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Hepatic lipid peroxidation and trace elements – nutritional status in streptozotocin-induced diabetic rats

A. Wachnik*, G. Biró, L. Biró, A. Gergely, and M. Antal

National Institute of Food-Hygiene and Nutrition, Budapest, Hungary
* National Research Institute of Mother and Child, Warsaw, Poland

Lipidperoxidation in der Leber und Ernährungsstatus von Spurenelementen bei Ratten mit Streptozotocin-induziertem Diabetes

Summary: The aim of this experiment was to study the interrelationships between nutritional status of chosen trace elements (Cu, Zn, Fe) and hepatic lipid peroxidation in streptozotocin-induced diabetic rats. Both copper accumulation and disruption of iron storage were observed in livers of diabetic rats. MDA 0' (baseline) and MDA 30' (produced) levels measured in the liver were negatively correlated with blood glucose levels. MDA 30' levels correlated positively with iron concentration in the liver. It is supposed that the hormonal lability during experimental diabetes caused changes in metabolism of trace elements, and subsequently influenced the rate of lipid peroxidation.

Zusammenfassung: Diese Arbeit hatte eine Überprüfung der gegenseitigen Abhängigkeit des Ernährungsstatus von ausgewählten Spurenelementen (Cu, Zn, Fe) und der Lipidperoxidation in der Leber von Ratten mit Streptozotocin-induziertem Diabetes vor. Es wurde eine Akkumulation des Cu in Leber und eine Speicherstörung des Fe bei Diabetes-Ratten beobachtet. Der MDA-0'-Spiegel wie auch der MDAS-30'-Spiegel korrelierten statistisch bedeutsam mit dem Zuckerspiegel des Blutes. MDA 30' korrelierte positiv mit dem Fe-Spiegel in der Leber. Man kann annehmen, daß die – während des experimentell hervorgerufenen Diabetes – hormonale Labilität Änderungen im Stoffwechsel der Spurenelemente verursachte, was in der Folge einen Einfluß auf die Intensivität der Lipidperoxidation ausübte.

Key words: Diabetes; Cu-, Zn-, Fe- metabolism; lipid peroxidation

Schlüsselwörter: Diabetes; Cu-, Zn-, Fe- Stoffwechsel; Lipidperoxidation

Introduction

A strong interaction between metabolism of trace elements and hormones involved in glucose metabolism was shown in several studies

Abbreviation index:
Malondialdehyde (MDA)
Body mass (bm)
Triglyceride (Tg)

which appeared recently (5, 6, 8, 11). Some scientists suggest that experimentally induced, insulin dependent diabetes leads to accumulation of copper, zinc and iron in the liver and kidney (5, 6, 8, 11).

In our former studies, we observed connections between hepatic malondialdehyde (MDA) and trace element status in copper-deficient rats (15) and in obese ones (3). Therefore, we found it interesting to investigate connections between lipid peroxidation and mineral metabolism in streptozotocin-induced diabetic rats. In this experiment, we used OETI rats predisposed to obesity due to impaired lipid metabolism (3). In this strain a pronounced lipid peroxidation was observed previously in comparison to H-Wistar rats and in relation to diet composition (14).

Materials and methods

Young, mature OETI male rats (250 ± 25 g) were fed ad libitum conventional laboratory chow (LATI, LATI Inc. Hungary). The protein, carbohydrate, and lipid contents of this food were about 21 %, 66 %, and 15 % of the total energy (1500 kJ), respectively. Animals were divided into two groups. One group (n = 20) was injected intraperitoneally with streptozotocin (Upjohn NDC 0009-0844-01, 60 mg/kg body mass) diluted in citrate buffer. Controls (n = 10) received the same quantity of citrate buffer. Thirty-two days after injection the animals were anesthetized with ether, blood was collected via the abdominal aorta, and the liver was quickly removed.

The trace elements were determined in livers of eight animals from each group.

The glucose level in blood was monitored with the Gluco GOD/POD test (Galenopharm: cat. nr. 1110–22).The copper and zinc contents of sera (after dilution), and copper, zinc, and iron concentrations of the liver (after washing at 450 ± 20 °C and nitric acid treatment) were determined using a Perkin Elmer 403 Atomic Absorption Spectrophotometer (HC lamp) (1). The level of triglyceride (Tg) in sera was measured with EnzGlycid GPO test (Organon Teknika, Freiburg). Hepatic lipid peroxidation was studied using spectrophotometrical determination of malondialdehyde-thiobarbituric acid complex (MDA-TBA) with Buckingham's

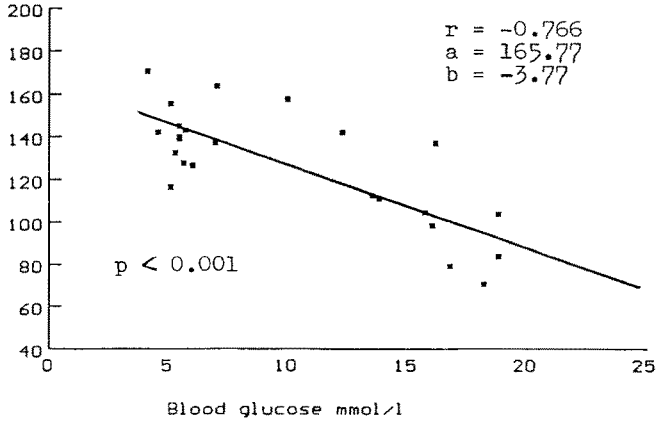

Fig. 1. The correlation between blood glucose levels (n = 24) and hepatic iron concentration (n = 24) in control and streptozotocin-induced diabetic rats.

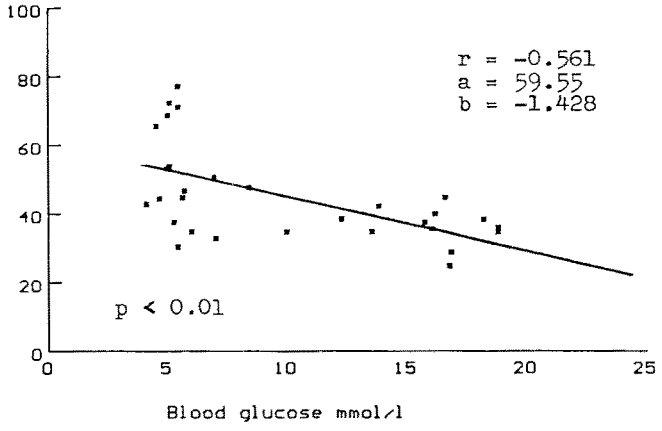

Fig. 2. The correlation between blood glucose levels (n = 30) and hepatic baseline MDA levels (n = 30) in control and streptozotocin-induced diabetic rats.

modified method. MDA-TBA complex was measured immediately after the liver was excised (0'), and after 30 min of incubation in a 37 °C water bath (4).

Results

The results are shown in Figs. 1–4 and in Table 1. Differences among results, determined by Student's *t*-test are presented in Table 2.

There was a negative correlation between blood glucose levels and hepatic iron, MDA 0' and produced MDA ($r = -0.766$; $r = -0.561$; $r = -0.661$, respectively) (Figs. 1, 2, 3). Hepatic MDA production positively correlated with hepatic iron levels ($r = 0.473$) (Fig. 4).

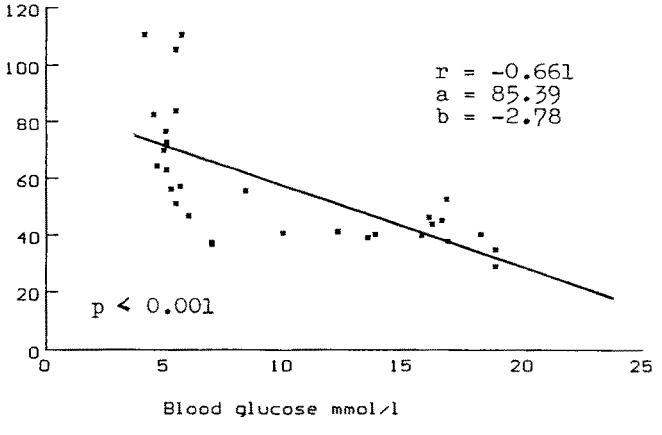

Fig. 3. The correlation between blood glucose levels (n = 30) and hepatic produced MDA levels (n = 30) in control and streptozotocin-induced diabetic rats.

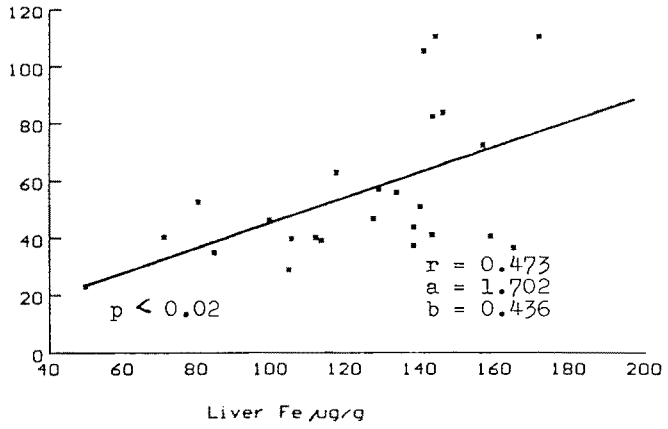

Fig. 4. The correlation between hepatic iron concentration (n = 24) and hepatic produced MDA levels (n = 24) in control and streptozotocin-induced diabetic rats.

Streptozotocin-treated rats were divided into two subgroups on the basis of blood glucose levels: group 1 (ND) – non diabetic (n = 10); group 2 (MD) – mild diabetic (n = 10); group 3 (SVD) – severe diabetic (n = 10).

The levels of copper, zinc, and triglyceride in sera did not differ between groups. The hepatic copper concentration was the lowest in the control non-diabetic group (Table 1).

Table 1. The parameters measured in sera and liver.

Parameter	(ND)		(MD)		(SVD)	
Body weight	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD
	n=10		n=10		n=10	
Initial g	256.8	24.4	251.2	29.0	262.6	26.0
Final g	299.9	26.4	247.4	31.5	233.9	19.8
Blood	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD
	n=10		n=10		n=10	
Glucose mmol/l	5.00	0.50	9.00	1.20	17.00	1.50
Sera	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD
	n=10		n=10		n=10	
Cu µg/ml	1.39	0.22	1.33	0.16	1.34	0.27
Zn µg/ml	1.25	0.21	1.28	0.15	1.33	0.12
Tg mmol/l	0.83	0.25	0.72	0.37	1.11	0.72
Liver	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD
	n=8		n=8		n=8	
Cu µg/g	3.40	1.52	5.30	0.83	5.30	0.65
Zn µg/g	38.90	5.73	34.80	5.60	35.60	3.90
Fe µg/g	142.20	24.03	141.20	16.30	90.40	38.50
	n=10		n=10		n=10	
MDA 0' nmol/g	58.28	14.70	40.84	8.30	36.72	5.96
MDA 30' nmol/g	77.80	18.90	53.40	22.70	41.56	6.48

Table 2. Statistical significances calculated by Student's *t*-test.

Parameter	1	2	3
Blood glucose	0.001	0.001	0.001
Serum Cu	NS	NS	NS
Serum Zn	NS	NS	NS
Serum Tg	NS	NS	NS
Liver Cu	0.02	0.01	NS
Liver Zn	NS	NS	NS
Liver MDA 0'	0.01	0.001	NS
Liver MDA 30'	0.02	0.001	NS

Notation:
NS = not significant
1 = significant differences between (ND) and (MD) groups
2 = significant differences between (ND) and (SVD) groups
3 = significant differences between (MD) and (SVD) groups

Discussion

The results confirmed that streptozotocin-induced diabetes leads to accumulation of copper in the liver. However, no significant changes were noticed in hepatic zinc content. At the same time, a very low hepatic iron level was observed in severe diabetic rats. The reason for these contradictory data may be due to an experimental period twice as long as that in other studies (5, 6, 8). Another explanation for the contradictory data may lie in strain differences. In all other studies Sprague-Dawley rats were used, whereas the present experiment was performed with OETI rats. As was mentioned above, this strain shows impaired lipid metabolism.

It is well known that the rate of lipid peroxidation depends on the presence of iron ions. The copper deficiency with increased hepatic iron accumulation led to higher production of MDA in the liver (15). The present study showed that, in severe diabetes, the lowest iron concentration of the liver corresponds to the lowest hepatic MDA level (blood glucose/hepatic iron $r = 0.766$). Additionally, the hepatic copper content increased significantly in both diabetic groups. Copper influences lipid peroxidation as part of antioxidant enzymes (ceruloplasmin EC 1.16.3.1 and superoxide dismutase EC 1.15.1.1), but also through its participation in iron transport and storage (13, 15). Since significant positive correlation was found between hepatic MDA production and iron accumulation, it seems that the hepatic iron concentration influences MDA production very strongly. This explanation may be responsible for the lower production of hepatic MDA in diabetic rats. Although there was no significant correlation between copper levels and levels of blood glucose and hepatic MDA, a slight positive tendency ($r = 0.40$) occurred within the first of the mentioned parameters, and a negative one ($r = -0.44$) within the second. These tendencies suggest that the influence of copper on hepatic MDA level is of lesser importance in the case of streptozotocin-induced diabetes. In another experiment (to be published) a similar but more significant

regularity between copper status and MDA level caused by estrogen action was observed.

Recently, increased attention has been paid to research concerning lipid peroxidation and the danger of free radical production (2–4, 15). However, Sevenian and Hochstein are inclined to the opinion that the lower rate of lipid peroxidation may lead to interruption of some physiological processes, e.g., disturbances in prostaglandin synthesis and decreased maturation of reticulocytes (12).

As was mentioned, the hepatic MDA level is lower in diabetic rats and also in female rats. Moreover, it was shown that both in diabetic and female rats the adrenal hormones are significantly higher (7–9). It should be noted that adrenocorticosteroids may also affect the process of lipid peroxidation. It also should be taken into consideration that streptozotocin as a toxic chemical could influence biochemical reactions in the rat and, subsequently, charges in the concentrations of measured substances. In our opinion, at least two possibilities require further investigation for a better understanding of lipid peroxidation in biological systems:

- influence of insulin and estrogen on trace element metabolism and, subsequently, on lipid peroxidation;.
- influence of adrenocorticosteroids (affected by the hormones mentioned above) on trace elements and lipid peroxidation.

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Author's address:

Dr. Anna Wachnik, The National Research Institute of Mother and Child, Dept. of Nutrition, Kasprzaka 17-a, 01-211 Warsaw, Poland