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Early High-Fat Feeding Induces Alteration of Trace Element Content in Tissues of Juvenile Male Wistar Rats

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Abstract The primary objective of the current study was to assess the influence of early high-fat feeding on tissue trace element content in young male Wistar rats. Twenty weanling male Wistar rats were divided into two groups fed standard (STD) or high-fat diet (HFD) containing 10 and 31.6 % of total calories from fat, respectively, for 1 month. Serum lipid spectrum, apolipoproteins, glucose, insulin, adiponectin, and leptin levels were assessed. The level of trace elements was estimated using inductively coupled plasma mass spectrometry. High-fat feeding significantly increased epididymal (EDAT) and retroperitoneal adipose tissue (RPAT), as well as total adipose tissue mass by 34, 103, and 59 %, respectively. Serum leptin levels in HFD animals were twofold higher than those in the control rats. No significant difference in serum lipid spectrum, apolipoproteins, glucose, adiponectin, and insulin was detected between the groups. HFD significantly altered tissue trace element content. In particular, HFD-fed animals were characterized by significantly lower levels of Cu, I, Mn, Se, and Zn in the liver; Cr, V, Co, Cu, Fe, and I content of EDAT; Co, Cu, I, Cr, V, Fe, and Zn concentration in RPAT samples. At the same time, only serum Cu was significantly depressed in HFD-fed animals as compared to the control ones. Hair Co, Mn, Si, and V levels were significantly increased in comparison to the control values, whereas Se and I content was decreased. HFD feeding induced excessive adiposity and altered tissue trace element content in rats without insulin resistance, adiponectin deficiency, and proatherogenic state. Hypothetically, trace element disbalance may precede obesity-associated metabolic disturbances.

Keywords Adiposity · Chromium · Vanadium · Adipose tissue · Obesity

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Introduction

Obesity is expanding worldwide and has more than doubled since 1980 [1]. In particular, in 2014 1.6 billion of adults were overweight whereas 600 million of them were obese [1]. Current projections indicate that the incidence of overweight and obesity will increase in 2030 to 2.2 and 1.1 billion, respectively [2]. At the same time, certain studies demonstrate that the prevalence of obesity is stabilizing [3]. Moreover, some authors predict the end of obesity epidemic [4]. Independently of the possible future trends, obesity remains a serious public health concern.

Special attention is also given to childhood obesity that has been significantly increased over the past three decades [5].

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According to WHO report, the number of overweight children under the age of five was 42 million in 2013 [1]. Childhood obesity has a significant impact on adult health [6]. Particularly, childhood obesity significantly increases the risk of metabolic syndrome in adults [7]. Recent studies demonstrated multiple causes of childhood obesity, including genetic, prenatal, early life, family factors, physical activity, and diet [8].

Trace elements play a significant role in human biology due to their signaling, cofactor, and structural functions [9]. Impairment of trace element status may have a high impact on the development of certain diseases [9]. In particular, trace elements like Cr, Zn, V, and Se are considered to be the key elements in glucometabolic disorders due to their insulin-mimetic and antioxidant effect [10]. Moreover, numerous studies also demonstrated alteration of iron homeostasis in obesity [11, 12] Multiple clinical [13–17] and experimental [18–22] studies demonstrated a tight interplay between trace element balance and obesity-related disorders. At the same time, the majority of experimental data are based on dietary intervention in adult animals, whereas data on the influence of dietary regimen on trace element status in early age are insufficient.

Therefore, the objective of the study is assessment of the influence of early high-fat feeding on trace element content in various tissues in young male Wistar rats.

Materials and Methods

Diet

A standard granulated chow ("Orenburg food mixture factory", Orenburg, Russia) containing 270 kcal/100 g (20 % protein, 70 % carbohydrate, 10 % fat) was used as a standard diet (STD). Lard-supplemented high-fat diet (HFD) contained 31.6 % calories from fat, 15.2 % from protein, and 53.2 % from carbohydrates. Mineral content of the diets is presented in Table 1.

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

Element	STD	HFD
Co	1.0 ± 0.1	0.95 ± 0.1
Cr	0.65 ± 0.05	0.55 ± 0.05
Cu	16 ± 2	15 ± 1
Fe	210 ± 20	195 ± 20
I	1 ± 0.1	0.95 ± 0.1
Mn	80 ± 10	75 ± 10
Se	0.20 ± 0.03	0.17 ± 0.02
V	0.40 ± 0.03	0.35 ± 0.03
Zn	30 ± 3	27 ± 3

Data expressed as mean values and the respective standard deviations

Animals

The protocol of 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 10 female 3-months-old Wistar rats were used in the current experiment. After 2-week acclimatization, the female rats were mated with 10 male 3-months-old Wistar rats. The presence of spermatozoa in vaginal smear was used as criteria of the pregnancy in rats. After mating, the dams were separated into the individual cages. All dams were fed STD during pregnancy and lactation (21 day postpartum). At weaning, all littermates were weighted and divided by sex. Male littermates were used in the study. Male rats were divided into two equal groups maintained at STD and HFD for 1 month in order to model early stages of obesity. All animals had free access to the respective diets and drinking water. 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).

At the end of experiment the animals were weighted. The visceral adipose tissue (AT) and liver were collected through a median laparotomic incision. In particular, epididymal (EDAT) and retroperitoneal adipose tissue (RTAP) depots were collected bilaterally after separation from the underlying tissues. Large blood vessels were removed from the fat pads. The obtained samples were used for trace element analysis. In turn, EDAT was used for histological staining and subsequent morphometry. Blood was collected from jugular vein whereas hair was collected from the cranial part of the spine.

Histological Study

The obtained adipose tissue samples were fixed in neutral buffered formalin and embedded in paraffin blocks. The blocks were sliced using microtome to obtain 5-μm-thick slices. The obtained 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. Analysis of adipocyte area was performed using ImageJ (NIH, Bethesda, MD, USA).

Biochemical Studies

The obtained blood serum was used for biochemical analysis. Serum was analyzed for total cholesterol (TC), triglycerides (TG), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), and glucose spectrophotometrically using the respective Roche kits. Serum levels of apolipoprotein A_1 ($ApoA_1$) and B (ApoB) were assessed using immunoturbidimetric method with specific antisera. All the above mentioned analyses were performed at an

automated biochemical analyzer "COBAS Integra 400 plus" (Roche Diagnostics Ltd., Switzerland).

Serum levels of insulin (AccuBind, Monobind, Inc., USA), leptin (Biovendor, Czech Republic), and adiponectin (USCN Life Science Inc., China) were assessed using enzyme-linked immunosorbent assay at Multiscan MS spectrophotometer (Labsystem Multiscan MS, Labsystem, Helsinki, Finland) according to the manufacturer's instructions. The obtained data on serum glucose and insulin levels were used for calculation of insulin resistance index by the homeostasis model assessment (HOMA-IR) as follows [23]: HOMA-IR = (glucose × insulin)/22.5.

Chemical Analysis

The obtained hair samples were washed with acetone and rinsed three times with distilled deionized water with subsequent drying at 60 °C on air. Blood serum was diluted with an acidified diluent (1:15 *v*/*v*) containing 1 % 1-butanol (Merck KGaA, Darmstadt, Germany), 0.1 % Triton X-100 (Sigma-Aldrich, Co., St. Louis, USA), and 0.07 % HNO_3 (Sigma-Aldrich, Co., St. Louis, USA) in distilled deionized water. Fifty milligram of all studied samples were introduced into Teflon tubes containing concentrated HNO_3 for microwave digestion in Berghof speedwave four (Berghof, Eningen, Germany) system for 20 min at 180 °C.

Hair, serum, liver, and adipose tissue trace element content was assessed using NexION 300D (PerkinElmer Inc., Shelton, CT 06484, USA) equipped with ESI SC-2 DX4 (Elemental Scientific Inc., Omaha, NE 68122, USA) autosampler. System's calibration was performed using 0.5, 5, 10, and 50 μg/l solutions of the studied trace elements prepared from Universal Data Acquisition Standards Kit (PerkinElmer Inc., Shelton, USA) by addition of distilled deionized water acidified with 1 % HNO_3. Internal online standardization was performed using yttrium (^{89}Y) isotope Yttrium Pure Single-Element Standard (PerkinElmer Inc., Shelton, USA). Laboratory quality control was performed using certified reference materials of hair (GBW09101; Shanghai Institute of Nuclear Research, Shanghai, China) and serum (ClinCheck Plasma Control, lot 129, levels 1 and 2; RECIPE Chemicals + Instruments GmbH, Germany). The recovery rates for all studied elements exceeded 80 %.

Statistical Analysis

The obtained data were processed using Statistica 10.0 (Statsoft, Tulsa, OK, US). Data normality was assessed using Shapiro-Wilk test. Normally distributed data were expressed using mean and the respective standard deviations. Group comparisons were performed using one-way ANOVA. Medians and the respective 25 and 75 percentile boundaries were used for expression of values not characterized by a normal distribution. Mann-Whitney *U* test was used for comparative analysis. All differences were considered significant at $p < 0.05$.

Results

The obtained data demonstrate that early HFD did not significantly alter body weight in juvenile rats (Table 2). At the same time, HFD-fed rats were characterized by higher adiposity. In particular, EDAT, RPAT, and total AT mass in early HFD-fed animals exceeded the respective control values by 34, 103, and 59 %. Histological examination of adipose tissue also demonstrated a significant 82 % increase in adipocyte area in HFD-fed animals as compared to the control ones (Table 2).

Data on adipokine spectrum were generally in agreement with the morphometric parameters (Table 3). In particular, circulating leptin levels and leptin-to-adiponectin ratio in HFD-fed animals significantly exceeded the control values by a factor of more than two. However, no significant changes in serum adiponectin were revealed. Early HFD feeding for 1 month also did not result in insulin resistance. Particularly, no significant changes were detected in serum glucose, insulin, and HOMA-IR.

Despite a 24 and 20 % increase in serum TG and HDL-C levels, these changes were not significant (Table 4). Oppositely, early HFD feeding resulted in a significant 32 % decrease in serum LDL-C concentrations as compared to the control values. Similarly, no significant difference in serum $ApoA_1$ and ApoB levels as well as apoA-to-apoB ratio was revealed.

Despite the absence of significant changes in biochemical parameters, early HFD feeding significantly decreased liver trace element content. In particular, the liver Cu, I, Mn, Se, and Zn levels were decreased by 14, 26, 21, 15, and 10 % in

Table 2 Body weight, adipose tissue mass, and adipocyte area in control and high-fat-fed rats

	STD	HFD	*p* value
Initial weight, g	70.7 ± 9.8	75.0 ± 11.3	0.461
Final weight, g	287 ± 32	289 ± 19	0.785
EDAT, g	2.62 ± 0.40	3.49 ± 0.78	0.018*
RPAT, g	1.52 ± 0.40	3.08 ± 0.68	<0.001*
Total AT, g	4.14 ± 0.76	6.57 ± 1.27	0.001*
AA, μm^2	1298 ± 561	2339 ± 978	<0.001*

Data presented as mean ± SD

EDAT epididymal adipose tissue, *RPAT* retroperitoneal adipose tissue, *AT* adipose tissue, *AA* adipocyte area

*The group difference is significant at $p < 0.05$ according to one-way ANOVA

Table 3 Serum adipokines, insulin, glucose, and HOMA-insulin resistance index in experimental animals

Parameter	STD	HFD	*p* value
Adiponectin, ng/ml	12.3 ± 1.8	13.2 ± 3.4	0.495
Leptin, ng/ml	88.1 ± 9.9	213.4 ± 54.7	<0.001*
Leptin/adiponectin ratio	7.2 ± 0.96	17.9 ± 8.3	<0.001*
Glucose, mmol/l	10.9 ± 1.2	11.6 ± 1.2	0.273
Insulin, mIU/l	3.6 ± 0.99	3.2 ± 0.88	0.247
HOMA-IR	1.73 ± 0.41	1.7 ± 0.50	0.793

Data presented as mean ± SD

*The group difference is significant at $p < 0.05$ according to one-way ANOVA

comparison to the control values, respectively (Table 5). No significant group difference in hepatic levels of other trace elements was detected.

The level of trace elements in EDAT was also significantly altered by HFD at weaning (Table 5). The most prominent decrease was detected in EDAT Cr and V content, being two-fold lower than that in the control animals. Epididymal fat pad Co, Cu, Fe, and I levels were characterized by a 30, 27, 48, and 40 % decrease in comparison to the control values. Despite a 31 % decline in EDAT Zn content in HFD-fed animals, these changes were not significant.

Early HFD feeding also resulted in a significant decrease in RPAT Cr, Co, and I content, being lower than that in the control animals by a factor of 3, 2.5, and 2, respectively (Table 5). RPAT Cu and V levels were also 37 and 38 % lower than the respective control values. Retroperitoneal fat pad Fe and Zn content was also decreased by 22 and 37 % as compared to the control values, respectively. However, these changes were not significantly different.

Table 4 Serum apolipoprotein profile in control and high-fat-fed animals

Parameter	STD	HFD	*p* value
TG, mmol/l	0.97 ± 0.23	1.1 ± 0.39	0.520
TC, mmol/l	1.9 ± 0.37	2.1 ± 0.32	0.226
HDL-C, mmol/l	1.5 ± 0.29	1.7 ± 0.25	0.082
LDL-C, mmol/l	0.48 ± 0.13	0.31 ± 0.08	0.004*
ApoB, g/l	0.03 (0.02–0.03)	0.02 (0.01–0.03)	0.304
$ApoA_1$, g/l	0.01 (0.01–0.02)	0.01 (0.01–0.01)	0.492
ApoA/ApoB ratio	0.5 (0.3–1.0)	0.5 (0.3–1.0)	0.968

Data presented as mean ± SD (normally distributed) and as median and 25–75 percentile boundaries (abnormally distributed)

TG triglycerides, *TC* total cholesterol, *HDL-C* high-density lipoprotein cholesterol, *LDL-C* low-density lipoprotein cholesterol, *ApoB* apolipoprotein B, *ApoA1* apolipoprotein A_1

*The group difference is significant at $p < 0.05$ according to one-way ANOVA

In contrast to tissue trace element distribution, early HFD feeding significantly altered only serum Cu levels, being 21 % lower than that in the control animals (Table 6).

Hair trace element content was also significantly altered by early HFD feeding (Table 6). However, the observed changes were rather distinct from those in the studied tissues. In particular, HFD at weaning resulted in a significant increase in hair Co, Mn, Si, and V levels, being 50, 73, 36, and 50 % higher than the respective control values. At the same time, hair levels of I and Se in the high-fat-fed animals were 48 and 10 % lower than those in the control group.

Table 5 Trace element content (μg/g) in rats' liver, epididymal, and retroperitoneal adipose tissue

Element	STD	HFD	*p* value
Liver			
Co	0.040 (0.030–0.040)	0.040 (0.030–0.040)	0.714
Cr	0.020 (0.010–0.020)	0.020 (0.020–0.030)	0.392
Cu	4.070 (3.860–4.300)	3.495 (3.100–3.780)	0.002*
Fe	64.960 (52.700–67.450)	63.250 (56.450–70.000)	0.791
I	0.095 (0.070–0.110)	0.070 (0.060–0.080)	0.029*
Mn	2.725 (2.670–2.780)	2.145 (2.060–2.320)	<0.001*
Se	0.890 (0.870–0.920)	0.755 (0.740–0.780)	<0.001*
V	0.0008 (0.0007–0.0009)	0.001 (0.0008–0.001)	0.375
Zn	30.960 (28.930–33.410)	27.805 (24.480–28.840)	0.017*
EDAT			
Co	0.001 (0.001–0.002)	0.0007 (0.0006–0.0010)	0.013*
Cr	0.040 (0.020–0.080)	0.020 (0.010–0.020)	0.014*
Cu	0.260 (0.240–0.340)	0.190 (0.170–0.220)	0.008*
Fe	6.460 (5.400–7.670)	3.305 (2.900–4.140)	0.007*
I	0.050 (0.030–0.060)	0.030 (0.020–0.040)	0.047*
Mn	0.050 (0.040–0.060)	0.040 (0.040–0.040)	0.177
Se	0.0100 (0.0039–0.0400)	0.0065 (0.0039–0.0300)	0.631
V	0.0008 (0.0005–0.0010)	0.0004 (0.0002–0.0005)	0.001*
Zn	2.850 (2.030–3.130)	1.980 (1.580–3.020)	0.170
RPAT			
Co	0.0010 (0.0007–0.0010)	0.0004 (0.0004–0.0009)	0.018*
Cr	0.030 (0.010–0.030)	0.010 (0.007–0.010)	0.032*
Cu	0.340 (0.270–0.380)	0.215 (0.200–0.240)	0.003*
Fe	4.050(3.480–5.900)	3.165 (2.710–3.810)	0.113
I	0.040 (0.030–0.050)	0.020 (0.009–0.040)	0.049*
Mn	0.050 (0.040–0.060)	0.040 (0.030–0.050)	0.159
Se	0.130 (0.004–0.230)	0.130 (0.004–0.140)	0.549
V	0.0008 (0.0006–0.0010)	0.0005 (0.0004–0.0007)	0.006*
Zn	2.890 (2.020–3.180)	1.830 (1.570–2.660)	0.170

Data presented as Median and 25–75 percentile boundaries

EDAT epididymal adipose tissue, *RPAT* retroperitoneal adipose tissue

*The group difference is significant at $p < 0.05$ according to Mann-Whitney *U* test

Table 6 Trace element levels in serum (μg/ml) and hair (μg/g) of experimental animals

Element	STD	HFD	*p* value
Serum			
Co	0.002 (0.001–0.002)	0.002 (0.001–0.002)	0.597
Cr	0.005 (0.004–0.006)	0.0045 (0.004–0.007)	0.678
Cu	1.310 (1.110–1.510)	1.040 (0.950–1.090)	0.026*
Fe	2.450 (1.810–3.740)	2.340 (1.340–3.510)	0.705
I	0.080 (0.070–0.090)	0.090 (0.080–0.100)	0.496
Mn	0.006 (0.005–0.007)	0.006 (0.005–0.008)	0.791
Se	0.595 (0.500–0.680)	0.535 (0.410–0.590)	0.199
V	0.00025 (0.0002–0.0003)	0.0002 (0.0002–0.0003)	0.791
Zn	1.330 (1.130–1.780)	1.330 (1.200–1.430)	0.970
Hair			
Co	0.020 (0.020–0.030)	0.030 (0.030–0.040)	0.007*
Cr	0.100 (0.080–0.180)	0.145 (0.130–0.180)	0.307
Cu	11.300 (10.160–12.530)	12.345 (11.100–14.130)	0.091
Fe	15.600 (13.020–21.130)	17.700 (14.940–25.530)	0.275
I	5.730 (4.410–6.740)	2.935 (2.440–3.300)	<0.001*
Mn	4.520 (2.880–5.740)	7.820 (5.650–11.750)	0.002*
Se	0.460 (0.430–0.500)	0.410 (0.400–0.450)	0.029*
V	0.010 (0.007–0.010)	0.020 (0.010–0.020)	0.012*
Zn	174.000 (160.000–199.000)	172.000 (161.000–179.000)	0.460

Data presented as median and 25–75 percentile boundaries

EDAT epididymal adipose tissue, *RPAT* retroperitoneal adipose tissue

*The group difference is significant at $p < 0.05$ according to Mann-Whitney *U* test

Discussion

The obtained data demonstrate that early high-fat feeding did not result in significant weight gain, although excessive adiposity was observed. Increased circulating leptin levels also correspond to expanded adipose tissue mass [24]. Surprisingly, HFD feeding did not result in significant weight gain. At the same time, certain studies revealed even a decreased body weight in weanling animals fed a high-fat diet [25]. The absence of significant changes in serum adiponectin levels also indicates that the experimental animals were not characterized by adipocyte dysfunction [26] that is characteristic for severe obesity [27]. Taking into account an anti-inflammatory and insulin-sensitizing role of adiponectin [28], the similarity of concentrations between the HFD-fed and control animals demonstrates the absence of insulin resistance. This supposition is also confirmed by unaltered values of serum glucose, insulin, and HOMA-IR, that is widely used for assessment of insulin resistance [29].

The observed situation may occur due to short period of dietary intervention. In particular, it has been demonstrated that feeding C57BL/6 J mice with a diet consisting of 20 % fat and 1 % cholesterol induces significant changes in serum glucose, insulin, and HOMA-IR values only after 16 weeks [30]. At the same time, significant diet-induced alteration of serum TC and LDL-C was detected after 6 weeks of dietary intervention [30]. Moreover, feeding of weanling male Sprague-Dawley with a high-fat diet containing 45 % of kilocalorie from fat did not result in a significant change in blood glucose levels after 4, 8, or 12 weeks [31]. In addition, early age of animals may also significantly affect the efficiency of HFD feeding [32]. Moreover, it has been demonstrated that even 6 months feeding of weanling Sprague-Dawley with a diet containing 32 % of calories from fat did not significantly affect glucose and carbohydrate metabolism [33].

Therefore, early HFD feeding in weanling male Wistar rats resulted in excessive adiposity without insulin resistance and atherogenic changes. Hypothetically, the observed situation is characteristic for early stages in obesity development. Despite the absence of significant changes in serum markers of insulin resistance, HFD feeding in juvenile rats significantly altered trace element status. In particular, the most prominent changes were detected in the case of Cu, Cr, I, V, and Se.

HFD feeding in juvenile rats significantly decreased Cu content in the liver, adipose tissue, and serum. These findings are in agreement with the existing data. In particular, it has been shown that high fructose feeding significantly decreases plasma and liver Cu content in animals maintained on both Cu-adequate and Cu-deficient diet [34]. Earlier studies also demonstrated significantly decreased liver Cu levels in obese

mice [35] and rats [36] as compared to the control animals. Song and the coauthors (2012) hypothesized that diet-induced Cu deficiency due to alteration of duodenal Ctr1 expression may be a possible mechanism of fatty liver development [34]. It is also supposed that alteration of Cu homeostasis may be involved in pathogenesis of non-alcoholic fatty liver disease due to its influence on Fe status [37]. Data on adipose tissue Cu content in response to HFD feeding are insufficient. In particular, in our previous study, we failed to detect a significant decrease in adipose tissue Cu content in adult female rats fed a high-fat diet for 3 months [38]. Clinical data are more contradictory indicating an increase [39], decrease [40], or the absence of changes [41] in blood Cu levels in obese individuals.

Decreased adipose tissue Cr and V content are in agreement with our previous observation also indicating a significant association between high-fat induced decrease in adipose tissue Cr and V content and metabolic parameters in rats with excessive adiposity [21]. Taking into account the role of chromium [42] and vanadium [43] in insulin signaling, we have also supposed that a decrease in Cr and V levels in adipocytes may at least partially mediate obesity-related insulin resistance [44]. In the present animal model, we observed adipose tissue mineral dyshomeostasis without any other obesity-related metabolic disturbances like insulin resistance and atherogenic lipid profile. It is also notable that liver Cr and V content were not altered significantly by high-fat feeding. These data may be indicative of not a total Cr and V deficiency but rather the redistribution of these elements and its local deficiency in adipose tissue. The obtained data allow to propose that altered trace element status may precede more common metabolic disturbances. However, additional studies are required to highlight the causal relationships between low adipose tissue Cr and V and obesity.

We observed a significant decrease in hepatic and hair Se, being indicative of selenium deficiency. Since obesity is associated with oxidative stress [45], one can suppose that the obese organism faces increased requirements in selenium that is characteristic for prooxidant state [46]. Despite the presence of multiple indications on selenium deficiency in blood [47], data on selenium content in the liver are contradictory. Previous study demonstrated the absence of the influence of high fat [48] and cholesterol [49] feeding on liver Se content. Despite the absence of insulin resistance in the present model of excessive adiposity, it should be noted that fructose-induced metabolic syndrome, being associated with insulin resistance, was accompanied by a significant decrease in liver selenium [50].

A significant decrease in iodine status was also observed in high-fat-fed animals. These data are generally in agreement with the earlier studies indicating a significant association between obesity and thyroid dysfunction [51]. At the same time, the intimate mechanisms linking obesity and iodine status are unknown. For example, no indications on the influence of high-fat diet on iodine absorption exist to date [52].

Significant diet-induced alteration of Zn levels was observed only in the liver and adipose tissue to a lesser extent. These data are in agreement with the existing data on lower hepatic zinc content in genetically obese nice [53]. High-fat feeding also decreased zinc concentration in the liver [54, 55] and adipose tissue [22, 54]. Our data do not correspond to the earlier indications of decreased blood zinc levels [56, 57]. At the same time, certain studies also failed to reveal a significant alteration of circulating zinc levels in obesity [58].

At the same time, we failed to detect a significant alteration of iron status in HFD-fed rats. These data are contradictory to the results of the earlier studies demonstrating that obesity is accompanied by iron deficiency in general and hypoferremia in particular [59]. However, we also failed to detect an increase in adipose tissue iron resulting from its sequestration [12]. At the same time, earlier data obtained by Suliburska demonstrated that 6-week diet high in fat, fructose, and salt did not significantly alter liver metal content [60]. Taking into account the absence of obesity-associated metabolic disturbances in the present animal model, we suppose that iron dyshomeostasis has not developed.

Therefore, the obtained data indicate a significant diet-induced decrease in trace element content in the liver, adipose tissue, serum, and hair with the most prominent changes observed for Cu, Cr, I, V, and Se. It is notable that alteration of trace element homeostasis was detected even without diet-induced metabolic disturbances like inflammation, atherogenic dyslipidemia, and insulin resistance. Further studies involving different dietary manipulations like high-fat high-carbohydrate diet are required to assess the rate of trace element dyshomeostasis in obesity and the related diseases associated with inflammation and insulin resistance.

Current findings support our earlier hypothesis that altered adipose tissue trace element levels may be used as early markers of obesity-associated metabolic risk [44]. To our knowledge, this is the first report of early high-fat diet-induced alteration of trace element content in tissues in an animal model of excessive adiposity without metabolic disturbances.

Compliance with Ethical Standards

The protocol of 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.

Conflict of Interest The authors declare that they have no conflict of interest.

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