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Junji Kaneshige*

*Okayama University,

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Abstract

For the purpose to study the citrate metabolism in liver diseases, blood citrate, blood glucose and serum non-esterified fatty acids (NEFA) in fasting state were measured in the subjects with chronic hepatitis and with liver cirrhosis. Citrate and glucose were measured by the enzymatic methods. NEFA was measured colorimetrically. Fasting blood citrate level was investigated in relation to the type and extent of these liver diseases. Results revealed the following: 1. Fasting blood citrate level rose with the severity of liver diseases, especially in decompensated liver cirrhosis. 2. No significant difference in fasting blood citrate level was found between the subjects with and without glucose intolerance. 3. Fasting blood citrate level had a closer correlation with serum NEFA level than with blood glucose level. From these results, it has been concluded that the increase in blood citrate level in liver diseases is due to the impaired uptake of citrate by the liver and the increased release of citrate from peripheral tissues.

KEYWORDS: citrate metabolism, liver injuries

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STUDIES ON CITRATE METABOLISM IN LIVER INJURIES
1. FASTING BLOOD CITRATE LEVEL IN CHRONIC
HEPATITIS AND LIVER CIRRHOSIS

Junji KANESHIGE

*Department of Internal Medicine, Okayama University Medical School,
Okayama, Japan. (Director: Prof. K. Kosaka)*

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Abstract: For the purpose to study the citrate metabolism in liver diseases, blood citrate, blood glucose and serum non-esterified fatty acids (NEFA) in fasting state were measured in the subjects with chronic hepatitis and with liver cirrhosis. Citrate and glucose were measured by the enzymatic methods. NEFA was measured colorimetrically. Fasting blood citrate level was investigated in relation to the type and extent of these liver diseases. Results revealed the following: 1. Fasting blood citrate level rose with the severity of liver diseases, especially in decompensated liver cirrhosis. 2. No significant difference in fasting blood citrate level was found between the subjects with and without glucose intolerance. 3. Fasting blood citrate level had a closer correlation with serum NEFA level than with blood glucose level. From these results, it has been concluded that the increase in blood citrate level in liver diseases is due to the impaired uptake of citrate by the liver and the increased release of citrate from peripheral tissues.

An increase in lactate, pyruvate and α -ketoglutarate in the blood of liver diseases has been reported by many authors. The increase in citrate of the blood of liver diseases was reported for the first time by SJÖSTRÖM (1) and it has been supported by the succeeding papers (2, 3). The methods applied in these papers, however, are reported to be not necessarily specific for citrate (4). Therefore, in the present study, blood citrate was measured by the enzymatic method with citrate-lyase highly specific for citrate (4) in order to acquire a further knowledge of the citrate metabolism in the patients with chronic hepatitis and liver cirrhosis. The attempt was made to investigate blood citrate level in relation to the type and extent of these liver diseases. As it has been reported that citrate metabolism is affected by diabetes mellitus (4, 5, 6, 7), blood citrate level was also investigated in relation to the presence of glucose intolerance.

MATERIALS AND METHODS

The subjects studied here consisted of 17 cases of chronic hepatitis, 18 cases of liver cirrhosis and five normal adults for comparison. All subjects except five normal adults were inpatients of our clinic and did not receive any drugs as adrenocorticosteroids and insulin which might have some effect on citrate metabolism. The diagnoses of chronic hepatitis and liver cirrhosis were made by both liver biopsy and peritoneoscopic examinations in all the subjects except five of the ten with decompensated liver cirrhosis, whose diagnosis was made by liver function tests and clinical features.

All subjects were fasted but allowed free access to water for 14 hours after dinner in the previous evening and remained recumbent for at least 30 minutes before testing. Blood sample was taken from a superficial arm vein without venous occlusion. Immediately after blood sample was taken, one ml of blood was added to three ml of ice-cold perchloric acid (6% w/v), deproteinized and centrifuged at $24,000 \times g$ for 10 min. After centrifugation, the supernatant was neutralized to pH 5 with 5M K_2CO_3 and used for the measurements of glucose and citrate after removing precipitated $KClO_4$. Glucose and citrate were measured enzymatically by the method described by BERGMAYER *et al.* (8) and DAGLEY (9), respectively. The rest of the blood was used for the measurement of serum nonesterified fatty acids (NEFA) by the method described by ITAYA and UI (10). 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.

Chronic hepatitis was classified into active type and inactive type according to the criteria established by Japan Society of Hepatology (11). Liver cirrhosis with at least one of the following signs was classified into decompensated stage, and one with none of the following signs was classified into compensated stage: (1) Elevation of serum bilirubin above 2 mg/dl serum; (2) drop of serum albumin below 3 g/dl serum; (3) presence of ascites or edema.

The definition of glucose intolerance was based on the criteria established by Japan Diabetic Society (12). All the subjects except five normal adults were classified into either normal type or abnormal type of glucose tolerance test (GTT) with border-line cases included in normal type.

Peritoneoscopic findings were classified into five groups from 100 to 500 in code number according to the classification of liver surface presented by SHIMADA *et al.* (13).

RESULTS

1. Blood citrate level in liver diseases :

Blood citrate level in the subjects with chronic hepatitis was slightly higher than that in normal adults. However, no significant difference of blood citrate level was found between active type and inactive type of

chronic hepatitis and between normal type and abnormal type of GTT in chronic hepatitis. On the other hand, blood citrate level in the subjects with liver cirrhosis was shown to be significantly higher, especially in those with decompensated liver cirrhosis and with glucose intolerance (Table 1).

TABLE 1 BLOOD CITRATE LEVEL IN LIVER DISEASES

normal (5)		60 ± 12	
C H (17)	67 ± 9	inactive	(6) 68 ± 7
		active	(11) 66 ± 10
		normal GTT	(8) 71 ± 8
		abnormal GTT	(9) 65 ± 10
L C (18)	**89 ± 21	compensated	(8) 71 ± 10
		decompensated	(10) **103 ± 15
		normal GTT	(9) *79 ± 17
		abnormal CTT	(9) **100 ± 19

The citrate levels are expressed as mean ± S.D. in nmoles/ml blood. Numbers in parentheses show the number of subjects. CH, chronic hepatitis; LC, liver cirrhosis; GTT, glucose tolerance test.

*0.01 < p < 0.05, and **p < 0.01; for differences from normal.

2. *Relation of blood citrate level to peritoneoscopic findings:*

Blood citrate level rose gradually with the development of nodularity of liver surface; namely, with the progress of liver diseases from chronic hepatitis to liver cirrhosis (Fig. 1).

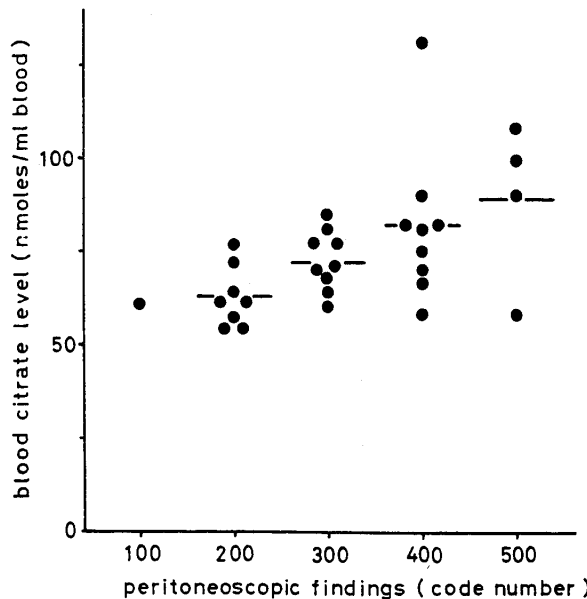


Fig. 1. Relation of blood citrate level to peritoneoscopic findings

3. Correlation of blood citrate level with blood glucose level and with serum NEFA level :

Correlation coefficient between blood citrate level and blood glucose level was 0.442 ($p < 0.01$) and 0.711 ($p < 0.001$) between blood citrate level and serum NEFA level (Fig. 2, Fig. 3). From these results, it is deduced

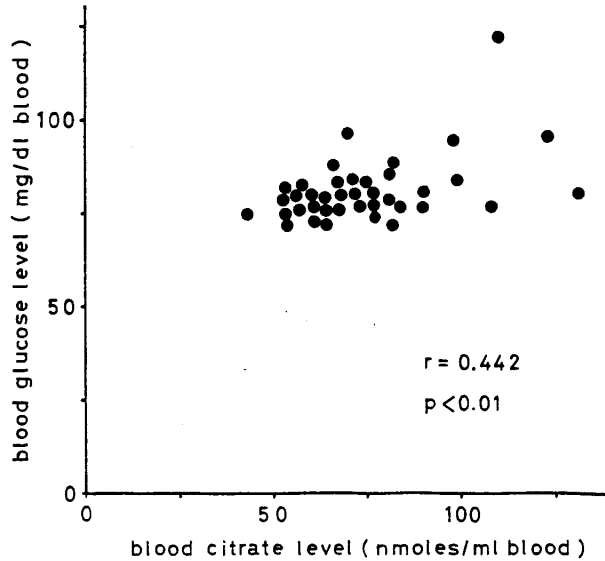


Fig. 2. Correlation between blood citrate level and blood glucose level

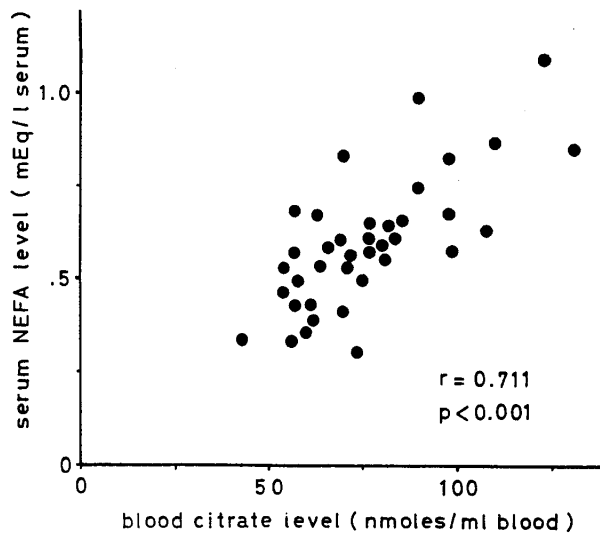


Fig. 3. Correlation between blood citrate level and serum non-esterified fatty acids (NEFA) level

that blood citrate level has a closer correlation with serum NEFA level than with blood glucose level.

DISCUSSION

Metabolic abnormalities of glucose (14, 15) and NEFA (16, 17, 18), the most important precursors of citrate, have been demonstrated in liver diseases. Furthermore, the metabolism of citrate in the liver itself is supposed to be disrupted in liver diseases (1, 2). Therefore, liver diseases are expected to be accompanied by some abnormality of blood citrate level.

The present study showed that blood citrate level was slightly higher in chronic hepatitis and greatly higher in liver cirrhosis, especially in decompensated liver cirrhosis. From these results, it is concluded that the blood citrate level rises with severity of liver diseases, i. e. with the progress of liver disease from chronic hepatitis to liver cirrhosis. This is also supported by peritoneoscopic findings that the blood citrate level rises with development of the nodularity of liver surface.

Comparing blood citrate levels in liver diseases reported by various authors (1, 2, 3), the previous measurements have revealed the results similar to the present study showing that the level rises roughly in proportion to the severity of liver diseases. However, the level obtained by the colorimetric method is higher than that obtained by enzymatic method, suggesting that the colorimetric method may include nonspecific reactions in the measurement of citrate as already pointed out by START *et al.* (4) (Table 2).

TABLE 2 COMPARISON OF BLOOD CITRATE LEVELS IN LIVER DISEASES REPORTED BY VARIOUS AUTHORS

method	normal	C H	L C	reporter
enzymatic (citrate de- hydrogenase)	106*	174*	181*	SJÖSTRÖM
colorimetric	203	323	562	TAKAOKA
colorimetric	83	81	105	MIYATA
enzymatic (citrate-lyase)	60	67	89	author

The citrate levels are expressed in nmoles/ml blood; *expressed in nmoles/ml serum. CH, chronic hepatitis; LC, liver cirrhosis.

As for mechanisms for the increase in blood citrate level in liver diseases, two possible explanations are conceivable. First, citrate is released into the blood from the peripheral tissues, i. e. muscle and adipose tissues, (19) and taken mostly in the liver (19, 20) and partly in the kidney (21). The liver has an ability to extract about 20% of citrate passing through it (19). Furthermore, it has been demonstrated that effective hepatic blood flow is

decreased by liver diseases roughly in proportion to their severities (22, 23). In view of these findings, it seems reasonable to suggest that the increased level observed in the subjects with liver diseases is partly due to a diminution in citrate uptake by the liver. Secondly, it has been also reported that the serum NEFA level is increased by liver diseases roughly in proportion to their severities, causes for which have been shown to be the diminished uptake and utilization of NEFA by the liver (16, 18). A high concentration of circulating NEFA causes an increased rate of oxidation of fatty acids in the tissues, leading to an increased supply of acetyl-CoA in the synthesis of citrate (24). In addition, it has been reported that the concentrations of acetyl-CoA and of citrate in the muscle are increased in alloxan-induced diabetic rats and in perfusions with fatty acids probably due to the increased rate of oxidation of fatty acids to acetyl-CoA in the tissue (24). Similar results have been obtained in the adipose tissue from diabetic animals (25). From these evidences, it seems likely that liver diseases, being accompanied by an increased concentration of circulating NEFA, increase the concentrations of acetyl-CoA and citrate in both tissues and accelerate a release of citrate into the circulating blood to raise the blood citrate level. NATELSON *et al.* (26, 27) have reported that blood citrate level is increased after epinephrine injection and decreased after administrations of glucose and insulin in normal adults. GORDON *et al.* (19) have reported that the peripheral release of citrate is decreased after glucose administration in cirrhotic subjects. These findings support the above explanation from the point of changes in circulating NEFA level under the influences of these hormones and glucose. The alternative explanation that the increase in blood citrate level is due to a release of citrate from the liver is unlikely for the reason that the net uptake of citrate by the liver is found even in cirrhotic subjects as well as in normal subjects (19).

It has been reported that blood citrate level is increased in animals (6) and human subjects (5) with diabetes mellitus. The present study showed no significant difference of the level in chronic hepatitis between the subjects with and without glucose intolerance. This is not consistent with the results reported previously (5, 6). The inconsistency with the previous results is supposed to be partly referred to the difference of subjects studied, because they were exclusively chemical diabetes in the present study in contrast with clinical diabetes in the previous report (5). Another explanation for the inconsistency may be referred to some pathogenetic differences between primary glucose intolerance, *i. e.* diabetes mellitus, and secondary glucose intolerance induced by liver diseases (14, 15). On the other hand, blood citrate level in the cirrhotic subjects was higher in those with glucose intolerance

than in those without glucose intolerance. Considering the results found in chronic hepatitis and the evidence that the majority of cirrhotic subjects with glucose intolerance (8 out of 9 cases) consisted of those with decompensated liver cirrhosis, it seems reasonable to suggest that the increased level in cirrhotic subjects with glucose intolerance is due to decompensated stage of the disease rather than glucose intolerance.

NEFA (28, 29) as well as citrate (19, 20) are taken in the liver from the blood passing through it, whereas glucose is released from the liver into the blood in fasting state. Moreover, NEFA is utilized in the muscle as energy source in preference of glucose in fasting state (30, 31), leading to the increased rate of oxidation of fatty acids and subsequent increase in peripheral release of citrate as discussed above. In view of these evidences, it seems likely that the blood citrate level has a closer correlation with serum NEFA level than with blood glucose level. This conclusion is supported by the present study which has revealed correlation coefficients of 0.442 between citrate and glucose and of 0.711 between citrate and NEFA.

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