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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 °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:

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

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