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Research report

The different satiating capacity of CHO and fats can be mediated by different effects on leptin and ghrelin systems

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ABSTRACT

Leptin and ghrelin are known to be the main hormones involved in the control of food intake, with opposite effects. Here we aimed to assess whether changes in leptin and ghrelin systems can be involved in the different satiating capacities of carbohydrates (CHO) and fat. Adult male Wistar rats were studied under 24 h fasting conditions and after 24 h fasting followed by a 12 h re-feeding period with 64 kcal of CHO or fat, consisting of a mixture of wheat starch and sucrose or bacon, respectively. Serum levels of leptin and ghrelin, and mRNA levels of leptin and ObRb in the retroperitoneal and inguinal adipose tissue and of NPY, POMC, ObRb and GSHR in the hypothalamus were measured. CHO re-feeding resulted in higher leptin mRNA expression levels in the retroperitoneal adipose tissue and in higher circulating leptin levels compared with those after fat re-feeding. Moreover, circulating ghrelin levels and ghrelin/leptin ratio were significantly higher after fat re-feeding compared with CHO re-feeding, and hypothalamic expression levels of ghrelin receptor increased after fat, but not after CHO, re-feeding. Hence, expression levels of hypothalamic neuropeptides involved in food intake control and regulated by these hormones, particularly the orexigenic NPY and the anorexigenic pro-opiomelanocortin (POMC)-derived α-melanocyte-stimulating hormone, were also differently affected by CHO and fat re-feeding, resulting in a significantly lower NPY/POMC ratio after CHO re-feeding than after fat re-feeding. In conclusion, different effects on the leptin and ghrelin systems can account, at least in part, for the lower satiating capacity of fat compared to CHO.

1. Introduction

Obesity is a multifactorial disorder affected by multiple genetic and environmental factors that increase the risk of type 2 diabetes, cardiovascular disease, and some cancers [1]. Although the importance of molecular genetic factors in determining individual susceptibility to obesity cannot be ruled out, the easy access to highly palatable, energy-dense foods, coupled with a sedentary lifestyle can also account for the current increase in the world-wide prevalence of obesity [2]. In addition to the amount, the composition of food eaten may also influence body-weight regulation [3]. High-fat diets, particularly those rich in saturated fatty acids, have been shown to induce obesity independently of total energy intake, and, under conditions of excess, dietary fat leads to a greater fat accumulation than does dietary carbohydrates (CHO) [3].

Leptin and ghrelin are antagonic hormones with main roles in the complex molecular mechanism integrating the regulation of food intake, energy expenditure and fat reserves [4]. Leptin is a hormone mainly produced and secreted by the adipose tissue, in proportion to the amount of fat stores, which contributes to the long-term regulation of body weight by decreasing food intake and increasing energy expenditure [5]. Leptin is also produced by the stomach [6–8], and released in response to feeding [9], suggesting that this hormone is also involved in the acute regulation of food intake, acting as a satiating hormone (reviewed in [10]). However, although the relationship between circulating leptin and the overall energy status is clear, little is known about the effects of specific nutrients on leptin production and secretion.

The stomach also produces another hormone with antagonic effects to those of leptin, namely ghrelin, which has been identified as the only known circulating orexigenic hormone [11,12]. In addition to stimulating overall food intake, ghrelin has been found to increase the preference for dietary fat [13] and to directly promote adipogenesis [14,15]. In rats, serum ghrelin levels rise just before the onset of the dark period and drop suddenly just after, in accordance with its role not only as a regulator of appetite, but also as a determinant of meal initiation [16]. Similarly, in humans,

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ghrelin levels also rise preprandially initiating voluntary meals [17]. In addition, gastric ghrelin expression has been shown to be dependent not only on the feeding status, but also on diet composition [18]. Notably, the decrease in ghrelin expression by feeding is more persistent when the ingested macronutrients are CHO compared with fat and, interestingly this can be related to the different satiating capacities of the ingested macronutrients, which is higher for CHO than for fat [18].

Leptin and ghrelin signals are integrated in the hypothalamus and other central areas, where they affect the expression and release of orexigenic and anorexigenic neuropeptides, including, respectively, neuropeptide Y (NPY) and α-melanocyte-stimulating hormone (αMSH), a cleavage product of pro-opiomelanocortin (POMC) [19]. Both NPY and POMC neurons express the long-form of the leptin receptor (ObRb) (a class I cytokine receptor) [5,20], and, through its receptor interaction, leptin stimulates the anorexigenic pathway and inhibits the orexigenic one [19]. The ghrelin orexigenic effects appear to be mediated, at least in part, by direct activation of NPY neurons, where ghrelin receptor (GSHR) is expressed [12], and, indirectly, by inhibition of POMC neurons [21]; in fact, a competitive interaction between ghrelin and leptin in feeding regulation has been suggested [12].

Although the different satiating capacities of CHO and fat is clear, little is known about the molecular basis of the mechanisms involved. Considering our previous results showing that the inhibition on gastric ghrelin production by a short (20 min) period of feeding is dependent on diet composition [18], we considered it interesting to further characterize whether the intake of CHO or fat for a longer period (12 h) may also produce changes in the orexigenic and anorexigenic signalling regulated by leptin and ghrelin systems, which may account for the different satiating capacities of these macronutrients.

2. Material and methods

2.1. Animals

Seven-month-old male Wistar Rats (Charles River Laboratories España, SA, Barcelona, Spain), housed at 22 °C with a period of light/dark of 12 h (lights on from 0800 to 2000) and fed a standard chow diet (Panlab, Barcelona, Spain) with free access to food and water, were randomly distributed under 3 different feeding conditions ($n = 5$ in each group): 24 h fasting and 12 h re-feeding after 24 h fasting with two different foodstuffs matched to a caloric value of 64 kcal: 16 g of a mixture of equal amount of wheat starch (Sigma) and sucrose (table sugar) as a carbohydrate meal, or 10.4 g of bacon (65.2% lipids, 7.62% proteins and 27.2% water) as a fat source. This amount of food offered to the animals corresponds to the amount of standard chow diet eaten normally by these animals during the dark period. We checked that all animals had finished the amount of food offered. All animals were killed at the beginning of the light phase.

After killing the animals by decapitation, the hypothalamus and two white adipose tissue (WAT) depots, one subcutaneous (inguinal) and the other internal (retroperitoneal), were rapidly removed. The hypothalamus was harvested by using the following landmarks, i.e., frontal edge of the optical chiasm, lateral sulci, caudal edge of the mammary bodies, and a depth of 2 mm. All samples were immediately frozen in liquid nitrogen and stored at −70 °C until RNA analysis. Blood was also collected and centrifuged at 1000 × *g* for 10 min to collect the serum, which was stored at −20 °C until analysis.

The animal protocol followed in this study was reviewed and approved by the Bioethical Committee of the University and guidelines for the use and care of laboratory animals of our University were followed.

2.2. RNA extraction

Total RNA was extracted from tissues by Tripure Reagent (Roche Diagnostic GmbH, Mannheim, Germany) according to the manufacturer's instructions. Isolated RNA was quantified using the NanoDrop ND-1000 spectrophotometer (NadroDrop Technologies Wilmintog, Delaware, USA) and its integrity confirmed using agarose gel electrophoresis.

2.3. RT-qPCR analysis

Real-time polymerase chain reaction was used to measure mRNA expression levels of NPY, POMC, ObRb and GSHR in hypothalamus and leptin and ObRB in both WAT depots studied.

0.25 μg of total RNA (in a final volume of 5 μl) was denatured at 65 °C for 10 min and then reverse transcribed to cDNA using MuLV reverse transcriptase (Applied Biosystem, Madrid, Spain) at 20 °C for 15 min, 42 °C for 30 min, with a final step of 5 min at 95 °C in a Applied Biosystems 2720 Thermal Cycler (Applied Biosystem, Madrid, Spain). Each PCR was performed from diluted (1/50) cDNA template, forward and reverse primers (1 μM each), and Power SYBER Green PCR Master Mix (Applied Biosystems, CA, USA). Primers, obtained from Sigma (Madrid, Spain), for the different genes are described in [22]. Real-time PCR was performed using the Applied Biosystems StepOnePlusTM Real-time PCR Systems (Applied Biosystems) with the following profile: 10 min at 95 °C, followed by a total of 40 two temperature cycles (15 s at 95 °C and 1 min at 60 °C). In order to verify the purity of the products, a melting curve was produced after each run according to the manufacturer's instructions. The threshold cycle (Ct) was calculated by the instrument's software (StepOne Software v2.0) and the relative expression of each mRNA was calculated as previously described [23].

2.4. Quantification of serum leptin and ghrelin levels

Leptin and ghrelin concentration in serum was measured with a mouse leptin enzyme-linked immunosorbent assay (ELISA) kit (R&D Systems, Minneapolis, MN, USA) and with a rat ghrelin enzyme immunosorbent assay (EIA) kit (Phoenix Europe, Karlsruhe, Germany), respectively. Values of intra- and inter-assay coefficient of variation of the kits are 3.8 and 5.8% for leptin, and 5–10 and <15% for ghrelin, respectively. Specificity values are 79% for leptin and 100% for ghrelin.

2.5. Statistical analysis

All data are expressed as the mean ± SEM. Differences between groups were assessed by one-way ANOVA followed by least significances difference (LSD) post hoc comparison to assess statistical differences between the groups. Single comparisons were assessed by Student's *t* test. The analyses were performed with SPSS for Windows (SPSS, Chicago, IL). Threshold of significance was defined at $p < 0.05$.

3. Results

3.1. Serum leptin and ghrelin levels

CHO or fat re-feeding for 12 h after fasting conditions did not result in changes in circulating leptin levels, although a tendency to higher levels was found after CHO intake; and, notably, circulating leptin levels were higher after CHO re-feeding compared with

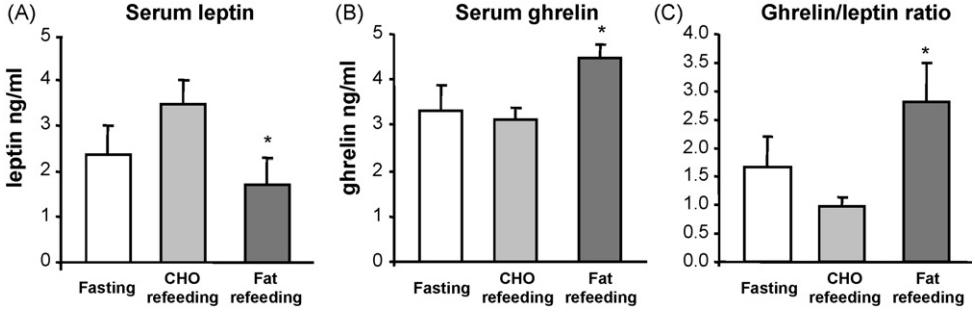

Fig. 1. Serum leptin (A) and ghrelin (B) levels and ratio of the ghrelin and leptin serum levels (C) of rats under different feeding conditions: 24 h fasting, and 12 h re-feeding after 24 h fasting with 64 kcal of a carbohydrate (CHO) or a fat meal. Data are means ± SEM ($n = 5$). *CHO vs fat re-feeding ($p < 0.05$, Student's *t* test).