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Nutrition

Evaluation of tissue metal and trace element content in a rat model of non-alcoholic fatty liver disease using ICP-DRC-MS

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ABSTRACT

The primary objective of the study was to assess the level of metals and trace elements in liver, serum, and hair of rats with diet-induced non-alcoholic fatty liver disease (NAFLD) using inductively coupled plasma dynamic reaction cell mass spectrometer (ICP-DRC-MS). 56 female 3-months-old Wistar rats divided into two equal groups were fed either standard (10% calories from fat) or high-fat high-carbohydrate diet (60% calories from fat in chow and 10% sucrose solution) for 6 weeks. Serum was examined for insulin resistance markers, lipid profile, and alanine aminotransferase (ALT) activity. Liver histology was assessed after hematoxylin and eosin staining. Metal and trace element concentrations were assessed by means of ICP-DRC-MS. Overfed animals were characterized by higher values of morphometric parameters. Liver examination revealed large and small droplet steatosis, hepatocyte ballooning and necrosis, being characteristic for NAFLD. Animals with NAFLD were characterized by insulin resistance, atherogenic changes of lipid profile and increased ALT activity. Significantly decreased hepatic Co, Cu, I, Li, Mn, Se, Zn levels were observed in rats with NAFLD. At the same time, only hepatic Mn and Se levels remained decreased after adjustment for total protein. Overfed animals were characterized by significantly lower I, Li, and Mn levels in blood serum, whereas concentration of Co, Se, V, and Sr exceeded the control values. In general, the results of the study demonstrate that NAFLD significantly affects metal and trace element status in experimental animals.

1. Introduction

Non-alcoholic fatty liver disease (NAFLD) is characterized by macrovesicular steatosis of more than 5% of hepatocytes in persons who do not consume alcohol. The prevalence of NAFLD is increasing worldwide reaching nearly 35% in some countries and being the most common liver disease [1]. NAFLD is often associated with obesity and adipokine dysregulation was supposed to play a key role in insulin resistance and NAFLD development [2]. Moreover, NAFLD is considered as a specific feature of metabolic syndrome with hepatic insulin resistance [3]. However, the causal relationship between NAFLD and metabolic syndrome is still questionable [4].

Like in the case of obesity [5] and metabolic syndrome [6], environmental pollution plays a significant role in NAFLD development [7]. It is supposed that heavy metals may play a significant role in NAFLD development. In particular, it has been demonstrated that cadmium [8], arsenic [9], and other heavy metal [10] exposure is significantly associated with NAFLD. A pathogenetic role of heavy metals in NAFLD development may be associated with their proinflammatory and prooxidant properties [11]. However, the existing

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Table 1
Trace element content in diets (μg/g).

Element	STD	HFHCD
Co	1.0 ± 0.1	0.96 ± 0.15
Cr	0.65 ± 0.05	0.53 ± 0.09
Cu	16 ± 2	13 ± 3
Fe	210 ± 20	190 ± 38
I	1 ± 0.1	0.95 ± 0.2
Li	0.13 ± 0.01	0.12 ± 0.03
Mn	80 ± 10	75 ± 14
Se	0.20 ± 0.03	0.16 ± 0.04
V	0.40 ± 0.03	0.38 ± 0.05
Zn	30 ± 3	25 ± 3
Al	68 ± 4	59 ± 8
As	0.35 ± 0.01	0.33 ± 0.05
Cd	0.05 ± 0.01	0.04 ± 0.02
Ni	2.66 ± 0.30	2.31 ± 0.37
Sr	72.1 ± 1.0	68.2 ± 2.6

STD – standard diet; HFHCD – high fat high carbohydrate diet.
Data expressed as mean ± SD.

data regarding the association between metal levels in the organism and NAFLD are inconsistent.

Essential trace elements also play a significant role in chronic liver diseases [12]. The most studied essential trace element in NAFLD is iron. In particular, it has been demonstrated that iron and copper dyshomeostasis may significantly contribute to NAFLD pathogenesis [13]. In particular, iron overload is tightly associated with NAFLD through activation of inflammation and oxidative stress [14]. However, certain contradictions exist. In particular, it has been demonstrated that both iron excess and deficiency may be associated with increased hepatic lipogenesis [15]. Experimental studies also revealed low hepatic iron in high fat fed animals [16]. Taking into account the role of insulin resistance [17] and oxidative stress [18] in NAFLD, other trace elements possessing insulin sensitizing (Cr, V, and Zn) and antioxidant properties (Se, Zn) [19] may also have a significant impact on the disease development. At the same time, data on the association between NAFLD and the level of these elements are still insufficient.

Nutritional strategies including trace element treatment are used for management of NAFLD [20]. Consequently, detailed data on mineral status of the organism in NAFLD are required for effective management of the disease.

Therefore, the primary objective of the study was to assess the level of metals and trace elements in liver, serum, and hair of rats with diet-induced NAFLD using inductively coupled plasma dynamic reaction cell mass spectrometer (ICP-DRC-MS).

2. Materials and methods

2.1. Experimental design

The protocol of the present investigation was approved by the Local Ethics Committee. All animal studies have been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments. A total of 56 female 3-months-old Wistar rats divided into two equal groups were used in the present study. The first group of animals (Control) obtained a standard diet (STD) containing 10% calories from fat (270 kcal/100 g, "Orenburg food mixture factory", Orenburg, Russia) and pure drinking water with a total mineralization of not more than 250 mg/l. Animals from the second group (NAFLD) were maintained on a lard-based high-fat diet containing 60% calories from fat (429 kcal/100 g) and also obtained a 10% sucrose solution instead of drinking water (40 kcal/100 ml). No significant difference in trace elements content was detected between the STD and HFHCD (Table 1). The earlier data demonstrate that high-fat high-carbohydrate diet (HFHCD) is an effective dietary model to induce NAFLD in laboratory rodents [21]. The animals had free access to food and drinking solutions throughout the experiment. The mean chow consumption in STD and HFHCD groups were 23.5 ± 8.7 g and 24.5 ± 6.3 g, respectively (p = 0.451). The temperature in the animal room was 22 ± 2 °C. The light and dark cycles in the animal room were 12 each (8.00–20.00). The total duration of dietary intervention was 6 weeks. At the end of the experiment liver was collected through a median laparotomic incision. Venous blood was collected from jugular vein with subsequent centrifugation to obtain serum. Hair samples were collected from the cranial part of the spine using ethanol-precleaned stainless scissors.

2.2. Morphometric and histological study

Body weight and naso-anal length (body length) were assessed in all animals. The values of thoracic circumference (TC) and abdominal circumference (AC) were used for the calculation of the AC/TC ratio.

The obtained liver samples (median lobe) were fixed in neutral buffered formalin. After embedding in paraffin, the blocks were sliced using microtome to obtain 5 μm-thick slices. The slices were stained with hematoxylin and eosin using standard techniques. The obtained sections were assessed and photographed using LOMO Micmed-6 (Lomo, Russia) microscope equipped with digital camera. Both periportal and centrilobular areas were assessed and photographed. Hepatocyte nucleus and lipid droplet areas were assessed using ImageJ software (NIH, Bethesda, MD, USA).

2.3. Blood biochemistry

The obtained serum was examined for glucose, total protein, albumin, triglycerides (TG), total cholesterol (TC), low density lipoprotein cholesterol (LDL-C), and high density lipoprotein cholesterol (HDL-C) concentrations and alanine aminotransferase (ALT) activity using the respective Olvex kits (St. Petersburg, Russia). Serum total lipid levels were assessed using Lachema kit (Lachema, Brno, Czech Republic) spectrophotometrically. Circulating insulin levels were estimated by means of enzyme-linked immunosorbent assay using Alpco kit (Alpco Diagnostics, Windham, NH). The values of serum glucose and insulin were used for calculation of insulin resistance index by the homeostasis model assessment (HOMA-IR) as follows: HOMA-IR = (glucose × insulin)/22.5 [22].

2.4. Oxidative stress biomarkers

Serum and liver samples were used for assessment of routine biomarkers of oxidative stress. Liver homogenization (1:10; w/v) for determination of total thiols (TSH) and thiobarbituric acid-reactive substances (TBARS) level was performed in an ice-cold 1/15 M phosphate buffer (pH = 7.4) with subsequent centrifugation (3000 × *g*, 10 min, 4 °C). The obtained supernatant was used for analysis. Serum and supernatant levels of TSH [23] and TBARS [24] were assessed spectrophotometrically at PD-303UV spectrophotometer (Apel, Japan). The obtained levels were expressed per mg of protein in a sample. The total protein concentration in liver homogenate was determined using Lowry-Folin method [25].

2.5. Metal and trace element analysis

The obtained samples were differentially prepared for trace elements analysis. Liver samples were rinsed with ice-cold distilled deionized water and separated from connective tissue using precleaned stainless instruments. The obtained hair samples were washed with acetone and rinsed thrice with distilled deionized