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KEYWORDS: citrate metabolism, in liver

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STUDIES ON CITRATE METABOLISM IN LIVER INJURIES 2. RESPONSE OF LIVER CITRATE TO GLUCOSE LOAD

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Abstract: The effect of glucose load on the levels of blood glucose, serum non-esterified fatty acids (NEFA) and liver citrate was investigated in carbontetrachloride-intoxicated (injured) rats and compared with non-intoxicated controls. The citrate level in the liver from injured animals showed 15-fold of the value of the control. Glucose load on these animals caused gradual decrease in the citrate level, whereas similar administration to the control caused inverse results. The serum NEFA levels were lowered by glucose load in both of injured and control animals. The pattern of changes in the citrate level after glucose load in the liver from injured animals was similar to that in the muscle from the control, suggesting a similarity on citrate metabolism between the injured liver and the muscle. The possible mechanisms for these results were discussed in relation to the difference in citrate metabolism between the liver and the muscle.

The preceding paper of this series (1) demonstrated that blood citrate level increases accompanied with the severity of liver diseases, especially in decompensated liver cirrhosis, and has a closer correlation with serum non-esterified fatty-acid (NEFA) level than with blood glucose level.

As is generally known, glucose and NEFA are easily metabolized to acetyl-CoA and can be utilized for the synthesis of citric acid in various animal organs. It is also well known that administration of glucose causes the changes in blood concentrations of glucose and NEFA. Furthermore, the enzymatic pattern concerned with glucose metabolism in liver injury induced by various hepatotoxins including carbontetrachloride (CCl_4) is altered similarly to that in the muscle, showing a decrease in liver specific glucokinase and an increase in muscle specific pyruvate-kinase-M (2, 3).

These results led the author to elucidate how glucose load affects the citrate metabolism in the liver injured by CCl_4 and to compare it with the metabolism in the liver and muscle from non-injured animals.

MATERIALS AND METHODS

Sprague-Dawley male rats, weighing between 200 and 250 g, were fed on a standard laboratory diet and divided into two groups, i.e. liver injured and

non-injured (control) animals. Liver injury was made by intraperitoneal administration of CCl_4 dissolved in liquid paraffin in a single dose of 2g/kg of body weight. Similar administration of liquid paraffin in an equivalent volume was made for the control. After these procedures, all rats were fasted but allowed free access to water for 24 hours, and were given glucose in a single dose of 6g/kg of body weight by a gastric tube. At 0 (before glucose load), 45, 90 and 180 minutes after the load, a portion of the liver was quickly excised and ground into powder in liquid nitrogen by the quick-freeze technique. At 0 and 90 min after the load, a portion of the abdominal muscle from control rats was quickly excised and ground into powder by the method identical with that of liver preparation. The powdered sample was deproteinized in three volumes of ice-cold perchloric acid (6% w/v), centrifuged at $24,000 \times g$ for 10 minutes, and the supernatant was neutralized to pH 5 with $5M$ K_2CO_3 and used for the measurement of citrate after removing precipitated $KClO_4$.

Blood sample was taken from the abdominal aorta and used for the measurements of blood glucose, blood citrate and serum NEFA after preparing by the methods described previously (1).

Glucose and citrate were measured enzymatically by the methods described by BERGMEYER *et al.* (4) and DAGLEY (5), respectively. NEFA was measured colorimetrically by the method of ITAYA and UI (6).

ATP, NADH and NADP were purchased from Sigma Chemical Co.. Lactate dehydrogenase (EC 1.1.1.27), malate dehydrogenase (EC 1.1.1.37), glucose-6-phosphate dehydrogenase (EC 1.1.1.49), hexokinase (EC 2.7.1.1) and citrate-lyase (EC 4.1.3.6) were purchased from Boehringer Mannheim.

RESULTS

1. *The changes in blood glucose and serum NEFA:* Before glucose load, the blood glucose level was lower in injured rats than in the control. But as shown in Fig. 1, glucose load significantly raised the level in both groups, showing a much greater rise in injured rats. On the other hand, the serum NEFA level before glucose load was high in injured rats as compared with the control. When glucose was loaded to these animals, the level was significantly lowered in both groups and showed minimum values 90 minutes after the load. The level once lowered, however, was recovered to about 65% of the initial value 180 min later in the control, whereas similar phenomenon was not observed in injured rats (Fig. 2).

2. *The changes in liver citrate:* As mentioned in the preceding paper (1), the blood citrate level showed a significantly high value in liver diseases as compared with the normal. Similar phenomena were also observed in the citrate level in the liver injured by CCl_4 (Fig. 3). As illustrated in the figure, the liver citrate level was markedly high in injured rats, showing about 15-fold of the control. After glucose load, the level rose slightly in the control, whereas it fell gradually in injured rats, revealing a significant fall 180

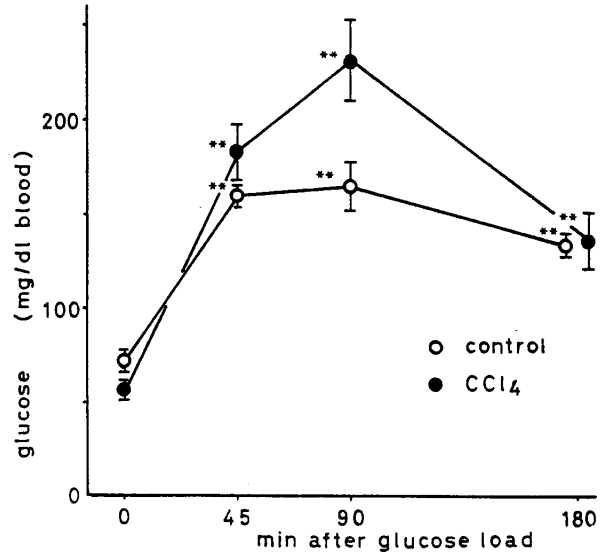

Fig. 1. Comparison of the changes in blood glucose level in CCl_4-intoxicated and control rats. Vertical line indicates standard error of the mean. $^{**}p<0.01$; for differences from the initial level.

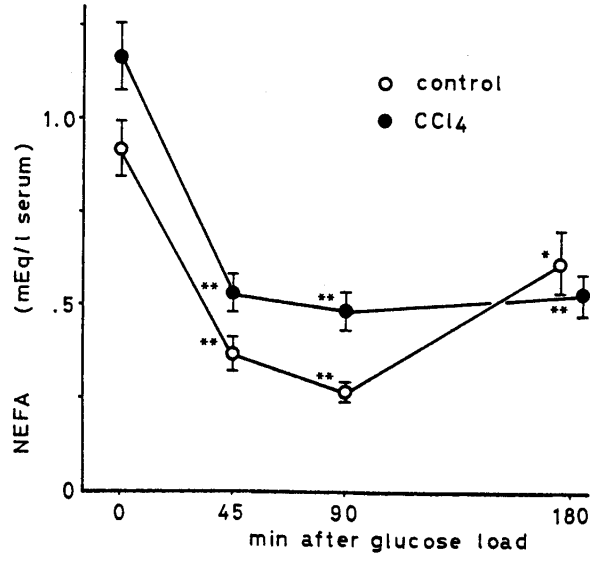

Fig. 2. Comparison of the changes in serum non-esterified fatty acids (NEFA) level in CCl_4-intoxicated and control rats. Vertical line indicates standard error of the mean. $^{**}p<0.01$, and $^{*}0.01<p<0.05$; for differences from the initial level.

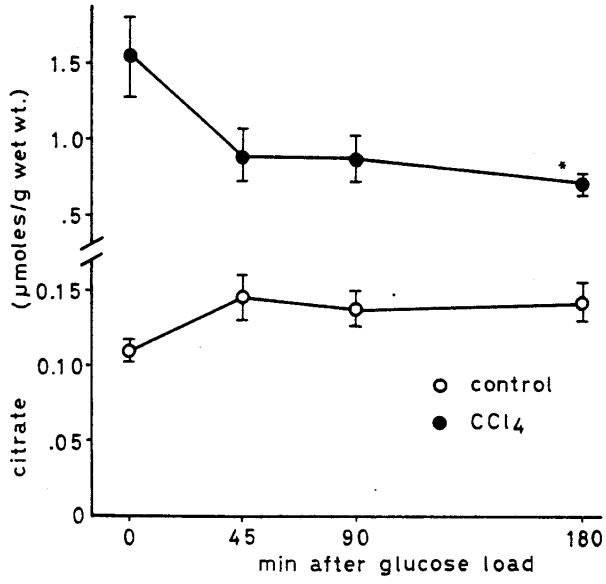

Fig. 3. Effect of glucose load on the liver citrate level in CCl_4-intoxicated and control rats. Vertical line indicates standard error of the mean. $*0.01<p<0.05$; for difference from the initial level.

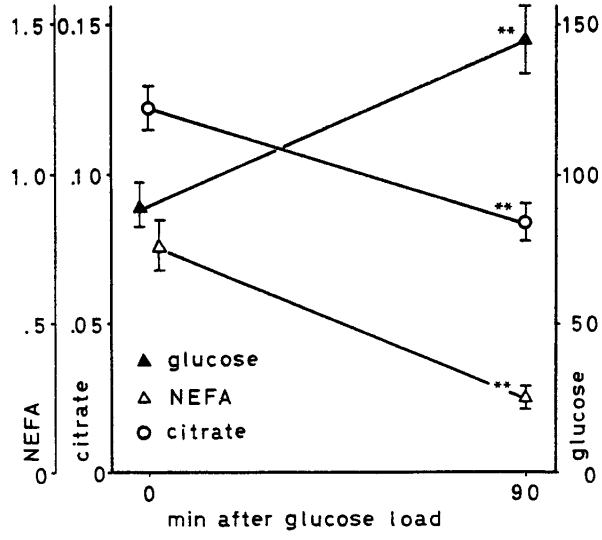

Fig. 4. Effect of glucose load on the blood glucose, serum non-esterified fatty acids (NEFA) and muscle citrate levels in control rats. Each values for glucose, mg/dl blood; NEFA, mEq/l serum; and citrate, μmoles/g wet wt. Vertical line indicates standard error of the mean. $**p<0.01$; for differences from the initial level.

minutes after the load.

3. *The changes in muscle citrate:* The muscle citrate level significantly fell after glucose load to 68% of the initial value. On the other hand, the levels of blood glucose and serum NEFA, as in the case of Figs. 1 and 2, significantly rose and fell, respectively, after the load (Fig. 4).

DISCUSSION

1. *The liver citrate level before glucose load:* As shown in Fig. 3, the liver citrate level is remarkably high in injured rats as compared with the control. This is consistent with the previous study (7). On the other hand, simultaneous measurements of blood citrate revealed the values of 0.149 ± 0.028 μmoles/ml blood in injured rats and 0.130 ± 0.014 μmoles/ml blood in the control (means $\pm$ S. D.), showing little difference between two groups. These data suggest that citrate, even in a high concentration in the injured liver, can be scarcely released into the circulating blood and that the liver citrate level may indicate more clearly the severity of liver injury than the blood citrate level.

The mechanisms for the increase in liver citrate in injured rats is presumed to be attributed not to an increased activity of citrate synthesis but to an impaired oxidation of citrate *via* Krebs cycle for the following evidences. About 80% of endogenous citrate has been reported to be localized in the mitochondrial fraction obtained by differential centrifugation of sucrose homogenate (8). The production of $^{14}CO_2$ from pyruvate-C^{14} (U) is markedly decreased in liver slices obtained from CCl_4-intoxicated rats, suggesting the decreased activity of oxidation of pyruvate *via* Krebs cycle (9). It has been also reported that the oxidations of NAD-linked substrates by liver mitochondria from injured rats proceed at a low rate (10) and activities of several mitochondrial enzymes including succinate dehydrogenase are decreased by CCl_4-intoxication (11). In view of these evidences, it seems reasonable to suggest that CCl_4-intoxication decreases the rate of oxidation of citrate *via* Krebs cycle and accumulates it in the mitochondria.

2. *The response of liver citrate to glucose load:* As for the changes in blood glucose and serum NEFA levels, it seems likely that glucose intolerance observed in injured animals is due to an impaired utilization of glucose by the liver (12, 13). In the high concentration of blood glucose, on the other hand, the rate of utilization of glucose by the muscle and adipose tissue is increased, leading to the increased capacity of reesterification for fatty acids in these tissues (14). The increased rate of reesterification, together with the inhibition of lipolysis by insulin (14, 15), contributes to the diminution in the rate of release of fatty acids from the tissues (especially

from the adipose tissue) into the circulating blood. From these evidences, the prompt recovery of serum NEFA in control rats, being accompanied by a lesser increase in blood glucose, may be due to the diminished effect of glucose on fatty acid metabolism in the tissues. The higher level of NEFA in injured rats than in the control before glucose load is probably due to the diminished uptake of circulating NEFA by the liver as discussed in the preceding paper (1).

The changes in liver citrate level after glucose load have not been reported hitherto, although the blood citrate level has been reported to be decreased after the load (16). The factor that contributes to the regulation of citrate synthesis in mitochondria is an availability of two precursors of citrate, i. e. acetyl-CoA and oxaloacetate (17, 18). SHEPHERD *et al.* (19) have reported the Km of liver citrate synthase for oxaloacetate to be 2 μM. WILLIAMSON *et al.* (20) have calculated the mitochondrial oxaloacetate concentration of intact liver to be in the range of 0.1 to 0.5 μM (assuming that μmoles/g wet wt of tissue corresponds to μmoles/ml), significantly below the Km of the enzyme for oxaloacetate. This situation indicates that slight changes in oxaloacetate concentration may result in marked changes in the rate of citrate synthesis. In addition, it has been reported that oxaloacetate concentration in intact liver mitochondria is significantly increased by glucose load, although a reverse phenomenon is observed with acetyl-CoA concentration (13). In view of these findings, it seems reasonable to conclude that the increase in citrate in the liver from control rats is due to the concomitant increase in oxaloacetate in the mitochondria. As for injured rats, it has been reported that oxaloacetate concentration in liver mitochondria is significantly decreased by glucose load (13). Therefore, the decrease in liver citrate in injured rats is presumed to be attributed to the concomitant decrease in oxaloacetate concentration in the mitochondria. In addition, the decrease in acetyl-CoA concentration in the liver after glucose load (13) may also contribute to this phenomenon.

It has been shown that concentrations of acetyl-CoA and of citrate are increased in the muscle in perfusions with fatty acids, suggesting that these changes in concentrations are due to the increased rate of oxidation of fatty acids to acetyl-CoA for the synthesis of citrate (21). Therefore, it seems likely that intramitochondrial concentration of acetyl-CoA is concerned more tightly with citrate synthesis in muscle mitochondria than in liver mitochondria. On the other hand, it is well known that glucose load inhibits the oxidation of fatty acids in various tissues including muscle (22) and presumably decreases the intramitochondrial concentration of acetyl-CoA. In view of these evidences, it seems reasonable to suggest that the decrease in muscle

citrate is, at least partilaly, due to a decreased availability of acetyl-CoA in citrate synthesis.

It has been reported that enzymatic pattern of the liver concerned with glucose metabolism is altered by liver injuries to show a decreased activity of liver specific enzymes and an increased activity of liver non-specific enzymes (2, 3). This observation, together with the similarity on citrate metabolism between injured liver and muscle, leads us to an interesting speculation that injured liver may lose its specificity in metabolic pattern as well as in enzymatic pattern.

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