Role of Glucose on Fatty Liver Formation in Pyridoxine-Deficient Rats

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Summary Studies were made on whether glucose starvation causes fatty liver in pyridoxine-deficient male Wistar rats. Pyridoxine deficiency resulted in significantly lower levels of liver glucose than in pair-fed controls but no significant change in the serum glucose concentration. In non-starving animals, serum immuno-reactive insulin (IRI) was significantly lower in pyridoxine-deficient rats than in pair- or ad libitum-fed controls. Liver glucokinase activity in pyridoxine-deficient rats was also significantly lower than in ad libitum-fed controls. The extent of insulin deficiency was evaluated by examining the effect of administration of insulin on pyridoxine-deficient rats. Administration of insulin had no effect on the activity of liver glucokinase in pyridoxine-deficient rats, but induced the enzyme in ad libitum-fed controls. In response to a decrease in the activity of liver glucokinase or hexokinase in the deficient group, glycolytic activity, estimated as lactate production from glucose in the liver supernatant spun at 100,000 × g, was reduced to half the control level in pyridoxine-deficient rats. The effects of glucose administration on the liver lipid content, serum insulin and serum glucose were investigated. The serum glucose concentration was not significantly different in pyridoxine-deficient and control rats at any time after the glucose load. The level of serum IRI after the load was similar in the two groups after 30 min but then gradually decreased in the deficient group. The liver lipid content of the deficient rats tended to decrease whereas that of the controls remained unchanged throughout the experiment. Thus glucose starvation in pyridoxine-deficient rats is one factor responsible for fatty liver formation. Possible mechanisms of this phenomenon are discussed.

Key Words pyridoxine deficiency, fatty liver, oral glucose load, liver glucose, serum immuno-reactive insulin, liver glucokinase

Okada and Ochi previously reported that rats fed on 70% casein pyridoxine-deficient diet for 3 to 5 weeks show marked accumulation of lipid in the liver (1–3).
This accumulation of liver lipid was decreased by administration of 20% casein pyridoxine-free diet for 2 days (4). The accumulation of lipid in the liver in pyridoxine-deficient rats could have been due to carbohydrate or glucose starvation of the liver cell, because the supply of amino acid carbon for gluconeogenesis became limited due to decreased amino acid metabolism (5-7). This possibility is supported by the facts that the concentrations of free serine, threonine and glycine in the liver and serum of deficient rats are increased several fold, and the concentration of free alanine in the liver is lower in pyridoxine-deficient rats than in pair-fed controls (8, 9). The facts that alanine is considered to be an important precursor in hepatic gluconeogenesis (10-12) and that its concentration is low in the liver of deficient rats prompted us to test the effect of pyridoxine deficiency on the concentrations of serum and liver glucose, liver glycogen and serum immunoreactive insulin (IRI). We then measured the activities of liver glucokinase and hexokinase, and the activity of glycolytic enzymes in deficient rats. Finally we studied the changes in serum glucose, liver lipid content, and insulin secretion after an oral load of glucose in these animals to determine whether glucose starvation of liver cells is responsible for hepatic lipid accumulation in pyridoxine deficiency.

**METHODS**

*Animals and diet.* Male Wistar-strain rats (50 g) were used. The method of feeding and the composition of the diet were as described previously (4).

*Determination of serum and liver glucose.* Animals were starved for 15 hr and then killed. The pair-fed controls and pyridoxine-deficient animals were killed by decapitation, and blood samples were obtained without added anticoagulant. The livers isolated after decapitation of the rats were immersed in liquid nitrogen within 20 sec. They were then weighed and homogenized in 3 volumes of cold 6% perchloric acid (13). The supernatant was neutralized with 1 N KOH and diluted to 10 volumes with 0.1 M phosphate buffer, pH 7.0. Serum and liver glucose concentrations were determined using a Glucosetat from Fujisawa Pharmaceutical Co.

*Determination of glycogen.* Glycogen was isolated from the livers of pyridoxine-deficient and pair-fed control rats with 30% KOH and 95% ethanol, as described by Hassid and Abraham (14). The glycogen was hydrolyzed in 0.6N HCl and the glucose in the hydrolysate was determined as described above.

*Determination of serum insulin concentration.* One group of rats was starved for 15 hr and then killed. Other animals killed at 12.00 and 24.00 hr were fed ad libitum until they were killed. The concentration of serum insulin was measured using a radioimmunoassay kit from Daiichi Chemical Co. Radioactivity was measured in an Aloka Malchmode Scala instrument, Model TDC 601.

*Measurement of glucokinase, hexokinase and lactate production.* Glucokinase and hexokinase activities in rat liver were assayed by the method of DiPietro and Weinhouse (15). Lactate production from glucose was measured in the liver 100,000 × g supernatant using the method of Lea and Weber (16). Insulin (Tori
Pharmaceutical Co.) was injected i.p. into rats at a dose of 2 units/100 g body weight 24 hr and 1.5 hr before the animals were killed.

Assay of insulin secretion after a glucose load. Rats were starved for 15 hr, and then 40% glucose (2 g/kg body weight) (17) was administered by oesophageal tube to animals under light anaesthesia with ether. Then serum IRI and glucose concentrations were determined at intervals using the method described above.

Measurement of lipid and protein concentrations. As described previously (4) the liver lipid concentration was determined using the method of Folch et al. (18). Protein concentration was determined using the biuret reaction (19).

RESULTS

Effects of pyridoxine deficiency on serum glucose, liver glucose and liver glycogen concentrations

As shown in Table 1, administration of pyridoxine-deficient diet resulted in no

Table 1. Effects of pyridoxine deficiency on serum glucose, liver glucose and liver glycogen concentrations.

|                          | Pyridoxine deficiency | Pair-fed controls |
|--------------------------|-----------------------|-------------------|
| Serum glucose (mg/100 ml serum) | 129.5 ± 5.4 (4)      | 120.8 ± 5.4 (4)   |
| Liver glucose (µg/g liver)    | 449.1 ± 73.1** (7)   | 1,022.4 ± 167.5 (7) |
| Liver glycogen (µg/g liver)   | 3,540.5 ± 2,187.5** (5)| 47,804.7 ± 2,736.1 (5) |

Values are mean ± SEM for samples determined in duplicate. Numbers in parentheses are numbers of rats per group. **Statistically significant at p<0.005.

Table 2. Effect of pyridoxine deficiency on serum IRI concentration in the rat.

|                          | Feeding |
|--------------------------|---------|
|                          | At 12.00 | At 24.00 |
| 15 hr fasting            |         |
| Pyridoxine deficiency     | 28.3 ± 2.4 (6) | 19.6 ± 2.7a,b (4) | 44.9 ± 7.6a (4) |
| Pair-fed control          | 35.4 ± 3.8 (6) | 51.8 ± 9.3 (4) | 97.7 ± 22.8 (4) |
| Ad lib.-fed control       | 91.5 ± 14.0 (4) | 227.1 ± 43.9 (4) |

IRI: Immuno-reactive insulin. Values are mean ± SEM of samples determined in duplicate. Numbers in parentheses are numbers of rats per group. a and b indicate statistically significant differences from the ad lib.-fed control and pair-fed control, respectively (p<0.05).
significant difference in the concentration of serum glucose after 15 hr starvation from that in pair-fed controls, but decreased the liver glucose concentration to 43.9% of that in controls. It also decreased the liver glycogen content significantly.

Effects of pyridoxine deficiency on serum IRI concentration

Table 2 shows the values of immuno-reactive serum insulin of ad libitum-fed controls, pair-fed controls and pyridoxine-deficient rats that had been fed on the experimental diet for 4 weeks. There was no significant difference in the serum IRI concentrations of pyridoxine-deficient and pair-fed controls after starvation for 15 hr, although the concentration of liver glucose was lower in deficient rats. When animals were not starved, the IRI values at 12.00 and 24.00 hr, were lower in the rats on pyridoxine-deficient diet than in pair-fed or ad libitum-fed controls. Thus at all times, the serum IRI level was lower in pyridoxine-deficient rats than in control animals when the rats were not starved.

Effect of pyridoxine deficiency on the activities of liver glucokinase and hexokinase

The concentration of IRI shown in Table 2 does not necessarily represent the physiological activity of insulin. To estimate the physiological level of insulin in pyridoxine-deficient rats, we assayed the activities of liver glucokinase (which is known to be regulated by insulin (20)) and hexokinase. The activities of glucokinase and hexokinase in deficient rats were both half the control values (Table 3). Thus the level of physiologically active insulin in pyridoxine-deficient rats is lower than that in controls. To evaluate the extent of insulin deficiency, we examined the effect of insulin administration on the activity of liver glucokinase in deficient rats. Administration of insulin to pyridoxine-deficient rats had no effect on the activity of liver glucokinase whereas it induced the enzyme in ad libitum-fed controls. In response to a decrease in the activity of liver glucokinase or hexokinase in the

Table 3. Effect of pyridoxine deficiency on liver glucokinase, hexokinase and lactate producing activities in the rat.

| Insulin       | Glucokinase (μmol of G-6-P formed/min/g liver) | Hexokinase (μmol of G-6-P formed/min/g liver) | Glycolytic activity (μmol lactate/mg prot./min) |
|---------------|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|
| Pyridoxine deficiency | (−) 0.068 ± 0.004<sup>a</sup> (7) 0.102 ± 0.006<sup>c,d</sup> (7) 0.055 ± 0.003 (4) |
|                | (+) 0.062 ± 0.011<sup>b</sup> (6) 0.142 ± 0.015<sup>c,e</sup> (6) 0.099 ± 0.023 (4) |
| Pair-fed control | (−)                                          |                                               |                                               |
| Ad lib.-fed control | (−) 0.124 ± 0.015<sup>b</sup> (6) 0.287 ± 0.022<sup>d</sup> (6) |
|                | (+) 0.169 ± 0.059<sup>b</sup> (6) 0.302 ± 0.016<sup>e</sup> (6) |                                               |                                               |

Values are mean ± SEM for samples determined in duplicate. Numbers in parentheses are numbers of rats per group. Insulin was injected i.p. at 2 U/100 g body weight. The same letters at the right of values show significant differences (p < 0.05).

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deficient group, glycolytic activity, estimated as lactate production from glucose in the 100,000 × g liver supernatant, was reduced in pyridoxine-deficient rats to half that in controls. These results suggested that the activity of the glycolytic pathway decreased in response to a low level of physiologically active insulin in the deficient rats.

Fig. 1. Serum IRI concentration (A) and serum glucose concentration (B) after an oral load of glucose in pyridoxine-deficient (●) and pair-fed control (○) rats. Vertical lines show standard errors of means. * Statistically significant difference (p < 0.05).

Fig. 2. Liver lipid content after an oral load of glucose in pyridoxine-deficient (●) and pair-fed control (○) rats. Vertical lines are standard errors of means.
Effects of oral glucose administration on insulin secretion, serum glucose, and liver lipid content in pyridoxine-deficient rats

The serum glucose levels in deficient and control rats were not significantly different at any time after glucose loading. The levels of serum IRI after loading (Fig. 1A) were also similar in the two groups after 30 min, but then slowly decreased in pyridoxine-deficient rats, whereas they remained high for at least for 2 hr in control rats. Figure 2 shows the change in total liver lipid after glucose treatment in the two groups. The lipid content tended to decrease in pyridoxine-deficient rats after injection of glucose, whereas in the control group it remained unchanged.

DISCUSSION

In pyridoxine-deficient rats the serum glucose concentration remains normal, although gluconeogenesis from amino acid is impaired as a result of diminished transamination (5, 6). The glucose contents of liver are 1,278.0 µg/ml tissue water in pair-fed controls and 598.8 µg/ml tissue water in pyridoxine-deficient rats (based on a total tissue water content of liver of 0.8 ml/g wet weight reported by Kalkhoff et al. (21)). Since glucose penetrates across the liver cell membrane freely (21), the reasons for low levels of glucose in pyridoxine-deficient liver are not clear but one factor may be starvation (22). The low concentration of liver glucose, marked decrease in liver glycogen, and normal level of serum glucose in pyridoxine-deficient rats (Table 1) suggest that glucose newly synthesized by either glycogenolysis or gluconeogenesis is preferentially released into the blood to maintain normal glucose levels. Hormones, such as insulin, glucagon, corticosterone and epinephrine are known to affect many reactions that regulate the level of blood glucose (23). In this work we examined the effect of pyridoxine deficiency on serum insulin concentration. In non-starved animals the serum IRI level at 12.00 and 24.00 hr was lower in pyridoxine-deficient rats than in pair-fed or ad libitum-fed controls. This finding is consistent with the result of Gershoff et al. (24, 25) who found lower insulin-like activity in the serum and pancreas in pyridoxine-deficient animals than in controls. Since we found that the glucokinase activity was decreased in the deficient rats possibly in response to high-protein feeding and 15 hr starvation before the experiment (15), we concluded that the concentration of physiologically active insulin was also reduced in pyridoxine-deficient rats. Thus a lower level of serum insulin decreases the utilization of glucose in liver cells and conversely, glucagon and other hyperglycemic hormones may promote secretion of glucose from liver cells of pyridoxine-deficient rats (26). In contrast to our findings that pyridoxine-deficient rats can secrete insulin after treatment with glucose, Gershoff et al. reported that vitamin B₆-deficient rats do not respond to a glucose load, judging from the levels of insulin in samples taken from the retro orbital venous plexus 10 min after intrajugular injection of glucose (25). The reasons for the discrepancy between their findings and ours are not evident but could be due to hormonal substances (27, 28) or duration of time after glucose loading, since we injected...
glucose by stomach tube and took blood samples by decapitation 30 min after loading. Therefore, we could not observe the slow disappearance of glucose from the serum of vitamin B6-deficient rats after glucose loading reported by others (17, 25). The level of serum insulin (Table 2) or lack of response of liver glucokinase to insulin in pyridoxine-deficient rats may have been due to increased removal of insulin from the serum as observed in Fig. 1A. Administration of glucose prevented deposition of lipid in the liver of pyridoxine-deficient rats (Fig. 2), but injection of saline had no effect. These observations are consistent with our previous finding that 20% casein pyridoxine-deficient diet significantly reduced the liver lipid content of the deficient rats that had been fed on 70% casein pyridoxine-deficient diet. Campanari-Visconti et al. reported that administration of glucose inhibited the increase of liver triglyceride caused by ethionine (29). The exact mechanisms involved in liver lipid accumulation in pyridoxine deficiency are unknown. But glucose effects reported here may be explained by a return to normal of the liver glucose concentration or by secretion of insulin into the serum, because the insulin-secretion capability in pyridoxine-deficient rats was not impaired (Fig. 1A) and liver glycogen content in pyridoxine-deficient rat was increased from 0.35% to 1.15%, 2 hr after a glucose load (not cited in table). The inhibition of fatty acid mobilization from the periphery to the liver by an increased serum glucose level probably does not contribute to the increased liver lipid content in pyridoxine-deficient rats, because the serum free fatty acid level tends to lower in pyridoxine-deficient rats compared to pair-fed controls. In response to low levels of serum IRI, fatty acid synthesis from [1-14C]acetate in pyridoxine-deficient liver is depressed (unpublished data). In addition, Beynen et al. recently reported that insulin stimulated the secretion of newly synthesized very-low-density lipoproteins from isolated hepatocytes (30). From the present findings we conclude that glucose may act by releasing a blockage of lipid transfer from the liver to the blood or an obstruction of fatty acid oxidation in the liver.

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