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The effect of peripheral chronic salsolinol administration on fat pad adipocytes morphological parameters

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Abstract: Salsolinol (1-methyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline) is thought to regulate dopaminergic neurons and to act as a mediator in the neuroendocrine system. We have previously reported that exogenous salsolinol evokes enteric neuronal cell death, leading to the impairment of myenteric neurons density and abnormal intestinal transit in rats. We also observed significant reduction of body weight, related to the disrupted gastrointestinal homeostasis.
The aim of current study was to evaluate the influence of prolonged salsolinol administration body weight, food intake, adipose tissue accumulation and fad pad adipocyte morphological parameters assessed by image analysis. Male Wistar rats were subjected to continuous intraperitoneal low dosing of salsolinol — 200 mg/kg in total with ALZET osmotic mini-pumps (Durtec, USA) for 2 or 4 weeks with either normal or high-fat diet. Appropriate groups served as the controls. Food intake, body weight were measured each morning. Both epididymal fat pads were dissected, weighted and processed for routine hematoxylin and eosin staining. The following parameters: cell area, perimeter, long and short axis, aspect ratio and circularity factor were assessed in stained specimens with the image analysis system (Multisoft, Polska).
Salsolinol administration significantly reduced total body mass with no differences in total food intake between the groups. The epididymal fat pad weight over final body mass ratio was lower in salsolinol treated rats on high fat diet in comparison with the control groups. The area, perimeter, short and long axis of the fad pad adipocytes were significantly decreased in salsolinol treated animals in comparison with relevant controls. Salsolinol targets some regulatory mechanisms concerned with the basic rat metabolism. Prolonged peripheral salsolinol administration in rats significantly decreases the adipocyte size, and such effect is related to the weight loss and reduced adipose tissue accumulation.

Key words: salsolinol, rat, epididymal fat pad, weight loss, adipocyte area, image analysis, morphometry, osmotic minipumps.

Introduction

In Parkinson's disease (PD) patients, a broad spectrum of non-motor symptoms have been reported, which in some circumstances may precede motor impairment. These symptoms include weight changes and gastrointestinal dysregulation, such as dysphagia, delayed gastric emptying, nausea, constipation and faecal incontinence [1]. Weight loss is frequent in patients with Parkinson's disease. Dysphagia, anorexia, sense of smell and appetite loss as well as gastrointestinal dysfunction may be possible causes of reduced energy intake; while rigidity, tremor, and levodopa-induced dyskinesia may increase energy expenditure. Moreover, some drugs e.g. levodopa may enhance glucose metabolism resulting in enhanced energy expenditure. Depression, antiparkinsonian drugs, medical complications such as pneumonia and malignancies may also facilitate weight loss in PD. Weight loss is associated with malnutrition and may precipitate infection, accelerate motor, behavioral and autonomic impairment in PD patients [2–4]. The mechanisms of weight loss in these patients, however, remain poorly understood.

In order to elucidate the possible mechanisms leading to weight loss in parkinsonian-like degenerations, we conducted previously few experimental studies using exogenously administrated salsolinol in rats [5–8].

The catechol isoquinoline derivatives are compounds widely present in the mammalian brain and the most investigated one is referred to as salsolinol (1-methyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline). It was detected for the first time in rat and human brain tissue samples [9, 10]. It exerts a wide range of effects on the catecholaminergic transmission and dopaminergic neurons. It has been documented that salsolinol can cause the release of stored catecholamines, the inhibition of catecholamine reuptake, the inhibition of monoamine oxidase, catechol-O-methyltransferase and tyrosine hydroxylase [11–13]. In addition, it has been implicated in the development of alcoholism, in the release of the hormone prolactin [14] and in the etiopathogenesis of Parkinson's disease [15, 16]. Salsolinol can be either endogenously synthesized from dopamine and acetaldehyde by salsolinol synthase and alternatively, from dopamine and pyruvic acid [17] or delivered exogenously with food [18]. Various toxins, tetrahydroizochinolines among them, may easily cross the intestinal barrier and reach the intestinal, neural pathways, initiate the processes of neurodegeneration in enteric nervous system and subsequently, via dorsal motor nucleus of the vagus nerve (dmX), in the central nervous system. Uptake of exogenous substances from the extraneuronal space is known to occur at the axon terminal and such material is to be transferred to the cell soma via retrograde axonal transport. Lots of neuroactive substances are taken up this way, usually through receptor-mediated endocytosis by the presynaptic membrane of the next nerve cell in the neuronal chain. And, such a neurotropic constituent might induce conformational changes in normal α-synuclein molecules, provoking their aggregation and formation of Lewy Bodies [19].

The vagus nerve consists of axons, which emerge from or converge onto three nuclei of the medulla, including the dorsal nucleus of vagus – which sends parasympathetic output to the viscera, especially the intestines. The vagus nerve contributes to the bidirectional communications between the gastrointestinal tract and central nervous system. Afferent neurons of the vagus nerve are important targets of gut hormones, particularly the hormones involved in controlling food intake. Vago-vagal reflexes are involved in feeding homeostasis, making neuromodulation an attractive method for obesity treatment [20].

In animal models with vagal nerve stimulation (VNS) decreased food intake and weight loss are considered as the result of the stimulation of brain centers, the peripheral action of vagal stimulation via short cholinergic reflexes, and the combination of central and peripheral signals [21]. It is also hypothesized that the VNS decreases both food intake and body weight gain by mimicking the "satiety" signals transmitted from the gut to the brain, leading to the activation of the hypothalamic neurons that initiate the state of satiety. VNS also exerts anorexigenic effects on food intake and body weight gain in rats with high-fat diet-induced obesity [22–24].

Body weight, food intake and body fat content are regulated by multiple factors [20, 25, 26]. Despite fluctuations in the amount of food consumed, body weight remains stable within a relatively narrow range. Food intake together with body mass is controlled by both short- and long-term regulatory mechanisms [27]. Hypoglycemia increases food intake and stimulates vagal nerve activity [28]. Food transported into the stomach and duodenum activates both chemo- and mechanoreceptors. These signals are also transmitted via the vagus nerve to the hind brain where they are integrated and play a major role in short-term regulation by limiting the size of the meal consumed [25]. The long-term control of food intake involves many mediators (ghrelin, leptin, nesfatin-1, orexins and neuropeptide Y) and structures (nucleus of the solitary tract, arcuate nucleus of vagus nerve and hypothalamus) [26].

Vagus nerve stimulation influences the brain centers controlling appetite, body weight and also the adipose tissue accumulation. In rodents, fat tissue is localized in different depots, and visceral fat accumulates in the epididymal fat pad. This visceral fat is well delimitated and easy to excise. Although the epididymal fat pad represents only a small portion of the total body weight, previous studies have shown that the epididymal fat pad weight calculated as a proportion of total body weight is highly correlated with total body fat in mice and rats [29–31]. Moreover, the caudal epididymal fat pad is under strong hormonal and nervous regulation [32–34]. Significant reduction of fat pad weight in rats following long-term VNS has been previously reported [22–24]. When the body composition was altered by electrical vagus nerve stimulation; epididymal fat pad mass relative to body weight was significantly lower in rats that received VNS compared with control animals.

In our previous experiments, we applied salsolinol given peripherally and continuously in rats to induce changes in the enteric nervous system, which might be similar

to those observed in PD patients. Surprisingly, we noted significant decrease in body weight of the animals during salsolinol exposure. We hypothesized that salsolinol given peripherally can diminish food consumption and body weight via vagus afferents since it targets vagus nerve fibers and other centers responsible for appetite controlling [5–7]. Furthermore, while lipid profile, postprandial blood glucose and hepatic enzymes levels remained indifferent, postprandial triglyceridemia was significantly lower in all salsolinol-treated rats in comparison with the control, which might be related to disturbed absorption. We also suggested that diminished body weight gain and lower adipose tissue accumulation in salsolinol-treated animals were due to delayed gastric emptying together with disturbed gut function resulting in absorptive dysfunction. In the current study, we assessed the fat pad adipocytes morphological properties using image analysis methods, in order to verify the influence of peripheral salsolinol administration on visceral fat accumulation in rats.

Material and methods

Study protocol

Thirty-two adult male Wistar rats were studied. The animals were housed in individual cages. All animals were housed in the same optimal conditions, and food and water were provided ad libitum. During experiment, rats were fed with either standard diet (2.86 kcal/g, Labofeed, Poland) or with obesity-inducing high-fat diet (4.34 kcal/g, Bento Kronen Products, Belgium). The temperature was maintained at 23 ± 2°C, and animals were placed on a 12:12 h dark/light cycle. The Jagiellonian University Bioethical Committee approved the care and use of the animals (ethical approval number — 67/2009).

The detailed methods of salsolinol administration were described previously [5–7]. Briefly, rats were either subjected to continuous dosing of salsolinol or served as control. Salsolinol (salsolinol hydrochloride, Sigma, USA) in the total dose of 200 mg/kg was dissolved in 200 μL of 0.9% solution of salt and delivered by ALZET osmotic mini-pumps (Durtec, USA) implanted intraperitoneally. Control groups were implanted with ALZET osmotic mini-pumps filled with physiological solution of salt.

Prior to implantation rats were randomly divided into the following eight groups (n = 4 each): 1) rats subjected to continuous dosing of salsolinol for two weeks (delivery rate 0.5 μL/h, S1 group) and fed with standard diet throughout the entire experimental period; 2) rats subjected to continuous dosing of salsolinol for two weeks (delivery rate 0.5 μL/h, SF1 group) and fed with high-fat diet throughout the entire experimental period; 3) rats subjected to continuous dosing of salsolinol for four weeks (delivery rate 0.25 μL/h, S2 group) and fed with standard diet throughout the entire experimental period; 4) rats subjected to continuous dosing of salsolinol for four weeks (delivery rate 0.25 μL/h, SF2 group) and fed with high-fat diet throughout the entire experimental

period; 5) a control group fed with standard diet for two weeks (C1 group); 6) a control group fed with high-fat diet for two weeks (CF1 group); 7) a control group fed with standard diet for four weeks (C2 group); 8) a control group fed with high fat-diet for four weeks (CF2 group). Rats were starved for 12 hours and operated under general anesthesia induced with sodium pentobarbital given intraperitoneally at a dose of 0.25 mg/kg (Vetbutal, Biowet, Poland).

During the study, daily food intake and body weight were measured each morning. At the end of the experiment (day 15 or 29, respectively), animals were euthanized by decapitation and both epididymal fat pads, located between the cauda epididymis and the distal extremity of the testis, were dissected from each rat and weighed. The epididymal fat pad/body weight ratio was calculated by dividing the fat pad weight by the final body weight.

Histological staining and morphometrical analysis

Fresh fat pad specimens were cut and rinsed thoroughly with PBS (phosphate-buffered saline, 0.01 mol/L, pH = 7.4). Tissue samples were then fixed in 4% phosphate-buffered paraformaldehyde, routinely processed and embedded in paraffin. Serial sections were cut and mounted on glass slides. The sections were deparaffinized in xylene, rehydrated through a graded series of alcohol, and transferred to PBS prior to staining. Histopathological examination and morphometrical analysis were performed after routine staining with hematoxylin and eosin (H&E).

The adipocytes were analyzed semi-quantitatively under light AXIOPHOT microscope (ZEISS, Germany) connected with ProgResC12 plus (Jenamed, Germany) CCD camera using image cytometry system (Multiscan v.18; Computers Scanning System, Warszawa, Poland). At least 100 cells were counted in each specimen. The following parameters were assessed: cell area, perimeter, long and short axis, aspect ratio A_R (calculated as the ratio of the largest diameter and the smallest diameter) and circularity factor *f* (calculated as follows: $f = 4\pi A/P^2$; A — area, P — perimeter).

All data were expressed as the mean and standard deviation (SD). The results were analyzed using a one-way analysis of variance (ANOVA) followed by a post hoc Tukey's test. All statistical tests were performed using STATISTICA software package (StatSoft, Tulsa, USA). Statistical significance was set at $p < 0.05$.

Results

Food intake, body weight gain and epididymal fat pad weight in rats after salsolinol administration are summarized in Table 1 and Table 2.

Table 1. Food intake, body weight gain and epididymal fat pad weight in salsolinol-treated (S1, SF1 — rats for two weeks and fed with standard or high-fat diet, respectively) and control rats (C1, CF1 — control rats fed with standard or high-fat diet, respectively). Asterisks (*) indicate significant differences between the salsolinol-treated and control groups, $p < 0.05$.

Group	S1	C1	SF1	CF1
Total food intake (g)	340.4 ± 15.2	360.2 ± 24.6	238.7 ± 26.6	253.5 ± 36.3
Initial body weight (g)	253.7 ± 9.9	251.5 ± 9.1	243.2 ± 4.7	220.0 ± 9.4
Final body weight (g)	312.8 ± 14.1	334.0 ± 8.9	314.1 ± 19.8	318.0 ± 4.9
Body weight gain (g)	59.1 ± 5.9*	81.6 ± 2.7	70.9 ± 18.2*	98.0 ± 13.9
Epididymal fat pad weight (EFP) (g)	3.4 ± 0.3	3.8 ± 0.4	4.1 ± 0.4*	5.1 ± 0.2

Table 2. Food intake, body weight gain and epididymal fat pad weight in salsolinol-treated (S2, SF2 — rats for four weeks and fed with standard or high-fat diet, respectively) and control rats (C2, CF2 — control rats fed with standard or high-fat diet, respectively). Asterisks (*) indicate significant differences between the salsolinol-treated and control groups $p < 0.05$.

Group	S2	C2	SF2	CF2
Total food intake (g)	644.3 ± 55.1	696.9 ± 39.1	429.3 ± 36.3	509.3 ± 24.1
Initial body weight (g)	23.9 ± 4.9	232.5 ± 11.9	234.2 ± 8.5	225.7 ± 7.6
Final body weight (g)	351.1 ± 33.5	366.8 ± 18.4	331.8 ± 24.9	387.5 ± 17.9
Body weight gain (g)	98.3 ± 30.5*	120.4 ± 19.7	97.6 ± 28.9*	161.8 ± 13.8
Epididymal fat pad weight (EFP) (g)	3.7 ± 0.5	5.1 ± 0.5	4.9 ± 1.3*	7.8 ± 1.0

Salsolinol did not influence the mean food consumption in both — 14 days and 28 days treated groups. However the mean weight gain was significantly diminished in the salsolinol-treated groups of rats. Similarly, the epididymal fat pad weight, which reflects visceral body fat content, was significantly diminished in the salsolinol-treated groups compared with the relevant controls: SF1 vs. CF1 ($p = 0.032$) and similarly SF2 vs. CF2 ($p = 0.0007$). There were no significant EFP differences between both salsolinol-treated groups of animals: group S1 vs. group SF1 and group S2 vs. group SF2.

The results of morphological analysis of the fat pad adipocytes measured in rats after salsolinol administration are presented in Table 3 and Figs 1 and 2. Fig. 3 shows fad pad adipocytes in hematoxylin and eosin stained specimen of the fat pad adipose tissue after two weeks of chronic salsolinol administration.

Table 3. Adipocyte area, perimeter, long and short axis, aspect ratio A_R and circularity factor *f* in salsolinol-treated and control rats. All data are expressed as the mean and standard deviation (SD). Asterisks (*) indicate significant differences between the salsolinol-treated and control groups $p < 0.05$. Groups: S2, SF2 — rats for four weeks and fed with standard or high-fat diet, respectively; C2, CF2 — control rats fed with standard or high-fat diet, respectively; S1, SF1 — rats for two weeks and fed with standard or high-fat diet, respectively; C1, CF1 — control rats fed with standard or high-fat diet, respectively.

Group	Area	Perimeter	Long axis	Short axis	Aspect ratio	Circularity
C1	1371.63 ± 482.92	165.08 ± 28.30	47.58 ± 9.09	33.51 ± 8.07	1.469 ± 0.323	0.616 ± 0.089
S1	1070.66* ± 387.65	144.86* ± 24.33	40.44* ± 8.17	29.21* ± 7.15	1.424 ± 0.286	0.622 ± 0.098
CF1	1687.66 ± 734.04	195.58 ± 41.69	54.18 ± 13.07	34.75 ± 10.55	1.638 ± 0.427	0.533 ± 0.132
SF1	997.13* ± 376.92	141.04* ± 24.30	40.36* ± 8.68	27.40* ± 6.60	1.525 ± 0.420	0.616 ± 0.108
C2	1077.41 ± 357.57	154.26 ± 25.27	44.61 ± 10.72	25.01 ± 6.40	1.856 ± 0.511	0.563 ± 0.100
S2	1218.23 ± 506.31	153.20 ± 30.35	45.67 ± 10.67	30.68 ± 7.82	1.537 ± 0.372	0.632 ± 0.117
CF2	1444.41 ± 615.77	180.91 ± 32.74	51.78 ± 13.77	30.45 ± 9.43	1.793 ± 0.520	0.532 ± 0.107
SF2	1277.09* ± 486.96	155.60* ± 23.69	46.44* ± 9.21	32.11 ± 7.63	1.494 ± 0.339	0.647 ± 0.149

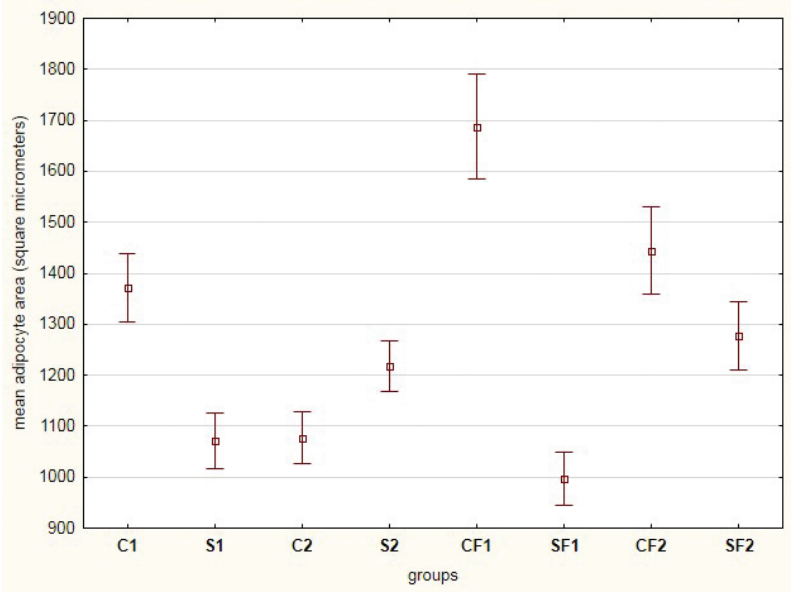

Fig. 1. Adipocyte area in salsolinol-treated and control rats. All data are expressed as the mean and standard deviation (SD). Groups: S2, SF2 — rats for four weeks and fed with standard or high-fat diet, respectively; C2, CF2 — control rats fed with standard or high-fat diet, respectively; S1, SF1 — rats for two weeks and fed with standard or high-fat diet, respectively; C1, CF1 — control rats fed with standard or high-fat diet, respectively.

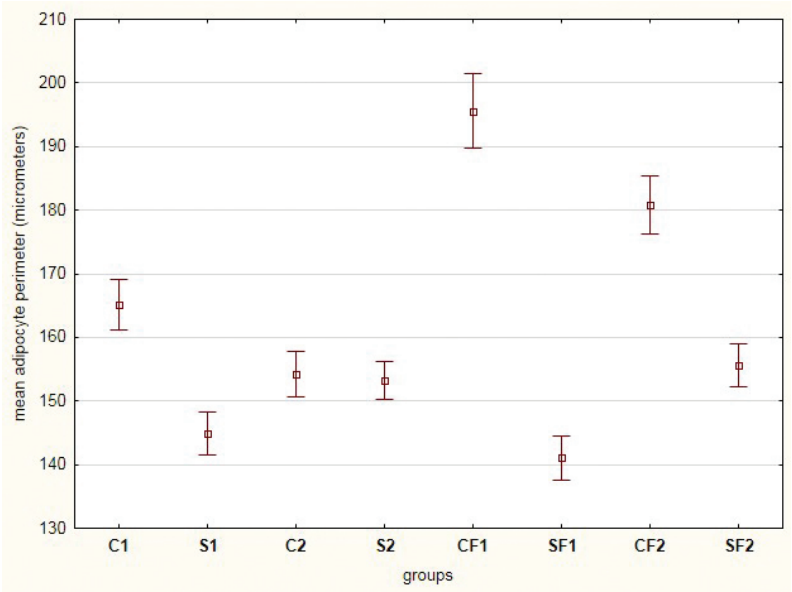

Fig. 2. Adipocyte perimeter in salsolinol-treated and control rats. All data are expressed as the mean and standard deviation (SD). Groups: S2, SF2 — rats for four weeks and fed with standard or high-fat diet, respectively; C2, CF2 — control rats fed with standard or high-fat diet, respectively; S1, SF1 — rats for two weeks and fed with standard or high-fat diet, respectively; C1, CF1 — control rats fed with standard or high-fat diet, respectively.

High fat diet significantly increased mean cell area, adipocyte perimeter, long and short axis in comparison with control diet. The mean adipocyte area, adipocyte perimeter, long and short cell axis were significantly decreased in all salsolinol-treated rats in comparison with the relevant controls. There was no clear statistically significant difference between the salsolinol-treated and control groups in the aspect ratio A_R and circularity factor *f* (Table 3).

Discussion

Adipocyte size reflects adipocyte physiology, increased size being associated with compromised metabolism and the detrimental effects of obesity and type 2 diabetes. Physiological status of adipose tissue can be estimated by morphometric analysis. Examining cells by microscopy has long been a primary method for studying cellular function. Besides available computational software are widely used for collecting data of numerous accurate measurements.

Adipose tissue is essential for glucose metabolism and energy production. Its endocrine function is no less relevant and modulated by the size of adipocytes in the body.

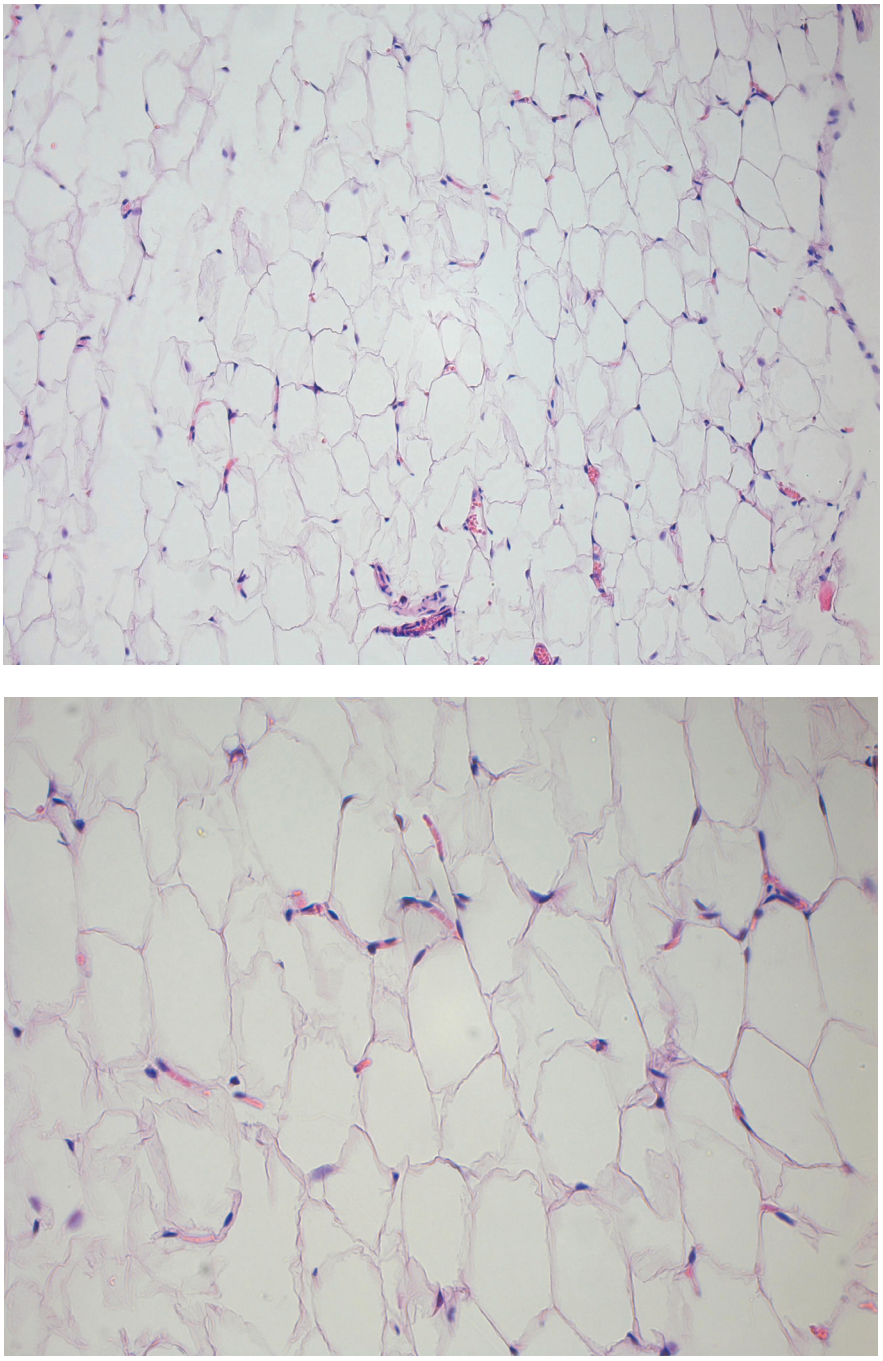

Fig. 3. Fad pad adipocytes in hematoxylin and eosin stained specimen from the fat pad adipose tissue after two weeks of salsolinol treatment. (A, magnification 200×) and (B, magnification 400×).

Adipocytes synthesize a number of cytokines and other bioactive molecules, together termed adipokines. In adipose tissue from obese animals or humans, several metabolic functions are subject to change with adipocyte size. These functions include storage and mobilization of lipids and secretion of both leptin and tumor necrosis factor a (TNF-α) [35]. This excess secretion has been hypothesized to cause insulin resistance. For this reason, no less important it is to measure essential parameters of adipocytes and compare it in different groups of subjects, than evaluate general distribution and amount of adipose tissue [36–38].

Quantifying the number and size of adipocytes in the development, deposition is essential in characterizing the phenotype of a given adipose tissue depot. Size of adipocytes might be evaluated as a predictor of metabolic disorders (type 2 diabetes) and in the same time as a result of different gene expression. Phosphodiesterase 3B (PDE3B) gene expression is generally reduced in large adipocytes of obese, insulin-resistant mice [39]. In cases of chronic positive energy imbalance adipocytes store surplus energy as triacylglycerols, expanding in cell size (hypertrophy) and in cell number (hyperplasia) as a consequence [40, 41]. Adipocyte hypertrophy results from a relative increase in lipid deposition versus lipolysis.

Despite in most studies the adipocyte size was observed, it is not clear known whether the function of adipose tissue cells vary with its size. In 1972 Salans *et al.* made an attempt to separate human adipocytes of different sizes from the same adipose tissue sample by exploiting differences in the flotation rates of large and small fat cells. Complicated analysis of different cells showed that triglyceride turnover increases with adipocyte size [42–44].

Methods that allow quick and factual determination of adipocyte size are important to studies of metabolism. Despite, not exists "gold standard" for adipocyte size measurement. Each methods has own positive and negative sights which might have an impact on results. In addition, no one of methods directly quantifies cell volume, which is the most significant predictor of endocrine function of adipocytes and also accurate parameter of cell size [37, 45].

The measurement of adipocyte size by microscopy is widely used, although the method is tedious and time consuming. Also manual techniques demand discrimination, counting and measurement of many hundreds of irregular events. This limits the number of fields that may be accurately counted in a timely fashion. More recently, manual quantification has been superseded by computational methods. Nowadays several programs for computer image analysis are available: STEPanizer (web-based tool), Adiposoft, CellProlifer etc. All of them are fully automated open-source software for the quantification of adipocyte cellularity in histological sections. All measurements are faster and independently of the user, thus providing more objective results than manually based methods [42, 45, 46]. The "interactive" methods and systems, however requiring the time consuming personal supervision, are still quite popular.

The most popular parameters usually estimated by microscopy with different magnification are adipocyte area, perimeter, volume, length and width (the major and minor axes of adipocytes) and shape factors. Human fat cells can change ~20-fold in diameter and several thousand-fold in volume [47]. Thus, relevant reference specimens and control groups are extremely important to obtain reliable morphometrical data.

In humans and lower mammals, fat is stored in the adipose tissue in various locations within the body such as intraabdominal depots around the omentum, intestines, and perirenal areas, as well as in subcutaneous depots in the buttocks, thighs, and abdomen. In addition, WAT (white adipose tissue) can be found in many other areas, including in the retro-orbital space, face and the bone marrow [48]. Adipose tissue is made principally of white adipocytes, which are the primary site of triglyceride/energy storage, but also brown adipocytes (brown adipose tissue – BAT), which are important in both basal and inducible energy expenditure in the form of thermogenesis [49] White and brown adipose tissues are regulated by multiple hormonal and neural mechanisms, thus they constitute a great point of interest for studying obesity and metabolic disorders. Although the epididymal fat pad represents only a small portion of the total body weight, previous studies have shown that the epididymal fat pad weight calculated as a proportion of total body weight is highly correlated with total body fat in mice and rats [29–31]. In addition to adipocytes, fat pads contain preadipocytes, vascular cells, nerves, macrophages, and fibroblasts. It has been shown the direct innervation of white adipose tissues by both sympathetic (SNS) and parasympathetic nervous system (PNS). Bartness *et al.* have shown direct neuro-anatomical contact of nerves to white adipose tissue [33, 50–52]. There are many reports regarding the influence of SNS onto the number and size of the adipocytes by proliferation and differentiation of white and brown adipocytes [53]. Kreier *et al.* have shown for the first time innervation to various different fat pads (retroperitoneal, mesenteric, epididymal, and subcutaneous inguinal fat) by nervus vagus [34], what can partly explain the weight changes of epididymal fat pad after performed vagotomies or other manipulations on the vagus nerve and the impact of the VNS on fat content can be one of mechanisms of VNS effect on weight loss [22–24].

In our experiment high fat diet significantly increased mean cell area, adipocyte perimeter, long and short axis in comparison with control diet. But the mean adipocyte area, adipocyte perimeter, long and short cell axis were significantly decreased in all salsolinol-treated rats in comparison with the relevant controls.

There are existing data of the decrease of adipose tissue in the patients with Parkinson's disease, and the reason of this decrease is unknown. In the experiments on rats Kuranuki *et al.* have stated, that epididymal and retroperitoneal fat and total intra-abdominal fat weights, expressed relatively to body weight, were significantly lower in the PD rats, treated with 6-OHDA group, than in the control group. This decreased intra-abdominal fat mass might be associated with lower insulin levels, which reduces

lipogenesis [54]. Meng *et al.* searching for the explanation of the reason for the adipose tissue decrease, in their experiments on rats with the PD model induced by 6-OHDA, have stated that retroperitoneal white adipose tissues weight and adipocyte surface area were significantly reduced in comparison to control rats. Their study demonstrates that degenerated dopaminergic neurons in the nigrostriatal system correlate with increases in sympathetic nervous system function, resulting in lipolysis and inhibition of fat cell differentiation [55].

Little is known about the general influence of salsolinol on short-term lipids' and carbohydrates' absorption, their utilization as well as on body weight and energy storing.

Kurnik *et al.* [5, 7] reported decrease in body weight and alteration in body fat contents of the animals during chronic peripheral salsolinol exposure. While lipid profile, postprandial blood glucose and hepatic enzymes levels remained indifferent, postprandial triglyceridemia was significantly lower in all salsolinol-treated rats in comparison with the control. Epididymal fat pad and body weight ratio were also significantly diminished in salsolinol-treated rats compared to the control animals. This observation correlated with results of the body weight assessment. Although salsolinol treated animals consumed similar amount of food there was significant decrease in body weight gain in animals exposed to salsolinol. Therefore, author concluded that salsolinol may not influence the appetite regulating mechanism and the reduction of body weight was due to others, probably peripheral effects. Diminished body weight gain and lower adipose tissue accumulation in salsolinol-treated animals were due to delayed gastric emptying together with disturbed gut functions resulting in absorptive dysfunction [5, 7].

The results from current study confirm these observations. We showed that chronic peripheral salsolinol administration significantly decreases the adipocyte size, and such effect is related to weight loss and reduced adipose tissue accumulation in rats.

We might speculate that absorptive dysfunction, rather peripheral than central in origin, resulted from delayed gastric emptying together with disturbed colon function, might be responsible for diminished body weight gain and slower adipose tissue accumulation in salsolinol-treated animals, but further evidence is needed to support this postulate.

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Conflict of interest

None declared.

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