

Nutritional parameters and carbohydrate and fat metabolism after prolonged salsolinol administration in rats fed normal or high-fat diets

Magdalena KURNIK-ŁUCKA, Krzysztof GIL, Andrzej BUGAJSKI, Agata FURGAŁA, Piotr THOR

Department of Pathophysiology, Jagiellonian University Medical College, Kraków, Poland

Correspondence to: Magdalena Kurnik-Łucka, MSc, PhD.
Department of Pathophysiology
Jagiellonian University Medical College
Czysta 18, 31-121 Kraków, Poland.
TEL: +48 12 633 39 47; FAX: +48 12 632 90 56; E-MAIL: magdalena.kurnik@uj.edu.pl

Submitted: 2016-07-26 *Accepted:* 2016-09-03 *Published online:* 2016-00-00

Key words: **salsolinol; Wistar rats; osmotic mini-pumps; high-fat diet; epididymal fat pads; GIP; GLP-1; CRF**

Neuroendocrinol Lett 2016; **37**(7):501-510 PMID: 28326744 NEL370716A05 © 2016 Neuroendocrinology Letters • www.nel.edu

Abstract

OBJECTIVES: Previous studies have reported that exogenous salsolinol might contribute to myenteric cell death and altered gastrointestinal motility. Because the entire gut mucosal, entero-endocrine and motor functions are integrated by the enteric nervous system, the aim of the present study was to investigate if prolonged intraperitoneal salsolinol administration alters basic metabolism and nutritional parameters in adult Wistar rats fed normal or high-fat diets.

METHODS: Male Wistar rats were subjected to continuous intraperitoneal low dosing of salsolinol with ALZET osmotic mini-pumps for 2 or 4 weeks and fed either a normal or high-fat diet. Appropriate groups served as the controls. Nutritional status (food intake, body weight, and epididymal fat pads weight), residual solid food in the stomach and biochemical parameters (GIP, GLP-1, CRF, glucose, TG, LDL, HDL) were assessed.

RESULTS: Prolonged salsolinol treatment significantly reduced total body mass and adipose tissue accumulation. The effects were more pronounced in the salsolinol-treated rats fed a high-fat diet. In salsolinol-treated rats, serum postprandial GIP levels were elevated, and serum postprandial GLP-1 levels were lower compared with the appropriate controls.

CONCLUSIONS: Salsolinol might influence the regulatory mechanisms of body weight and epididymal fat pad accumulation through neurohormonal pathways.

Abbreviations:

aVTA	- anterior part of ventral tegmental area
CRF	- corticotropin-releasing factor
1,2-DMDHIQ+	- 1,2-dimethyl-6,7-dihydroxyisoquinolinium ion
GIP	- glucose-dependent insulinotropic polypeptide
GLP-1	- glucagon-like peptide-1
HDL	- high-density lipoproteins
i.p.	- intraperitoneal
LDL	- low-density lipoproteins
MPTP	- 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine
NAc	- nucleus accumbens
pVTA	- posterior part of ventral tegmental area
Salsolinol	- 1-methyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline
TG	- triglycerides

INTRODUCTION

Salsolinol (1-methyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline) is an adduct of dopamine and acetaldehyde and belongs to a family of tetrahydroisoquinolines that are widespread in nature (McNaught *et al.* 1998; Rommelspacher & Susilo 1985). Salsolinol is believed to act as an inhibitor of tyrosine hydroxylase and monoaminoxidase type A (Heikkila *et al.* 1971, Storch *et al.* 2000). It might regulate the function of catecholaminergic neurons (especially dopaminergic) through its specific binding sites (Naoi *et al.* 2004) and may also act at the level of sympathetic ganglia (Mravec *et al.* 2004). Moreover, it was postulated that salsolinol might be a selective and potent stimulator of prolactin secretion without affecting the secretion of other pituitary hormones (Homickó *et al.* 2003; Tóth *et al.* 2002).

The chemical structure of salsolinol resembles the well-recognised and studied exogenous neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and is able to form 1,2-dimethyl-6,7-dihydroxyisoquinolinium ions (1,2-DMDHIQ⁺) analogous to highly neurotoxic 1-methyl-4-phenylpyridinium ions (Maruyama *et al.* 1997; Naoi *et al.* 1989; 1994). Możdżeń *et al.* (2015) suggested that salsolinol can exhibit opposing biological actions depending on its concentration, either neuroprotective or pro-apoptotic. Thus, prolonged exposure to its high concentration might indeed cause apoptotic nerve cell death and could be one of the aetiological factors of neurodegenerative disease. It was previously reported that Parkinsonian patients treated with L-DOPA and chronic alcoholics showed significant elevations in the concentration of salsolinol in their cerebrospinal fluid and urine (Cohen & Collins 1970; Collins *et al.* 1979; Moser & Kompf 1992; Sandler *et al.* 1973), whereas a low concentration of salsolinol

was detected in normal human cerebrospinal fluid (Moser & Kompf 1992), brain and urine (Dostert *et al.* 1989; Sjöquist *et al.* 1981). However, this difference could be a result of its impaired unidentified metabolic routes instead of its direct involvement in the process of neurodegeneration. It is unknown whether the neurotoxic effect of salsolinol in the brain is solely associated with dopaminergic structures, and it is still debatable and inconclusive whether salsolinol is able to cross the blood-brain barrier. Furthermore, salsolinol has been detected in various food products, such as alcohol beverages, cheese and bananas (Duncan *et al.* 1982; 1984; Rigglin *et al.* 1976), but its influence on peripheral neurotransmission and metabolism is poorly understood and addressed in the literature.

Recently, we reported that exogenous salsolinol induced myenteric neuronal cell (the inhibitory motor neurons) death (Kurnik *et al.* 2015), which might contribute to altered gastrointestinal motility (Banach *et al.* 2005; 2006). Because the entire gut mucosal, entero-endocrine and motor functions are integrated by the enteric nervous system, the aim of the present study was to investigate whether and how prolonged intraperitoneal salsolinol administration alters basic metabolism and body composition in adult Wistar rats fed normal or high-fat diets. We decided to evaluate nutritional parameters (body weight gain and epididymal fat pads weight), residual solid food in the stomach and biochemical parameters corresponding to incretins produced in the small intestine, including postprandial serum levels of glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1), corticotropin-releasing factor (CRF) postprandial glucose, triglycerides (TG), and LDL and HDL (low- and high density lipoproteins, respectively), after salsolinol administration to address our question.

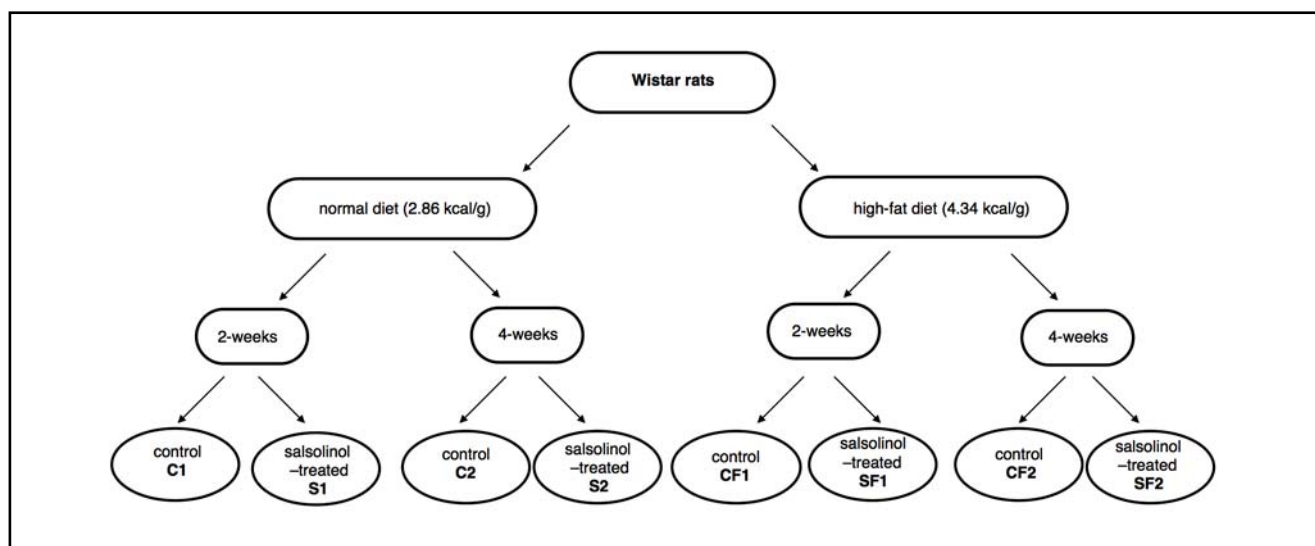


Fig. 1. The experimental design. S1, SF1 – 2 weeks of salsolinol i.p. administration in rats fed a standard or high-fat diet, respectively; S2, SF2 – 4 weeks of salsolinol i.p. administration in rats fed a standard or high-fat diet, respectively; C1, CF1 – control rats fed a standard or high-fat diet for 2 weeks, respectively; C2, CF2 – control rats fed a standard or high-fat diet for 4 weeks, respectively.

MATERIAL AND METHODS

Animal housing, diet and surgery

Adult male Wistar rats (n=48) were housed in individual transparent cages allowing for visual contact, with food and water provided *ad libitum*, temperature maintained at 23±2 °C and under a 12:12 h dark/light cycle. During the experiment, rats were fed either a standard diet (caloric distribution of the diet: protein 25%, fat 8%, carbohydrates 67%, metabolizable energy 2.86 kcal/g, Labofeed B, Kcynia, Poland) or an obesity-inducing high-fat diet (caloric distribution of the diet: protein 25.1%, fat 38.8%, carbohydrates 36.1%, metabolizable energy 4.34 kcal/g; Perform, Bento Kronen Products, Belgium). Rats were either subjected to continuous dosing of salsolinol or used as controls. Salsolinol (salsolinol hydrochloride, Sigma, USA) at a total dose of 200 mg/kg was dissolved in 200 µL of 0.9% NaCl and delivered using ALZET osmotic mini-pumps (Durect, USA) implanted i.p. Control groups were implanted with ALZET osmotic mini-pumps filled with 0.9% NaCl. Rats were starved for 12 hours, and mini-pumps were implanted under general anaesthesia induced with sodium pentobarbital given i.p. at a dose of 0.25 mg/kg (Vetbutal, Biowet, Poland). The Jagiellonian University Bioethical Committee approved the experiment (ethical approval number – 67/2009).

Thus, prior to pump implantation, rats were randomly divided into the following experimental groups (n=6 rats in each group): (1) rats subjected to continuous dosing of salsolinol for two weeks (ALZET delivery rate 0.5 µL/h and fed a standard diet throughout the entire experimental period (S1 group)); (2) rats subjected to continuous dosing of salsolinol for two weeks (ALZET delivery rate 0.5 µL/h) and fed a high-fat diet throughout the experiment (SF1 group); (3) rats subjected to continuous dosing of salsolinol for four weeks (ALZET delivery rate 0.25 µL/h) and fed a standard diet throughout the experiment (S2 group); (4) rats subjected to continuous dosing of salsolinol for four weeks (ALZET delivery rate 0.25 µL/h) and fed a high-fat diet throughout the experiment (SF2 group); (5) a control group fed a standard diet for two weeks (C1 group); (6) a control group fed a high-fat diet for two weeks (CF1 group); (7) a control group fed a standard diet for four weeks (C2 group); (8) a control group fed a high-fat diet for four weeks (CF2 group).

Nutritional status and biochemical determinations

The general health status and motor function of the experimental animals were evaluated daily during handling and by observing their in-cage behaviour. Food intake (g) and body weight (g) were measured each morning during the study. Daily and total food intake and daily and total weight gain were calculated. The feed efficiency ratio was calculated according to the following equation: [body weight gain (g) / total food intake (g)] × 100.

At the end of the experiment (either day 15 or day 29), following 12 h overnight fasting, the rats were allowed free access to pre-weighed food for sixty minutes. Thirty minutes later, the animals were euthanized *via* decapitation (between 9 and 11 a.m.) and their stomachs, epididymal fat pads and blood samples were collected.

Each stomach was excised and weighed. The amount of food in the stomach was calculated as the difference between the total weight of the stomach with its contents and the weight of the stomach after the contents were removed (adapted from a method described in Greene *et al.* 2009). The food pellets were weighed before and after the feeding period to determine the amount consumed by each rat and adjusted to the body mass of rats (mg/g of body weight). The residual solid food in the stomach after 90 minutes was determined according to the following equation: residual solid food in the stomach = [dried gastric content (g) / food intake (g)] × 100.

Both epididymal fat pads, located between the *cauda epididymis* and the distal extremity of the testis, were dissected from each rat and weighted (Bugajski *et al.* 2007; Gil *et al.* 2013). The epididymal fat pad/total body weight gain ratio was calculated by dividing the weight of the fat pads (g) by the total body weight gain (g) and expressed as percentage (%) values. The epididymal fat pad weight over the total food intake ratio was calculated by dividing the weight of the fat pads (g) by the total food intake (g) and expressed as per mille (‰) values.

Blood samples were collected from the jugular vessels in plastic tubes and incubated at least 30 minutes at 4 °C to induce clot formation. After centrifugation at 1500 ×g for 20 min at 4 °C (Megafuge 1.0R, Heraeus Instruments, Germany), serum samples were collected and kept frozen at –80 °C until further analysis. Serum postprandial levels of GIP, GLP-1 and CRF were assayed by quantitative ELISA kits (Phoenix Pharmaceuticals, USA) according to the manufacturer's instructions. Serum levels of postprandial glucose, TG, LDL and HDL were measured using a chemistry immune-analyser (Olympus AU600, USA). All measurements were performed in duplicate.

Statistical analysis

Results were analysed (GraphPad Prism 7.0a, USA) using a one-way analysis of variance (ANOVA) followed by a post hoc Tukey's test and are expressed as the mean±standard deviation (SD). Statistical significance was set at *p*<0.05.

RESULTS

Food intake, body weight and epididymal fat pad weight

None of the salsolinol-treated or control rats died or showed any visible disturbances of gross motor function. There were no significant differences in the mean

daily food intake (g) between the salsolinol-treated groups and their controls, except for SF2 = 30.66±2.59 vs. CF2 = 36.38±1.72 ($p=0.0011$). In addition, there were no significant differences in the mean daily energy intake (kcal) between the salsolinol-treated groups and their controls, except for SF2 = 66.53±5.63 vs. CF2 = 78.93±3.73 ($p=0.028$) (Figure 2A). However, salsolinol treatment led to significantly reduced body weight by the end of the experiment (data not shown). The mean daily body weight gain (g) was lower in all salsolinol-treated rats: S1 = 4.221±0.48 vs. C1 = 5.828±0.21 ($p=0.077$); SF1 = 5.061±1.30 vs. CF1 = 7.000±0.997 ($p=0.047$); S2 = 3.512±1.13 vs. C2 = 4.299±0.72 ($p=0.523$); and SF2 = 3.485±1.03 vs. CF2 = 5.777±0.49 ($p=0.011$) (Figure 2B). The mean feed efficiency ratio (%) was significantly lower in the salsolinol-treated animals fed a high-fat diet: SF1 vs. CF1 ($p=0.004$) and SF2 vs. CF2 ($p=0.004$) (Figure 2C). The mean feed effi-

ciency ratio (%) was also significantly different for S1 vs. SF1 ($p=0.0001$), S2 vs. SF2 ($p=0.009$), C1 vs. CF1 ($p<0.0001$) and C2 vs. CF2 ($p<0.0001$) (Figure 2C).

The mean epididymal fat pad weight over the total food intake ratio (‰) was significantly lower in SF1 compared with CF1 ($p=0.032$) and in SF2 compared with CF2 ($p=0.0007$) salsolinol-treated rats (Figure 2D). The mean epididymal fat pad weight over the total weight gain ratio (%) was not significantly different in the salsolinol-treated rats compared with the corresponding control groups: S1 = 5.43±0.66 vs. C1 = 4.584±0.60 ($p=0.32$), SF1 = 6.098±1.64 vs. CF1 = 5.263±0.80 ($p=0.53$), S2 = 3.295±0.67 vs. C2 = 3.804±0.37 ($p=0.40$), and SF2 = 5.047±0.34 vs. CF2 = 4.793±0.33 ($p=0.53$). There were no significant differences between rats fed a normal diet and a high-fat diet for both salsolinol-treated and control animals, except for S2 vs. SF2 ($p=0.012$).

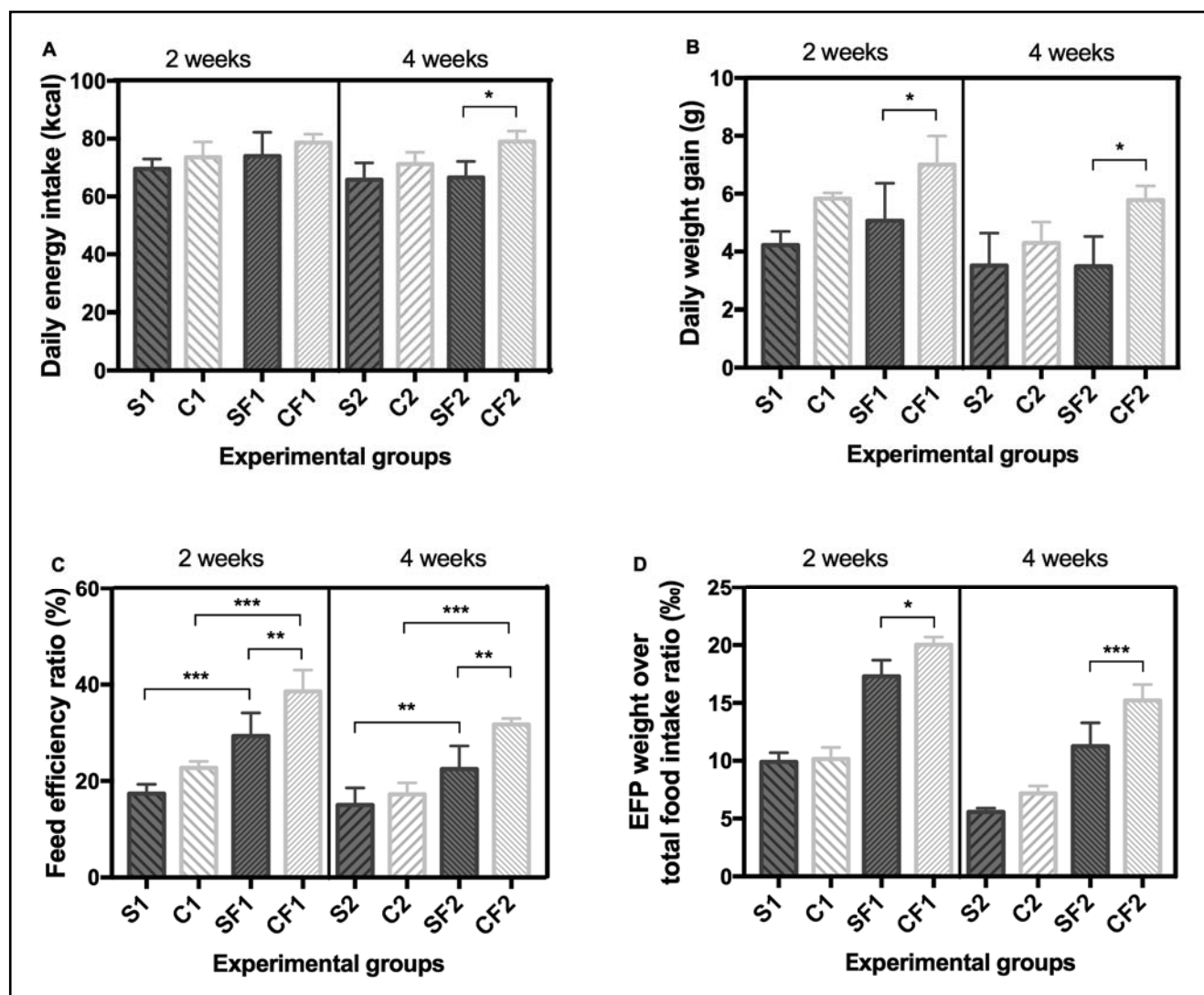


Fig. 2. (A) The mean daily food intake (kcal); (B) the mean daily body weight gain (g); (C) the mean feed efficiency ratio (%); (D) the mean epididymal fat pad (EFP) weight over the total food intake ratio (‰). Statistical significance: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. S1, SF1 – 2 weeks of salsolinol i.p. administration in rats fed a standard or high-fat diet, respectively; S2, SF2 – 4 weeks of salsolinol i.p. administration in rats fed a standard or high-fat diet, respectively; C1, CF1 – control rats fed a standard or high-fat diet for 2 weeks, respectively; C2, CF2 – control rats fed a standard or high-fat diet for 4 weeks, respectively.

Residual solid food in the stomach

The mean amount of food consumed within the one-hour testing period (mg/g of body weight) was not significantly different in the salsolinol-treated rats compared with the appropriate control groups: S1 = 16.4 ± 3.5 vs. C1 = 19.57 ± 2.49 ($p=0.97$), SF1 = 20.05 ± 5.03 vs. CF1 = 19.46 ± 3.10 ($p=0.99$), S2 = 14.77 ± 6.99 vs. C2 = 15.41 ± 2.23 ($p=0.99$), and SF2 = 15.78 ± 8.06 vs. CF2 = 7.89 ± 1.83 ($p=0.25$). The mean residual solid food in the stomach (%) was significantly higher in the S1 group = 80.2 ± 6.0 compared with the C1 group = 68.4 ± 6.1 ($p=0.033$). However, there were no significant differences in the mean residual solid food among the other groups: SF1 = 76.1 ± 11.0 vs. CF1 = 71.3 ± 2.8 ($p=0.99$), S2 = 81.2 ± 4.4 vs. C2 = 73.6 ± 6.8 ($p=0.91$), and SF2 = 78.6 ± 17.5 vs. CF2 = 69.1 ± 6.7 ($p=0.79$).

Biochemical analyses

The mean postprandial serum GIP levels (ng/ml) were elevated in salsolinol-treated rats, especially in S2 = 0.4194 ± 0.06 vs. C2 = 0.042 ± 0.02 ($p=0.0008$), excluding S1 = 0.0225 ± 0.001 compared with C1 = 0.3206 ± 0.12 ($p=0.0047$) (Figure 3A). The mean postprandial serum GLP-1 levels (ng/ml) were lower in the salsolinol-treated rats compared with controls, especially for S1 = 0.01275 ± 0.012 vs. C1 = 0.03738 ± 0.016 ($p=0.0148$). There were no significant differences between rats fed normal and high-fat diets among both the salsolinol-treated and control animals (Figure 3B). The mean serum CRF levels (ng/ml) were comparable in the salsolinol-treated rats and their respective controls: S1 = 0.09888 ± 0.054 vs. C1 = 0.08783 ± 0.068 ($p>0.99$), S2 = 0.07388 ± 0.010 vs. C2 = 0.1505 ± 0.129 ($p=0.75$), SF1 = 0.1139 ± 0.032 vs. CF1 = 0.1273 ± 0.102 ($p>0.99$),

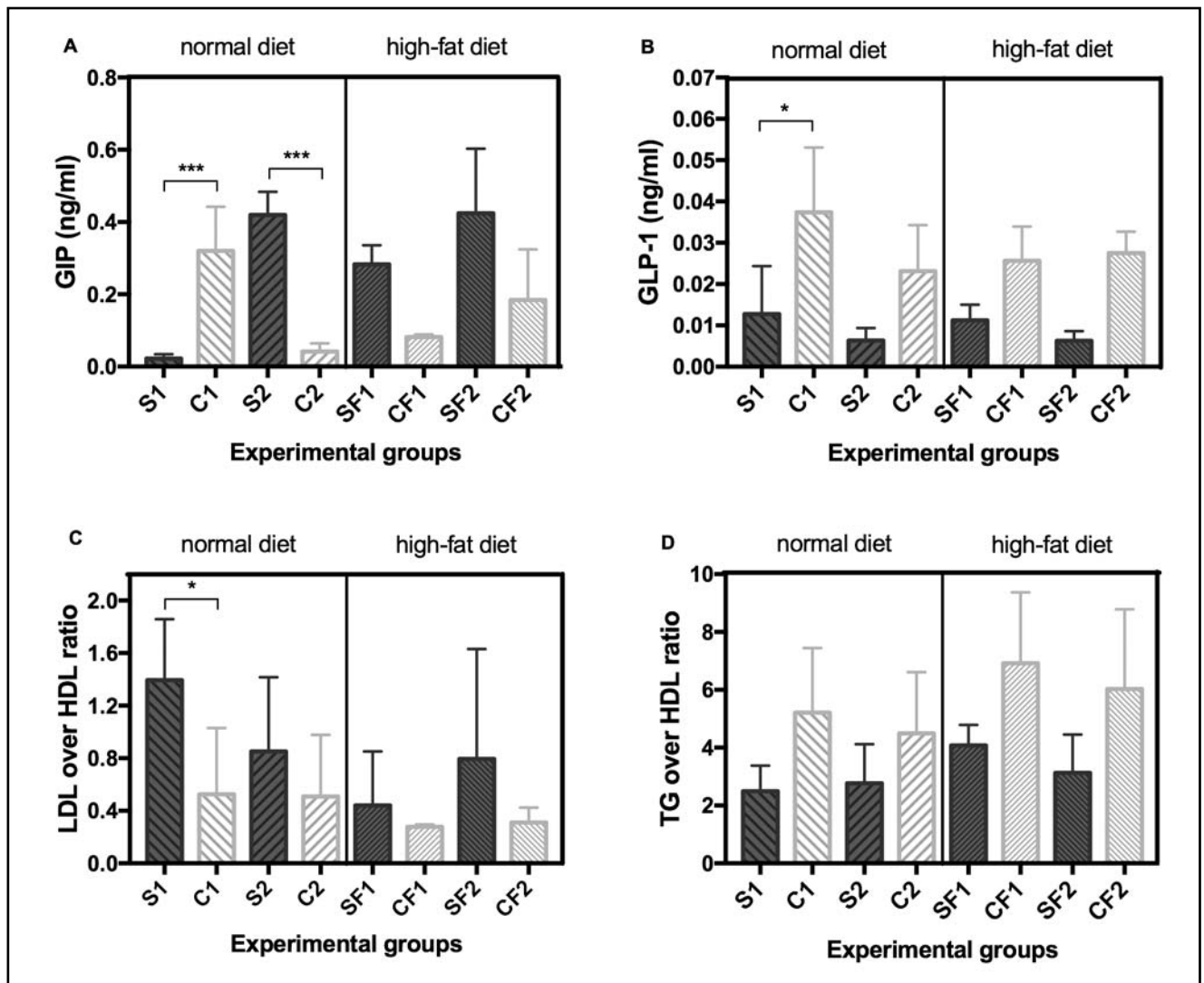


Fig. 3. (A) The mean postprandial GIP levels (ng/ml); (B) the mean postprandial GLP-1 levels (ng/ml); (C) the mean postprandial LDL over HDL ratio; (D) the mean postprandial TG over HDL ratio. Statistical significance: * $p < 0.05$, *** $p < 0.001$. S1, SF1 – 2 weeks of salsolinol i.p. administration in rats fed a standard or high-fat diet, respectively; S2, SF2 – 4 weeks of salsolinol i.p. administration in rats fed a standard or high-fat diet, respectively; C1, CF1 – control rats fed a standard or high-fat diet for 2 weeks, respectively; C2, CF2 – control rats fed a standard or high-fat diet for 4 weeks, respectively.

and SF2 = 0.1260 ± 0.045 vs. CF2 = 0.1280 ± 0.023 ($p > 0.99$). In addition, there were no differences between rats fed normal and high-fat diets among both the salsolinol-treated and control rats.

The mean postprandial serum glucose levels (mg/dl) were not statistically different between the salsolinol-treated and control rats: S1 = 134.6 ± 15.0 vs. C1 = 122.1 ± 28.7 ($p = 0.79$), S2 = 131.9 ± 11.9 vs. C2 = 119.7 ± 14.9 ($p = 0.76$), SF1 = 117.4 ± 7.7 vs. CF1 = 107.1 ± 3.2 ($p = 0.98$), and SF2 = 124.6 ± 8.4 vs. CF2 = 126.3 ± 10.6 ($p > 0.99$). There were no significant differences between rats fed normal and high-fat diets among both salsolinol-treated and control rats.

The mean postprandial serum levels of both LDL and HDL (mmol/l) were not significantly different between the salsolinol-treated rats and the appropriate control rats. However, the mean LDL over HDL ratio was increased in all salsolinol-treated rats compared with their controls, especially in S1 = 1.394 ± 0.47 compared with C1 = 0.525 ± 0.51 ($p = 0.021$) (Figure 3C). The mean postprandial serum TG levels (mmol/l) were not significantly different between the salsolinol-treated rats and the appropriate control rats, while the mean TG over HDL ratio was slightly increased in all salsolinol-treated rats compared with their controls, although this increase was not significant (Figure 3D).

DISCUSSION

Salsolinol attracted considerable attention in the 1980s because of its possible involvement in the etiopathogenesis of Parkinson's disease. Salsolinol can be formed *in vivo* by non-enzymatic Pictet–Spengler condensation of dopamine and acetaldehyde, leading to the racemic mixture, or by enantioselective formation of (R)-salsolinol by the enzyme (R)-salsolinol synthase (Naoi *et al.* 1996), although the exact site of biosynthesis remains unknown. Another important question that has been thoroughly explored is its metabolism. In the brain, salsolinol metabolism occurs through N-methylation and oxidation (Maruyama *et al.* 1992). The distribution of the N-methylated and oxidised derivatives seems to follow a specific pattern in the human nigro-striatal pathway (Maruyama *et al.* 1997), which might suggest that these derivatives may be involved in the function of dopamine neurons under physiological and/or pathological conditions. Primarily neurotoxic effects have been attributed to these metabolites (Maruyama *et al.* 1992; Naoi *et al.* 2004), although they might also possess neuroprotective properties depending on their concentrations (Maruyama *et al.* 1995; Możdżeń *et al.* 2015).

Our selected dosage regimen was based on a literature review (Table 1) and unpublished data from our department. We hypothesized that intraperitoneal administration of a low dose of salsolinol hydrochloride (total dose of 200 mg/kg) using ALZET osmotic mini-pumps for two (approximately 14 mg/kg salsolinol daily) or four (approximately 7 mg/kg salsolinol

daily) weeks would be optimal. The exposure of the enteric nervous system would be constant and repeatable and would eventually cause mild but stable changes within the gut wall. The intraperitoneal administration route is used for experimental purposes to deliver drugs to small laboratory animals to achieve successful outcomes due to its accuracy, reliability, and convenient delivery, which yields reproducible results rendered by a large absorbing surface area. The majority of the drug is absorbed by the veins of the mesentery and then gathered into the portal vein of the liver, which is similar to an oral gavage. However, continuous i.p. administration with an osmotic mini-pump recreates a disease state much better than an i.p. injection and causes less acute toxicity and less animal distress (Alvarez-Fisher *et al.* 2008; Fornai *et al.* 2005).

Dosage and route of administration of salsolinol, the initial body weights of male Wistar rats and the authors of original articles published since the year 2000 based on a PubMed database search (“salsolinol and Wistar rats and English”).

The primary finding of our experiment is that prolonged salsolinol treatment decreased body weight gain and adipose tissue accumulation in Wistar rats. The effect was more pronounced in salsolinol-treated rats fed a high-fat diet. There were no significant differences between C1 and CF1 or between C2 and CF2, but clearly two to four weeks were not sufficient to develop obesity in control rats, which is in agreement with previous experiments (Gil *et al.* 2013). Prolonged salsolinol treatment decreased the feed efficiency ratio despite the same genotype, sex, age, and thermal environment of the animals in our experiment. The decreased feed efficiency ratio was associated with decreased epididymal fat pad weight over total food intake ratio in salsolinol-treated rats fed a high-fat diet, especially after four weeks of treatment. Therefore, the questions arise as to whether i.p. administered salsolinol is metabolised and whether our results are mediated peripherally or centrally. We previously reported that salsolinol was not detected (the limit of detection was set at 0.86 ng/l) in serum samples in a similar experimental model, which suggests that salsolinol did not reach the systemic blood (Kurnik *et al.* 2012). However, the blood samples were collected 24 h after the last delivery, and other related metabolites were not measured. It was demonstrated that salsolinol competitively inhibited the activity of debrisoquine 4-monooxygenase (CYP2D1) and parkinsonism-related psychotropic drugs, the molecular structures of which may correspond not only to the active site of CYP2D1 but also to dopamine receptors (Iwashita *et al.* 1993). Lee *et al.* (2010) demonstrated that a single administration of salsolinol (10 µg) by gavage resulted in a significant elevation of rat (Sprague-Dawley) plasma salsolinol levels, which sharply declined to near basal levels by 14 hours. The mean plasma concentrations of (S)- and (R)-salsolinol at 1 hour after administration were 650 ± 46 and 614 ± 42 pg/ml, respectively.

The mean basal (S)- and (R)-salsolinol levels were 11 ± 4 and 10 ± 1 pg/ml, respectively. A single intake of 3 g banana (corresponding to 75 μ g of salsolinol) also increased the plasma salsolinol concentration. Despite the increases observed in plasma salsolinol or dopamine levels after banana ingestion, the levels were not changed in the striatum or NAc (Lee *et al.* 2010). It is well recognised that most catecholamines in the plasma are not incorporated into the central nervous system due to their inability to cross the blood–brain barrier. The salsolinol detected in the brain is likely derived from *in situ* synthesis (Origitano *et al.* 1981), but some authors argue such a hypothesis (Sjöquist & Magnusson 1980; Song *et al.* 2006). However, it was found that N-methylsalsolinol and N-methyl-norsalsolinol penetrated from the periphery into the brain (Lorenc-Koci *et al.* 2008; Thumen *et al.* 2002). Although no studies have demonstrated a mechanism by which salsolinol might cross the blood-brain barrier, a sodium-independent organic cation transporter has been recognised as a possible active transporter of salsolinol (Taubert *et al.* 2007). Several authors have also reported that systemically administered salsolinol is capable of altering behaviour (Antkiewicz-Michaluk *et al.* 2000a; Matsuzawa *et al.* 2000; Vetulani *et al.* 2001), which indirectly suggests that it could cross the blood-brain barrier. In our experiment, we did not observe any behavioural changes in the animals, and we did not observe any changes in CRF levels between experimental groups. However, we did observe an increase in the percentage of mean residual solid food in the stomach, suggesting reduced gastric emptying, which remains in agreement with the findings of Banach *et al.* (2005, 2006). They reported that intraperitoneally administered salsolinol altered gastric motility and might have a direct effect on intramuscular interstitial cells of Cajal and neuronal

pathways of reflex mechanisms located within the gastrointestinal intestinal wall or the vagal nerves (Banach *et al.* 2005, 2006). We also reported a decrease in large intestine transit and a marked decrease in the water content of faecal matter (Kurnik *et al.* 2015). Abnormal gastrointestinal motility and absorption might contribute to slower body weight gain and adipose tissue accumulation in salsolinol-treated rats.

Enteric reflexes are initiated by stimulation of sensory nerve endings located in the mucosa or within the muscle layers projecting from nerve cell bodies mainly in the myenteric plexus but also in the circular muscle and submucosal plexus (Kunze & Furness 1999). Those reflex circuits are located within the enteric nervous system, which is composed of small ganglia residing along the gastrointestinal tract. The ganglia contain neurons and glial cells and in many aspects are similar in structure to the central nervous system, except for the lack of connective tissue elements and blood-enteric nervous system barrier (Gershon 2005). In addition to the classical neurotransmitters, acetylcholine and adrenaline/noradrenaline, nerve cells can express a number of other putative transmitters. Dopamine is indeed an important mediator of gastrointestinal secretion, absorption, and motility and is the predominant catecholamine neurotransmitter of the central and peripheral nervous systems. Therefore, there is a chance that salsolinol might affect enteric dopaminergic neurotransmission. Unfortunately, characterization of dopamine and dopamine receptors in the gastrointestinal tract is challenging for several reasons. First, dopamine can produce inhibitory and excitatory effects on gastrointestinal motility. Second, localization of dopamine receptors has been hampered by the identification of dopamine receptors in locations that appear to be species specific. Third, studies

Tab. 1. Dosage and route of administration of salsolinol in experimental studies.

Dosage	Route of administration	Initial body weight of male Wistar rats	Authors
100 mg/kg daily in 0.9% NaCl (single dose or 14 days)	i.p.	220–240 g	Wąsik <i>et al.</i> 2015
200 mg/kg in total – osmotic mini-pumps –14 or 28 days	i.p.	~235 g	Kurnik <i>et al.</i> 2012, 2013, 2015
0.03, 0.3, 1 or 3 μ mol in artificial cerebrospinal fluid	pVTA	350–400 g	Deehan <i>et al.</i> 2013
50 mg/kg daily (21 days)	i.p.	180–220 g	Gil <i>et al.</i> 2011
30 pmol in artificial cerebrospinal fluid	pVTA	~300 g	Hipólito <i>et al.</i> 2011
0.3, 3, 30, 300 and 3,000 pmol in artificial cerebrospinal fluid	pVTA	300–320 g	Hipólito <i>et al.</i> 2010
0.1, 5 and 25 μ mol in artificial cerebrospinal fluid	NAc	300–320 g	Hipólito <i>et al.</i> 2009
0.03, 0.1, 0.3, 1 or 3 μ mol in artificial cerebrospinal fluid (multiple self-infusions)	pVTA or aVTA	250–320 g	Rodd <i>et al.</i> 2008
50 mg/kg daily (21 days)	i.p.	~200 g	Banach <i>et al.</i> 2005, 2006
10 mg/kg in 0.9% NaCl (single dose)	i.p.	220–250 g	Vetulani <i>et al.</i> 2001
100 mg/kg in 0.9% NaCl (single dose)	i.p.	220–240 g	Antkiewicz-Michaluk <i>et al.</i> 2000a
100 mg/kg daily in 0.9% NaCl (single dose or 18 days)	i.p.	190–220 g	Antkiewicz-Michaluk <i>et al.</i> 2000b

of dopamine in the gastrointestinal tract motility have often used pharmacologic amounts of this agonist, and interpretation of results has been confounded by the ability of dopamine to activate adrenergic receptors at high doses (Kurnik *et al.* 2015; Li *et al.* 2004).

However, the gastrointestinal tract is not only under neural but also under extensive endocrine control. Chemosensing of gut luminal contents plays a critical role in the control of functions, such as digestion, pancreatic secretion, food intake and metabolic regulation. Glucose-dependent insulinotropic polypeptide and glucagon-like peptide-1 are peptide hormones released from the gastrointestinal tract into circulation in response to glucose absorption. GLP-1 stimulates insulin secretion, increases β -cell mass, inhibits glucagon secretion and delays gastric emptying. GIP stimulates insulin secretion when glucose levels are elevated and decreases glucagon-stimulated hepatic glucose production. Dietary lipid is also a strong stimulant for GIP secretion. GIP is considered to increase the volume of adipose tissue by two major pathways: directly, by binding to GIP receptors located on adipocytes, and indirectly, by accelerating fat deposition and expansion of fat depots by increasing insulin secretion. There are both acute mechanisms of GIP secretion in response to a single administration of fat and chronic mechanisms for hyperproduction of GIP under high-fat feeding (Yamane *et al.* 2016). Interestingly, although prolonged salsolinol treatment increased postprandial GIP levels, the effect was not associated with increased levels of adipose tissue weight in our experiment. Conversely, postprandial GLP-1 levels were decreased after salsolinol treatment, especially after two weeks (higher daily dose), which was associated with increased residual solid food in the stomachs and an increased postprandial LDL to HDL ratio.

Peripheral signals from the gut and adipose tissue constitute feedback mechanisms allowing for the maintenance of a steady body weight, despite daily variations in energy expenditure and nutrient intake (Sam *et al.* 2012). Over the long term, white fat mass reflects the net balance between energy expenditure and energy intake. Adipose tissue is innervated mainly by sympathetic fibres. The neurotransmitter involved is norepinephrine, which binds to different noradrenergic-receptor subtypes depending on the fat pad and the species. The role of this afferent system is still not well understood, but it could be very important in the overall regulation of energy balance in the body. Catecholamines are the main hormones involved in the control of lipolysis (Pénicaud *et al.* 2000). It was postulated that salsolinol might affect plasma catecholamine levels by interfering with epinephrine and norepinephrine release from their terminal sites (Bodnár *et al.* 2004). Thus, we hypothesised that salsolinol might indirectly exert lipolytic effects, which is reflected by lowered epididymal fat pad weight over the total food intake ratio (Aleksandrovych *et al.* 2016).

Salsolinol might influence regulatory mechanisms associated with body weight and fat accumulation through neurohormonal pathways. Further studies are still needed to address these complex issues.

ACKNOWLEDGEMENTS

The research project was fully sponsored by the Jagiellonian University Medical College statutory grants K/ZDS/004607 and K/DSC/002098.

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