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NUTRITION

Adipose tissue chromium and vanadium disbalance in high-fat fed Wistar rats

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ABSTRACT

The primary objective of the current study is to investigate the relationship between adipose tissue chromium and vanadium content and adipose tissue dysfunction in a model of diet-induced obesity. A total of 26 female Wistar rats were fed either standard or high-fat diet (31.6% of fat from total caloric content) for 3 months. High-fat-feeding resulted in 21 and 33% decrease in adipose tissue chromium and vanadium content, respectively. No change was seen in hair chromium or vanadium levels. Statistical analysis revealed a significant inverse correlation of adipose tissue Cr and V with animal morphometric parameters and adipocyte size. Significant inverse dependence was observed between adipose tissue Cr and V and serum leptin and proinflammatory cytokines' levels. At the same time, adipose tissue Cr and V levels were characterized by positive correlation between serum adiponectin and adiponectin/leptin ratio. Adipose tissue Cr and V were inversely correlated ($p < 0.05$) with insulin and homeostatic model assessment insulin resistance index (HOMA-IR) levels. Cr and V concentrations were not correlated with serum glucose in either high-fat fed or control rats; however, both serum glucose and HOMA-IR levels were significantly higher in high-fat fed, compared to control, rats. The results allow to hypothesize that impairment of adipose tissue Cr and V content plays a certain role in the development of adipose tissue endocrine dysfunction in obesity.

Introduction

Chromium and vanadium are redox metals taking part in a number of cellular functions [1]. Chromium was considered to be essential for humans more than 50 years ago [2,3], however at the current moment a significant contradiction on chromium essentiality exists [4]. Vanadium is essential metal for several organisms [5], while its essentiality for mammals and humans in particular is unknown. At the same time, the role of numerous chromium and vanadium compounds as potent insulinomimetics has been demonstrated during the last decades. It is shown that the use of these compounds improves insulin sensitivity both in experimental and clinical studies [6–8]. Moreover, data on antiobesity potential of chromium and vanadium compounds exist [9,10]. Although experimental studies indicate a decrease in adipose tissue content in chromium-treated animals [11], clinical data remains controversial [12,13]. Nevertheless, a number of chromium and vanadium compounds can be considered as perspective preparations for metabolic syndrome treatment. At the same time, single indications of vanadium and chromium imbalance in obesity exist. In particular, a decrease in serum chromium was observed in a cohort of patients suffering from obesity [14]. Therefore, the primary objective of the current study is to investigate the relationship between adipose tissue (AT) chromium and vanadium content and adipose tissue dysfunction in a model of diet-induced obesity.

Abbreviations: STD, standard diet; HFD, high-fat diet; BMI, body mass index; TC, thoracic circumference; AC, abdominal circumference; AI, adiposity index; AT, adipose tissue; TNFα, tumor-necrosis factor-α; IL-6, interleukine-6; ELISA, enzyme-linked immunosorbent assay; MCP-1, macrophage chemoattractant protein-1; A/L, adiponectin/leptin ratio; HOMA-IR, homeostatic model assessment insulin resistance index; MinFeret, minimal Feret diameter; ANOVA, analysis of variance; NF-kB, nuclear factor kappa B.

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Materials and methods

Animals and treatment

This research was approved by the Local Ethics Committee of the Orenburg State Medical Academy. The rats had been acclimatized to the laboratory conditions with the temperature of 20 ± 2 °C during the two weeks prior to the experiment. The light and the dark cycles in the animal room were 12 h each.

A total of 26 rats was divided into two groups with equal body weight ($n = 13$). The rats in group I were fed STD (Control), whereas the animals from group II were maintained on HFD.

A 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). High-fat diet (HFD) was based on lard supplementation with 31.6% of fat from total caloric content. Both diets were not supplemented with chromium and vanadium. Basal chromium content in the experimental diets (STD and HFD) was 0.65 ± 0.05 and 0.57 ± 0.05 mg/kg, whereas vanadium content was 0.40 ± 0.03 and 0.35 ± 0.03 mg/kg, respectively, as estimated by ICP-MS analysis. The rats were fed ad libitum. Food consumption was measured daily at the same time. The total duration of the experiment was 3 months (90 days).

Animals' morphometric studies

Morphometric parameters were evaluated at the end of the experiment. The values of body mass and naso-anal length were used for body mass index (BMI) calculation (g/cm^2). The values of thoracic circumference (TC) and abdominal circumference (AC) were used for the calculation of the AC/TC ratio. After the morphometric studies, the dissection was performed through a median laparotomic incision. The parametrial adipose tissue (MAT) pads were dissected and weighted. The values of fat pad weight were used for adiposity index (AI) calculation [15]. The samples of adipose tissue were used for the subsequent analysis.

Chromium and vanadium assay

Adipose tissue vanadium and chromium content in the harvested samples was analyzed using ICP-MS spectrometer Elan 9000 (Perkin-Elmer, USA).

Biochemical analysis

The rats' serum was examined for circulating adipokines, proinflammatory cytokines, insulin and glucose. Serum glucose concentration was established using Roche kit. For determination of tumor-necrosis factor-α (TNFα) and interleukine-6 (IL-6) eBioscience enzyme-linked immunosorbent assay (ELISA) rat kits were used according to the manufacturer's instructions. The serum circulating adiponectin and macrophage chemoattractant protein-1 (MCP-1) concentrations were measured by commercial ELISA rat kits (USCN Life Science Inc.). Serum rat insulin and leptin were estimated with AccuBind and Biovendor ELISA rat kits, respectively. The values of serum insulin and glucose levels were used for calculation of HOMA-IR according to the existing formula [16]. Adiponectin and leptin concentrations were used for calculation of adiponectin/leptin ratio (A/L) being an important parameter of insulin resistance and metabolic syndrome [17].

Adipocyte cytometry

The PMAT samples were fixed in neutral buffered formalin and subsequently embedded in paraffin blocks. The blocks were sliced using freezing microtome to obtain 5 μm thick slices. The samples were stained with hematoxylin and eosin using standard techniques. The obtained AT sections were examined and photographed using LOMO Micmed-6 (Lomo, Russia) microscope and ×150 zoom. Subsequent morphometry of adipocytes was assessed using the ImageJ software from the U.S. National Institutes of Health [18]. Area, perimeter and minimal Feret diameter (MinFeret) of fat cells were calculated.

Table 1
Influence of the diet type on morphometric parameters.

	STD	HFD	*p*-value
Body mass, g	289.15 ± 21.24	330.31 ± 16.94*	0.000013
Length, cm	20.73 ± 0.59	20.58 ± 0.60	0.354856
TC, cm	15.58 ± 0.76	16.31 ± 0.83*	0.027829
AC, cm	18.15 ± 0.97	19.46 ± 1.01*	0.002508
AC/TC	1.17 ± 0.05	1.19 ± 0.05	0.171063
BMI	0.67 ± 0.06	0.78 ± 0.07*	0.000218
AT, g	6.95 ± 2.37	14.35 ± 3.63*	0.000002
AI, %	2.58 ± 0.73	4.23 ± 0.96*	0.000013

Data presented as mean ± SD.
* Significant differences between STD and HFD values according to one-way ANOVA. Individual *p*-values of the statistical difference are indicated.

Statistical analysis

The obtained data are expressed as mean values ± standard deviation (mean ± σ). One-way ANOVA was used for data processing. Fisher's least significant difference test was used for the group mean comparisons at the significance level $\alpha = 0.05$. Pearson's correlation coefficient was used for correlation analysis. All statistical analyses were performed using Statistica 10 (StatSoft Inc., 2011).

Results

Animal morphometric parameters and food consumption

HFD-feeding resulted in increased values of animal morphometric parameters (Table 1). In particular, HFD-fed rats were characterized by significantly increased values of body mass, AC and BMI by 14, 7 and 16% in comparison to the control group, respectively. It is important to note that changes in body length and AC/TC ratio were not significant between the groups. HFD consumption resulted in more than twofold increase in adipose tissue mass. The values of AI were also increased by 64% when compared to the control animals.

Analysis of feeding behavior failed to reveal significant difference between the daily values of food consumption of STD (19.11 ± 2.23 g/day) and HFD-fed (22.26 ± 4.58 g/day) animals; however, the latter had a tendency to hyperphagia.

Adipocyte cytometry

Adipocyte cytometry revealed a significant increase in adipocyte size after HFD-consumption (Table 2). In particular, chronic HFD-feeding resulted in a 143% increase in adipocyte area. The values

Table 2
Influence of the diet type on adipocyte size.

	STD	HFD	*p*-value
Area, μm^2	1116.27 ± 787.22	2719.10 ± 1944.23*	0.000001
Perimeter, μm	163.22 ± 50.96	250.28 ± 85.87*	0.000001
MinFeret, μm	31.33 ± 10.19	47.06 ± 16.49*	0.000001

Data presented as mean ± SD.
* Significant differences between STD and HFD values according to one-way ANOVA. Individual *p*-values of the statistical difference are indicated.