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Original Research Article

Up-regulation of orexigenic and down-regulation of anorexigenic neuropeptide gene expression in rat hypothalamus after partial lipectomy

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Abbreviations:
AgRP, agouti related protein
CART, cocaine–amphetamine-regulated transcript peptide
α-MSH, α-melanocyte stimulating hormone
MC4R, melanocortin receptors
NPY, neuropeptide Y
POMC, pro-opiomelanocortin
WAT, white adipose tissue

ABSTRACT

Leptin down-regulates orexigenic and up-regulates anorexigenic neuropeptide gene expression in hypothalamus. Surgical removal of adipose tissue leads to decrease in circulating leptin concentrations in rats. In the present study, we tested: (a) regulation of neuropeptide gene expression in hypothalamus, (b) food intake, and (c) standard growth rate after removal of adipose tissue in rats. Partial lipectomy caused an approximately 10-fold reduction of subcutaneous, retroperitoneal and epididymal adipose tissue weight (at the end of experiments adipose tissue weight was 1.5 ± 0.9 in lipectomy and 15 ± 3.9 g in control rats; statistically significant). Compared to control rats, the animals subjected to lipectomy presented increased food intake, standard growth rate, and decreased serum leptin concentrations (2.6 ± 0.8 vs. 3.7 ± 1.2 ng/mL in the controls, statistically significant). These changes were associated with approximately twofold increase in neuropeptide Y, threefold increase in agouti-related peptide (orexigenic neuropeptides) and about 50% decrease in pro-opiomelanocortin and cocaine–amphetamine-regulated transcript peptide (anorexigenic neuropeptides) mRNA levels in the hypothalamus. These results suggest that partial lipectomy, leading to a decrease in circulating leptin concentrations, may exert an effect on hypothalamic orexigenic and anorexigenic neuropeptide gene expression, and consequently modulate food intake and standard growth rate in rats.

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Introduction

Liposuction, a cosmetic procedure of removing fat from various anatomical regions of human body, is frequently performed in overweight women (Mordon and Plot, 2009). However, the consequences of this procedure to human health are still poorly understood and the reported results are inconclusive (Danilla et al., 2013; Klein et al., 2004; Martinez-Abundis et al., 2007; Giugliano et al., 2004). Also the data from animal experimental models used to study the effect of removal (Gabriely et al., 2002; Shi et al., 2007) or deprivation (Moitra et al., 1998) of white adipose tissue on insulin resistance and circulating metabolite concentrations are inconclusive. Biomedicine, which applies biological principles to clinical practice, including experimental neuroscience, laboratory diagnostic, molecular and cell biology (Berger, 2011), may help to resolve some issues related to the effect of lipectomy on human health.

Adipose tissue is the largest endocrine organ, synthesizing and secreting many adipokines (including leptin), free fatty acids and steroid hormones that play an important role in control of feeding, energy homeostasis, carbohydrate and lipid metabolism, neuroendocrine function and many other processes (Filer, 2004; Swierczynski, 2006; Swierczynski and Sledzinski, 2012). Thus, removal of adipose tissue may likely lead to a decrease in the serum/tissue concentrations of the above-mentioned substances, and consequently, may influence many important functions and processes, including hypothalamic expression of orexigenic and anorexigenic neuropeptide genes. Hypothalamus is a major center regulating feeding behaviors and body weight (Bray, 1992). Leptin, a protein secreted mostly by adipocytes, is one of the most important signal molecules, regulating energy intake and expenditure. After reaching the hypothalamus, leptin causes a decrease in food intake and an increase in energy expenditure (Swierczynski, 2006; Zhang et al., 1994; Friedman and Hallas, 1998). Leptin down-regulates orexigenic and up-regulates anorexigenic neuropeptide gene expression (Korner et al., 2001; Takahashi and Cone, 2005). Both our previous studies (Nogalska et al., 2009) and the experiments conducted by other authors (Gabriely et al., 2002) showed that surgical removal of adipose tissue is reflected by a decrease in the amount of circulating leptin in rats. As the assessment of changes in orexigenic and anorexigenic neuropeptide gene expression in human hypothalamus after adipose tissue removal is not possible, we examined this phenomenon in an animal model. The aim of this study was to evaluate whether partial surgical removal of white adipose tissue (WAT) in rats (which may simulate liposuction in humans) has an impact on hypothalamic orexigenic and anorexigenic neuropeptide gene expression and, consequently, on food intake, body mass gain and standard growth rate.

Materials and methods

Animals and white adipose tissue removal

Three-month-old male Wistar rats were obtained from the breeding colony at Tri-City Central Animal Laboratory – Research and Service Centre, Medical University of Gdansk, Gdansk (Poland). The rats were kept under controlled temperature and humidity (60%) with a 12 h day/12 h night cycle throughout the experiment, with lights on at 7:00 a.m. All the procedures involving the animals and their care were approved by the Institutional Ethics Committee. The rats were kept individually in stainless-steel standard wire-mesh cages with free access to tap water. The rats were fed ad libitum, with the commercial chow provided by Labofeed Chow Manufacturer Morawski (Poland). The rats were randomly divided into two groups. The first group, referred to as the lipectomy rats ($n = 6$), underwent resection of both epididymal and retroperitoneal WAT. The second group, referred to as the control animals ($n = 10$), underwent anesthesia and incision of the skin and muscles, but without removal of the epididymal and retroperitoneal WAT (sham surgery). The animals from the two groups were anesthetized with an intraperitoneal injection of ketamine (60 mg/kg body weight) and xylazine (6 mg/kg body weight). After 4 weeks, the lipectomy rats were anesthetized again (as described above), and subcutaneous WAT was removed. At the same time, control animals underwent anesthesia and incision of the skin without removal of subcutaneous WAT (sham surgery). The amount of WAT removed from the lipectomy rats was 7.7 ± 0.6 g (subcutaneous = 3.8 ± 0.3 g, epididymal = 2.0 ± 0.4 g, and retroperitoneal = 1.9 ± 0.4 g). Lipectomy was performed in two steps to reduce perioperative mortality. We decided to remove epididymal, retroperitoneal and subcutaneous WAT as these fat depots are easy to locate and manipulate in live, anesthetized rats. Surgeries were performed carefully in order to avoid bleeding.

Food intake was monitored every other day, between 08:15 a.m. and 09:00 a.m. Food intake was calculated by subtracting the residual food from the total food. After 90 days from the first surgery, all rats were anesthetized and killed by decapitation (between 08:00 a.m. and 10:00 a.m.). Serum samples were collected. Fragments of abdominal (epididymal and retroperitoneal adipose tissue) and subcutaneous WAT from the control animals, as well as the remnant pieces of WAT from the lipectomy animals, were removed and weighted. Mesenteric WAT, as well as the hypothalamic portion of the brain (as a whole), were also collected from all the animals. Immediately after obtaining, the tissues were rapidly frozen in liquid nitrogen for subsequent analyses of gene expression. The material was stored at $-80\,^{\circ}C$ until analysis.

Determination of a standard growth rate

Standard growth rate was determined as described previously (Dreon et al., 2010; Burrells et al., 1999). Briefly, body weight was determined daily for the control and lipectomy rats. The standard growth rate (SGR) was calculated according to the formula:

$$SGR = \left\{ \left(\frac{W_{to}}{W_t} \right)^{1/t} - 1 \right\} \times 100$$

where W_{to} is the initial body weight, W_t is the final body weight, and t is the time in days.

RNA isolation

Total cellular RNA was isolated from the frozen hypothalamic portion of the brain and mesenteric WAT by a guanidinium isothiocyanate–phenol/chloroform method (Chomczynski and Sacchi, 1987). The RNA concentration was determined from the absorbance at 260 nm; all the samples showed 260/280 nm absorbance ratio of about 2.0.

cDNA synthesis

First strand cDNA was synthesized from 1 μg of total RNA (RevertAid^{TM} First Strand cDNA Synthesis Kit – Fermentas, International Inc., Canada). Prior to the reverse transcription, RNA sample was treated with RNase-free DNase I (Fermentas, International Inc., Canada).

Determination of mRNA levels by real-time RT-PCR

Real-time RT-PCR, using Chromo4 Real-Time Detection System (Bio-Rad Laboratories Inc., USA), quantified NPY, AgRP, POMC, CART, leptin and β-actin mRNA levels. The primer sequences used in this study are presented in Supplementary Table 1. Real-time PCR amplification was performed in a 20 μl volume using Maxima SYBR Green qPCR Master Mix (Fermentas, International Inc., Canada). Each reaction included 0.3 μM of each primer. The samples were incubated at 95 °C for 10 min for an initial denaturation and polymerase activation, followed by 40 PCR cycles of amplification (95 °C for 30 s, 53 °C for 30 s, and 72 °C for 60 s). Control reactions, with omission of the RT step or with no template cDNA added, were performed during each experiment. All the samples were run in duplicate. β-Actin mRNA was used as an internal standard to compensate for any differences in the amount of RNA/cDNA, and/or the efficiency of the reverse transcription. Relative quantities of the transcripts were calculated using the $2^{-\Delta\Delta CT}$ formula (Livak and Schmittgen, 2001). The results are shown as relative expression ratios of a target gene to the β-actin gene. The amplification of specific transcripts was further confirmed by obtaining the melting curve profiles.

Supplementary Table S1 related to this article can be found, in the online version, at doi:10.1016/j.jab.2015.01.003.

Serum leptin analysis

Serum leptin was assessed using a commercially available ELISA Kit (Mouse and Rat Leptin ELISA kit provided by BioVendor – Laboratorní medicína a.s., Czech Republic). The sensitivity of the assay was 0.50 pg/mL.

Statistical analysis

Statistical analysis was conducted using a MS Excel 2010 spreadsheet (Microsoft). All the data were expressed as mean values for the control and lipectomy group ± SD. To verify the significance of differences in the analyzed parameters of the control and lipectomy rats Student's *t*-test was performed at the significance level $2\alpha = 0.05$.

Results

The weight of subcutaneous (inguinal), retroperitoneal and epididymal WAT determined at the end of the experiment, as well as the cumulative weight of the abovementioned fat depots in the control and lipectomy rats, are presented in Fig. 1. When compared to the control rats, lipectomy resulted in a complete reduction of the subcutaneous and approximately 80% reduction of the retroperitoneal and epididymal adipose tissue weight. Consequently, the cumulative weight of subcutaneous, retroperitoneal and epididymal adipose tissue was reduced by about 90% in the lipectomy rats. At the beginning of the experiment, mean body weight of the control and lipectomy rats was essentially similar (Fig. 2A). After the first surgery, mean body weight of the lipectomy rats decreased by approximately 13 g (Fig. 2A). Subsequently, the body weight of both the control and lipectomy rats increased constantly, although the lipectomy rats gained body weight faster than the controls (Fig. 2A). Essentially similar changes were found after the second surgery (Fig. 2A). At the end of the experiment, final body weights of the control and lipectomy rats were 403 ± 21 g and 407 ± 17 g respectively. During the first 70 days of the experiment, the lipectomy rats displayed a significantly greater standard growth rate than the controls (Fig. 2B). This phenomenon was probably at least in part due to greater food ingestion by the lipectomy rats, especially up to 40 days after the first surgery (Fig. 3). This intergroup difference in standard growth rate was no longer observed on subsequent days of the experiment, when the standard growth rates of the lipectomy and control rats were essentially similar (Fig. 2B). Three months after the surgery, serum leptin concentrations of the lipectomy rats were significantly lower than in the controls (Fig. 4A). Also the expression of leptin in mesenteric WAT of the lipectomy rats tended to be lower than in the controls, but this difference did not prove to be statistically

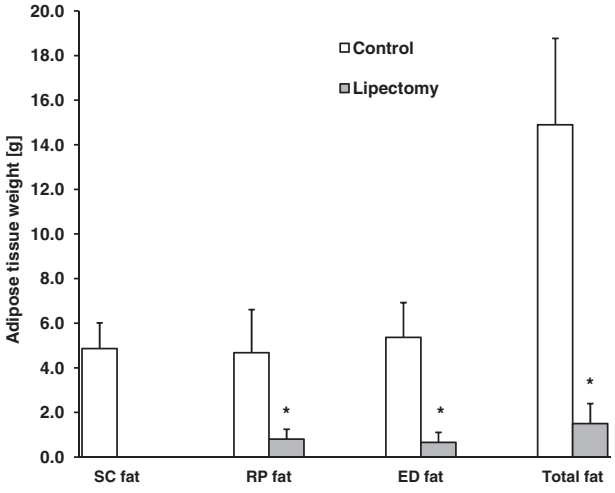

Fig. 1 – The weight of subcutaneous (SC), retroperitoneal (RP) and epididymal (ED) white adipose tissue and total weight of those three fat depots in the control and lipectomy rats. Subcutaneous adipose tissue was completely removed in the lipectomy rats. *Statistically significant.

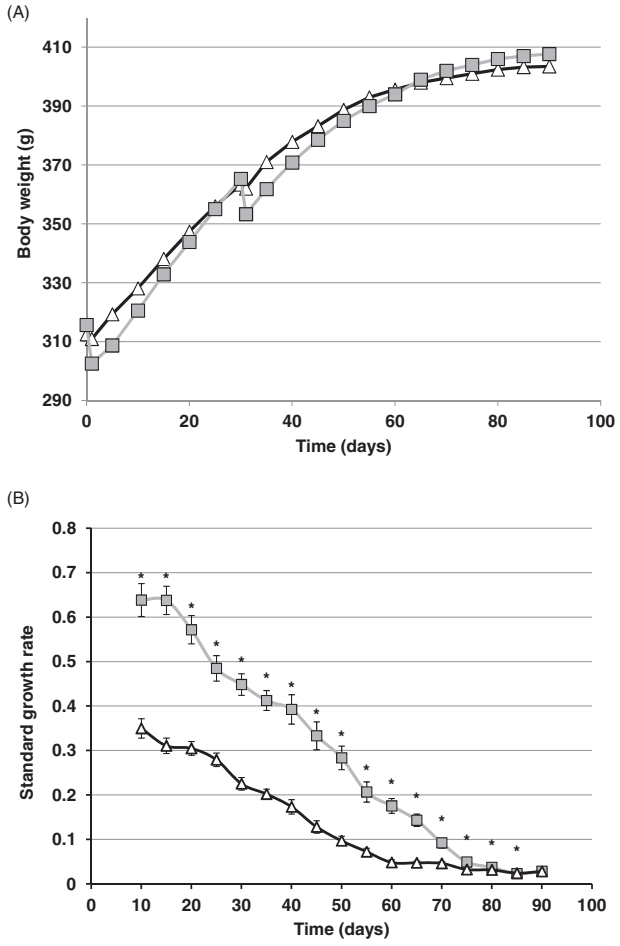

Fig. 2 – Average body weight of the lipectomy (gray squares) and control rats (white triangles) (A) and average growth rates of the lipectomy rats (gray squares) as compared to the controls (white triangles), calculated per each five-day period (B). The data are presented as mean ± SD. Symbols as in Fig. 1.

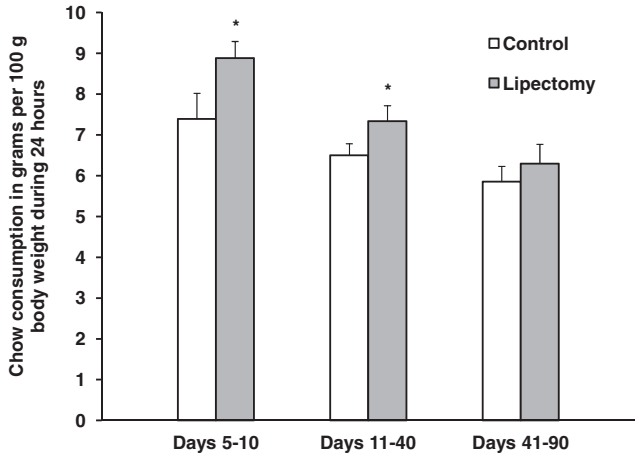

Fig. 3 – Effect of lipectomy on food consumption. Average chow consumption is shown in grams consumed per 24 h per 100 g animal body weight. The bars represent mean daily food consumption in periods of the experiment indicated below. Symbols as in Fig. 1.

significant (Fig. 4B). Both hypothalamic NPY (Fig. 5A) and AgRP (Fig. 5B) mRNA levels in the lipectomy rats were significantly higher than in the controls, possibly due to the decrease in the serum leptin concentration. In contrast, the mRNA levels of hypothalamic anorexigenic neuropeptides, POMC (a precursor of α-melanocyte stimulating hormone – α-MSH) (Fig. 6A), and CART (Fig. 6B) were reduced in the lipectomy animals when compared to the controls. Since NPY and AgRP enhance, and α-MSH (which acts on melanocortin receptors – MC4R), and CART reduce food intake, one would expect an increase in this parameter in the lipectomy rats.

Discussion

The principal finding of this study is that the removal of adipose tissue has an impact on food intake, standard growth rate, serum leptin concentration and hypothalamic neuropeptide gene expression of rats. The decrease in serum leptin concentration and leptin mRNA level in subcutaneous WAT after removal of visceral fat was previously reported in Zucker Diabetic Fatty rats (Gabriely et al., 2002). This suggests that the decrease in serum leptin levels may result from a reduction of

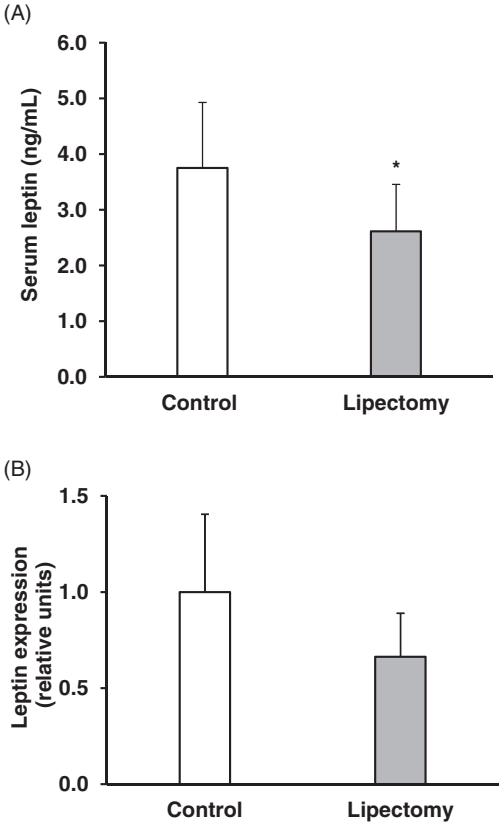

Fig. 4 – Serum leptin level of the lipectomy rats as compared to the controls (A); the relative transcript level of leptin in mesenteric WAT (B). Symbols as in Fig. 1.

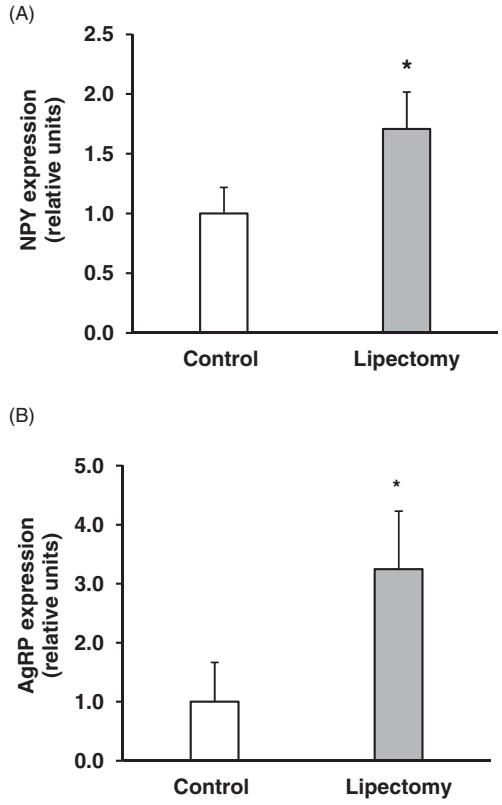

Fig. 5 – The relative transcript abundance of hypothalamic NPY (A) and AgRP (B) in the lipectomy and control rats. Symbols as in Fig. 1.

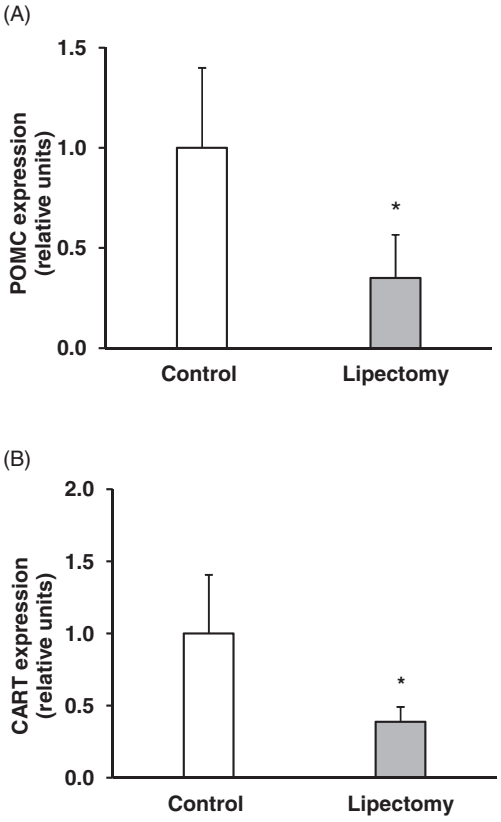

Fig. 6 – The relative transcript abundance of hypothalamic POMC (A) and CART (B) in the lipectomy and control rats. Symbols as in Fig. 1.

fat mass and, at least in part, from reduced synthesis of leptin. In this context, the hereby presented findings (Fig. 4A and B) are consistent with the previously reported results (Gabriely et al., 2002). The reduction of fat content in our rats resulted in the decrease in circulating leptin concentration, determined 3 months after the first and 2 months after the second surgery. This time point was chosen to avoid potential confounding effects of stress and inflammatory response to surgery (and associated anesthesia) on serum leptin concentrations, hypothalamic neuropeptide gene expression and food intake.

An increase in hypothalamic NPY and AgRP mRNA levels, as well as a concominant decrease in POMC and CART mRNA levels, was reflected by slightly greater food intake. To the best of our knowledge, this is the first report on the impact of adipose tissue removal on the hypothalamic expression of orexigenic and anorexigenic neuropeptide genes. The post-lipectomy increase in food intake can be explained by the fact, that NPY and AgRP are the most potent known orexigenic neuropeptides, whereas POMC and CART are considered the most potent anorexigenic neuropeptides. However, contrary to our findings, several previous studies showed no effect of lipectomy on food intake in mice with genetic alterations of leptin and its receptor (Harris et al., 2002), Siberian hamster (Shi and Bartness, 2005) and rats (Ling et al., 2014; Bueno et al., 2011, 2005). One possible explanation for these discrepancies is a different effect of lipectomy on circulating leptin concentration. We found a significant post-lipectomy decrease in circulating leptin concentration, whereas other authors showed that lipectomy exerts no effect on this parameter (Ling et al., 2014; Bueno et al., 2011). Too small amount of removed WAT may be one reason behind the lack of post-lipectomy changes in circulating leptin concentration and, consequently, food intake. In this study, the total amount of removed WAT exceeded 2.5% of total body weight, as compared to 1.5–2.5% in previous experiments (Ling et al., 2014; Bueno et al., 2011). Moitra et al. (1998) showed that transgenic mice (A-ZIP/F-1 mice), which had no WAT and presented with lower circulating leptin concentrations, consumed almost twice as much food as their littermate controls. This supports our hypothesis that the amount of removed fat plays a key role in the control of food consumption, possibly via regulation of the hypothalamic neuropeptide gene expression. The role of leptin and its receptor in the control of food intake is also supported by the results of a study analyzing the effects of lipectomy or fat transplantation in leptin-receptor deficient mice (*db/db* mice) (Erion et al., 2014). Although lipectomy resulted in a decrease (similar to our experiment) and fat transplantation to an increase in the circulating leptin concentration, no changes in food intake were observed in this study (Erion et al., 2014). Probably, the expressions of

orexigenic and anorexigenic neuropeptide genes did not change after lipectomy or fat transplantation due to the lack of leptin receptor in *db*/*db* mice, and thus their food intake remained unchanged.

In this study, we removed WAT from three main anatomical locations. In turn, abdominal WAT was the only fat depot removed in the previous experiments (Ling et al., 2014; Bueno et al., 2011). Schreiber et al. (2006) found that the circulating leptin concentration after liposuction of subcutaneous fat in obese Zucker rats is higher than in sham operated animals. Also the removal of subcutaneous WAT in mice fed high-fat diet resulted an increase in serum leptin level (Foster et al., 2013). Furthermore, the transplantation of subcutaneous WAT into visceral cavity led to a decrease in serum leptin concentration (Foster et al., 2013). Altogether, these data suggest that anatomical location of the removed WAT may be another factor modulating the effect of lipectomy on serum leptin concentration in rodents.

The increase in food intake (Fig. 3) and standard growth rate (Fig. 2B) associated with overexpression of orexigenic neuropeptide genes (Fig. 5) and down-regulation of anorexigenic neuropeptide genes (Fig. 6) may partially explain regeneration of non-excised adipose tissue that was observed after lipectomy (Mauer et al., 2001; Faust et al., 1977). It was suggested that a decrease in energy expenditure may also contribute to the compensatory increase in fat mass after liposuction (Benatti et al., 2012). Lower concentration of circulating leptin and changes in the hypothalamic neuropeptide gene expression favors the post-lipectomy decrease in energy expenditure (Busetto et al., 2008; Joly-Amado et al., 2014). Thus, it was the decrease in the energy expenditure which likely contributed, at least in part, to the accelerated body weight gain and increase in standard growth rate observed in our experimental model.

It is unclear whether liposuction induces similar changes in blood leptin concentration, orexigenic and anorexigenic neuropeptide gene expression in humans. Busetto et al. (2008) showed that surgical removal of subcutaneous adipose tissue in obese women led to a decrease in circulating leptin concentration and concominant decrease in energy expenditure. Also D'Andrea et al. (2005) observed a decrease in plasma leptin concentration and improvement in basal metabolism within 90 days after large-volume liposuction conducted in obese women. Thus, one may suppose that surgical removal of adipose tissue in obese women is associated with changes in the hypothalamic neuropeptide gene expression that favor a decrease in energy expenditure. A significant decrease in circulating leptin concentration was also observed after liposuction of subcutaneous abdominal fat in obese women (Robles-Cervantes et al., 2007). The post-liposuction decrease in circulating leptin concentration was associated with a diminished appetite (Robles-Cervantes et al., 2007).

The reduction of peripheral fat content in healthy women leads to a decrease in serum leptin concentration that persists for at least one week after liposuction, and then gradually returns to its baseline level (Talisman et al., 2001). Theoretically, the lower serum concentrations of leptin observed after liposuction should stimulate the appetite center via the leptin receptor and lead to an increase in appetite and decrease in energy expenditure. However, despite the decrease in concentration of circulating leptin, most lipectomized women were shown to lost appetite for 1–3 weeks post-surgery, and displayed about 7% reduction in body weight (Talisman et al., 2001). These discrepancies between the results of rat experiments and the data from healthy and obese humans after liposuction can be hardly explained based on the putative adipostatic role of leptin. Therefore, one can suppose that the loss of appetite observed in subjects after liposuction is not attributable to leptin itself.

Similarly to rodents, the effect of lipectomy on circulating leptin concentration in humans is proportional to the amount of removed fat. For example, Davis et al. (2006) showed that small-volume suction lipectomy leads to a decrease in circulating leptin concentration immediately after operation, but this effect is no longer observed one month after the procedure. Shcheglova et al. (2004) demonstrated that while a small-volume liposuction is associated with an increase in circulating leptin concentration, the large-volume procedures leads to an initial decrease of this parameter and its subsequent normalization at the baseline level.

Our findings suggest that accelerated body weight gain and increased standard growth rate observed after partial lipectomy in rats resemble compensatory increase of visceral fat after abdominal liposuction in healthy, normal-weight women (Benatti et al., 2012). Unfortunately, we did not determine accurately the weight of mesenteric WAT or fat from other anatomical locations. Lipectomy seems to result in compensatory increase in the volume of mesenteric WAT and fat depots in other anatomical regions of a body. However, further research needs to be conducted to elucidate this problem.

In conclusion, we revealed that partial lipectomy leads to up-regulation of orexigenic and down-regulation of anorexigenic hypothalamic neuropeptide gene expressions in rats, and consequently affects appetite regulation and standard growth rate, possibly via decreasing serum leptin concentration. As the changes in orexigenic and anorexigenic hypothalamic neuropeptide gene expressions may favor the decrease in energy expenditure in rats, one should not exclude that the latter also contributes to increased standard growth rate.

Conflict of interest

We have no conflict of interest to disclose.

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