenic in origin. The later rise in [F-2,6-P_2] likely represents a key signal for the attenuation of gluconeogenic carbon flow into glycogen as the latter approaches repletion levels.

Introduction

The question of how the mammalian organism converts exogenous glucose into liver glycogen when a fast is terminated has become a subject of renewed interest. The reason stems from recent observations, which were made both in experimental animals and in man, that point to the inadequacy of the conventional, direct pathway (i.e., glucose → glucose-6-P → glucose-1-P → UDPG → glycogen) to account quantitatively for the rate of hepatic glycogen deposition during the fasted to refed transition (1–3). It now appears that the operative mechanism is largely indirect and involves the initial metabolism of glucose at some unidentified site(s) to a three carbon intermediate (probably lactate), with the latter rather than glucose itself, serving as the proximate precursor of liver glycogen (2, 4, 5).[1] Presumably, amino acids are also contributory.

A central feature of this formulation is that carbon flow through the gluconeogenic reactions of liver is not acutely suppressed upon glucose ingestion (as is widely believed), but must remain active for at least several hours into the postprandial period. Accordingly, a number of intriguing questions are raised. For example, since hepatic glucose output is attenuated with glucose loading, how is gluconeogenically derived glucose-6P diverted away from free glucose formation and into the pathway of glycogen formation? This issue has been addressed elsewhere (12). The subject of the present communication has to do with the role of fructose-2,6-bisphosphate (F-2,6-P_2)[2] in the regulation of hepatic glucose metabolism. The liver content of F-2,6-P_2 is much higher in fed than in

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1. Interestingly, the early studies of Boxer and Stetten (6) also pointed to the operation of an indirect mechanism for the glucose-to-liver glycogen conversion. However, its contribution relative to the direct pathway was not firmly established at the time and, in fact, was subsequently judged by others to be minimal (7–11). As noted elsewhere (5), we suspect that much of the confusion in this area has probably arisen from differences in experimental design used by the various laboratories. On the basis of recent studies (1–5), we believe that, under normal refeeding conditions, the indirect pathway makes a substantial, perhaps major, contribution to overall hepatic glycogen synthesis.

2. *Abbreviations used in this paper:* F-2,6-P_2, fructose-2,6-bisphosphate.

fasted rats (13–15), a pattern consistent with its known ability to activate phosphofructokinase-1 and to inhibit fructose-1,6-bisphosphatase (F-1,6-P_2ase) (16–18). The prediction from in vitro studies is that the low fasting level of hepatic F-2,6-P_2 should rise acutely upon exposure of the tissue to elevated concentrations of glucose and insulin (19–22). But if this were to occur in vivo in response to refeeding after a fast, a paradox is evident. How could the reaction sequence: lactate → glucose-6-P → glycogen operate efficiently in the face of a high F-2,6-P_2 concentration?

To clarify this point, we infused glucose intragastrically into fasted rats and examined the temporal sequence of changes in the plasma glucose concentration and liver content of glycogen and F-2,6-P_2. The same parameters were measured in fasted rats that were allowed to eat a solid diet ad lib. The results establish that the rise in liver F-2,6-P_2 level is in fact a late event, beginning only after several hours of glucose administration or refeeding. Attainment of high, fed values appears to coincide with tissue glycogen repletion. Thus, it seems likely that the delayed increase in hepatic F-2,6-P_2 concentration is a key factor in allowing the operation of the indirect pathway for glycogen synthesis in the immediate postprandial state.

Methods

Animals. Male Sprague-Dawley rats weighing 100–160 g were used. They were maintained either on a high sucrose-low fat diet (diet A) as described previously (23) or on regular lab chow (Rodent Blox #8604-00; Wayne Pet Food Division, Chicago, IL) (diet B) with lighting from 3:00 pm to 3:00 am.

Infusion studies. Animals maintained on diet A were fitted with intragrastric, intravenous, and arterial catheters at 1:00 pm on day 1. They were then placed in restraining cages with water but no food available. At 9:00 am on day 2, i.e., after a 20-h fast, a glucose solution was infused intragastrically at a rate of 20 μl/min to deliver 167 mg of glucose/100 g body wt per h. At the indicated times, an arterial blood sample was taken for analysis of plasma glucose using a conventional glucose oxidase method. Immediately thereafter, anesthesia was rapidly induced (<15 s) with intravenous sodium pentobarbital (7.5 mg/100 g body wt) and the liver was quickly removed and freeze-clamped (within 1 min).

Refeeding studies. On day 1, animals maintained on diet B were fitted with a rodent tethering harness No. 510 (Spalding Medical Products, Arroyo Grande, CA) and placed in individual cages to adapt to the device. On day 3, a PE-10 polyethylene catheter was inserted into a femoral vein. The free end of the tubing was fed subcutaneously across the dorsal region and emerged through a small incision at the back of the neck. It was then passed through the central channel of the tethering lead and the end was sealed. At 3:00 pm on day 4, food was removed from the cages. At 9:00 am on day 5, the food was replaced and the animals were allowed to eat ad lib. At intervals thereafter, they were anesthetized with intravenous sodium pentobarbital and the livers were quickly removed as described above. A separate series of rats was used to obtain arterial blood samples. Using this procedure, it was possible to circumvent handling and injection of the animals, and, thus, to minimize spurious stress-related changes in tissue F-2,6-P_2 levels.

Analytical procedures. After pulverizing the liver under liquid N_2, a portion was taken for analysis of glycogen (24). The remainder was used for extraction of F-2,6-P_2 using a slight modification of the procedure described by Hue et al. (14). Thus, 500 mg of powdered tissue was homogenized for 5 min at 80°C in 3 ml of medium containing 10 mM Hepes (pH adjusted to 9.25 at room temperature), 50 mM KF, and 2 mM EGTA. The resultant mixture was immediately cooled in ice and, after centrifugation at 27,000 *g* for 10 min, the supernatant was analyzed for F-2,6-P_2 content. The assay procedure adopted was basically that used by Uyeda and co-workers (20, 25), but with the following modifications. First, the rabbit muscle phosphofructokinase-1 was obtained as an ammonium sulphate suspension from Boehringer-Mannheim, Indianapolis, IN. The ammonium sulphate was replaced by a buffer containing 50 mM Tris-phosphate (pH 8), 2 mM dithiothreitol, 1 mM EDTA, and 20% (wt/vol) glycerol by repeated centrifugation and washing using an Amicon YMT membrane filtration unit (Amicon Corp., Danvers, MA). Second, the final concentrations of fructose-6-P and ATP in the assay mixture were 1.1 and 4.5 mM, respectively. A standard curve over the range of 2 to 40 pmol of F-2,6-P_2 was constructed for each series of samples tested. The method routinely yielded 95–97% recovery of authentic F-2,6-P_2 added before the liver extraction step.

Materials. Enzymes were purchased from Boehringer-Mannheim. Authentic F-2,6-P_2 was from Sigma Chemical Co., St. Louis, MO).

Results

As seen from Fig. 1, intragastric infusion of glucose into fasted rats had the expected effects on circulating glucose and liver glycogen levels. The former rose sharply from a starting value of 4.9 mM to the region of 8–9 mM within 30 min and remained at this level throughout the experiment. Liver glycogen was deposited at an approximately linear rate of 10 mg/g of liver per h during the first 3 h, but thereafter this declined as what appeared to be a maximal storage capacity of ~60 mg/g of tissue was approached. In contrast to these changes, liver F-2,6-P_2 content remained very low (~0.3 nmol/g) over the initial 3-h period, but in the subsequent 2 h it increased eightfold. Presumably, this trend continued such that, at some point in the 5–10 h interval, a maximal concentration in the region of 9 nmol/g was achieved.

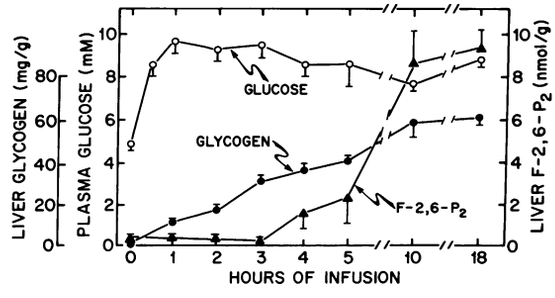

Figure 1. Effects of intragastric glucose infusion in fasted rats. Fasted rats received glucose intragastrically at a rate of 167 mg/100 g body wt per h. Analyses were carried out as described in Methods. Values are means±SEM for 3–7 animals at each time point.

A similar profile emerged when these experiments were carried out under more physiological conditions, i.e., in fasted animals allowed to eat ad lib. (Fig. 2). Although in this case there was a small elevation in F-2,6-P_2 levels at 1 h (possibly due to gorging which might be expected to elicit a much stronger insulin response than would slow glucose infusion), values had returned to basal by the 2 h time point and began to rise substantially only after 4 h of refeeding.

From the plot of F-2,6-P_2 vs. glycogen content of the liver (Fig. 3), it is evident that, regardless of the method used for refeeding, F-2,6-P_2 levels remained low until liver glycogen reached ~35 mg/g. Further increments in glycogen content were associated with a dramatic rise in the concentration of F-2,6-P_2.

Discussion

Although the absolute values for hepatic F-2,6-P_2 content in fed and fasted rats seen here are similar to those reported by other laboratories (13–15), to our knowledge this is the first study of the temporal sequence of changes in this parameter during the fasted to fed transition. We consider the results to be important in several respects. In the first place, they establish that, in the intact rat, the liver content of F-2,6-P_2 does not rise acutely in response to ingested glucose, at least in fasted animals given glucose intragastrically or refed with regular chow. Such a finding would not have been expected on the basis of studies in hepatocytes where exposure of the cells to glucose, even in the absence of insulin, caused a marked increase in F-2,6-P_2 levels within minutes (19, 20). An explanation for the difference between the in vivo and in vitro responses is not readily apparent. It might relate to differences in the concentration of glucose to which the tissue was exposed in the two types of experiments. Note that, in the in vitro studies cited (19, 20), large increases in F-2,6-P_2 concentration were seen only in hepatocytes exposed to glucose levels of 20 mM or greater, values far in excess of those present postprandially in the in vivo situation (Figs. 1 and 2 and References 2, 5, 23). Interestingly, lactate was shown to completely suppress the glucose-induced rise of F-2,6-P_2 in hepatocytes from fasted

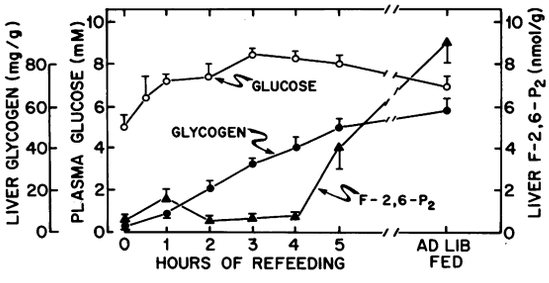

Figure 2. Effects of refeeding in fasted rats. Fasted rats were allowed to eat regular lab chow ad lib. Analyses were carried out as described in Methods. Values are means±SEM for 3–5 animals at each time point.

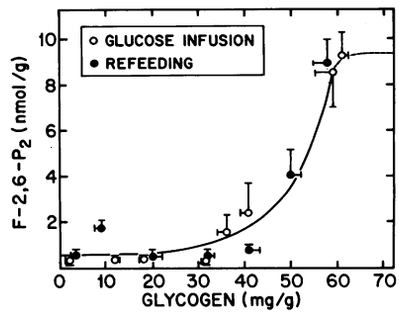

Figure 3. Relationship between hepatic glycogen content and F-2,6-P_2 levels in the rat. The data are taken from Figs. 1 and 2. The broken line represents our speculation that F-2,6-P_2 content approaches a maximum value as tissue glycogen stores reach saturation.

rats (26). Although the mechanism of this effect remains obscure, a similar phenomenon might have occurred in vivo since lactate is invariably present in the circulation and generally increases in concentration in response to glucose absorption (2, 23).

Regardless of the basis for the in vivo/in vitro discrepancy, the present findings promise to shed new light on a puzzling question central to the control of hepatic glucose metabolism. Thus, it is now clear that a substantial, if not major, fraction of the glycogen deposited in liver when a fast is terminated is derived from glucose-6-P generated via the glucogenic pathway (1–5). This would be difficult to reconcile with a high liver content of F-2,6-P_2, which should act to suppress carbon flow from F-1,6-P_2 to fructose-6-phosphate (7, 18, 27). The observation that F-2,6-P_2 levels in fact remained low for at least several hours into the postprandial period offers a plausible resolution to this metabolic paradox, i.e., it removes a theoretical obstacle to continued carbon flow through the F-1,6-P_2ase reaction. That regulatory enzymes proximal to this step in gluconeogenesis, such as pyruvate carboxylase and phosphoenolpyruvate carboxykinase, also remain active during this time frame has recently been established (4, 5).

There is good reason to believe that the fall in hepatic F-2,6-P_2 content that accompanies starvation or acute insulin deficiency is triggered in large part by concomitant elevation of the circulating [glucagon]:[insulin] ratio, which in turn causes a cyclic AMP-dependent inhibition of phosphofructokinase-2 and activation of F-2,6-P_2ase (22). Precisely how these events are reversed during the fasted to fed transition is not yet clear. It seems reasonable to conclude, however, that acute elevation in the circulating glucose concentration, coupled with a presumed sharp increase in [insulin] and decrease in [glucagon], is not a sufficient stimulus to reinstate high, fed levels of F-2,6-P_2. Of interest is the fact that liver F-2,6-P_2 content began to rise substantially only when the tissue glycogen stores were largely replete. While this in itself does not establish a causal relationship, it could provide clues to operative mechanisms. For example, since liver appears to have a limited capacity to store glycogen, it would seem logical that, when these stores have been largely refilled, some signaling device comes into play to prevent excessive glycogen accumulation. This might be achieved by a simultaneous glycogen-induced inhibition of glycogen synthase and activation of phosphorylase

(28). If, at the same time, F-2,6-P_2 synthesis was turned on [perhaps through increased glucose-6-P and fructose-6-P levels, as suggested by Hers et al. (27)], the effect would be to facilitate carbon flow through glycolysis and to suppress the opposing pathway of gluconeogenesis. Should food consumption still continue, any further lactate formed in nonhepatic tissues, together with pyruvate generated through hepatic glycolysis (and from amino acids), might then be expected to be shunted at an accelerated rate into fatty acid and triglyceride biosynthesis. Evidence for this sequence of events is currently being sought.

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