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PROCEEDINGS

Role of leptin present in maternal milk in the control of energy balance during the post-natal period

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Leptin is a hormone mainly produced by the adipose tissue [1], but also by the stomach [2–4], placenta [5, 6] and mammary epithelial cells [7]. The stomach can produce and store leptin and release it in response to food intake [3, 4, 8]. Also leptin is produced by the placenta and potentially plays a role in fetal and neonatal growth. Leptin is present in maternal milk and this leptin could be absorbed by the immature gastric mucosa of neonatal rats [9, 10]. Previous results of our laboratory show that during the first 15 days of life, maternal milk-borne leptin is the main source of gastric leptin and the increase of endogenous leptin expression in the stomach of neonates is related to the change of diet from milk to a solid chow diet [10].

The biological role of milk-borne leptin is not known. Neither is the biological role of milk-borne leptin or its implication in post-neonatal development known. However, there is large epidemiological evidence that food during early life does have implications in the further development of obesity [11].

Firstly, our aim was to check whether a single oral dose of leptin to 4-day-old suckling rats is absorbed and affects food intake. Previously, leptin concentration in rat milk was determined in order to know the physiological milk leptin levels and its variations during lactation. Thus, milk samples were collected on day 7, 14 and 21 of lactation and milk leptin levels were measured by ELISA [12]. Afterwards, 4-day-old rats were treated with a single oral dose of 4 ng of leptin (equivalent to five times the amount of leptin ingested normally from maternal milk) or the vehicle, and were sacrificed at different times: time 0, just before leptin administration, and 4 h after leptin or vehicle administration. Gastric content was weighted and samples of serum and stomach were collected for the measurement of leptin levels by ELISA. In order to check the endogenous or exogenous origin of gastric leptin, immunohistochemistry studies were performed.

The next step entailed studying the effect of a daily oral leptin administration during the suckling period. Daily and during the whole lactating period (21 days), suckling rats received the vehicle or an oral dose of leptin, equivalent to five times the amount of leptin ingested normally from maternal milk. At the end of lactation, suckling rats were sacrificed. Serum and gastric leptin levels were determined by ELISA and mRNA leptin expression in adipose tissue depots (epididymal, retroperitoneal, mesenteric and inguinal) and in the stomach were determined by northern blot and RT-PCR, respectively. UCP1 expression and its protein levels in BAT were also studied.

The administration of a single oral dose of leptin to 4-day-old rats produced, an increase in leptin levels in the stomach and in serum and a decrease in the weight of the gastric contents (Fig. 1). Immunohistochemistry studies show that the leptin-positive signal was mainly located at the apex of the superficial epithelial cells and at different levels of the gastric glands, suggesting that the leptin supplied is being absorbed by the gastric mucosa [12].

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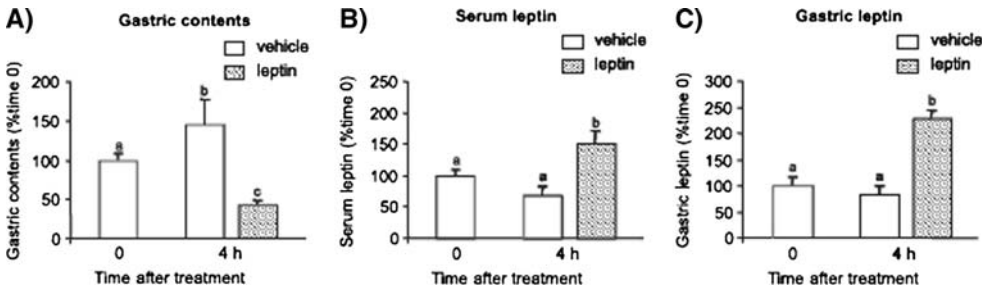

Fig. 1 Weight of gastric contents (**a**) and serum (**b**) and gastric (**c**) leptin levels in 4-day-old rats treated with an oral dose of leptin or vehicle at time 0 and 4 h later. Five different litters were used to perform the experiment and pups from each litter were randomly assigned into the different groups. The values from each litter are expressed as a percentage of its time 0 group. Within each graph, bars not sharing a common letter (*a*, *b*, *c*,) are significantly different. Adapted from [12] (Copyright 2005, The Endocrine Society)

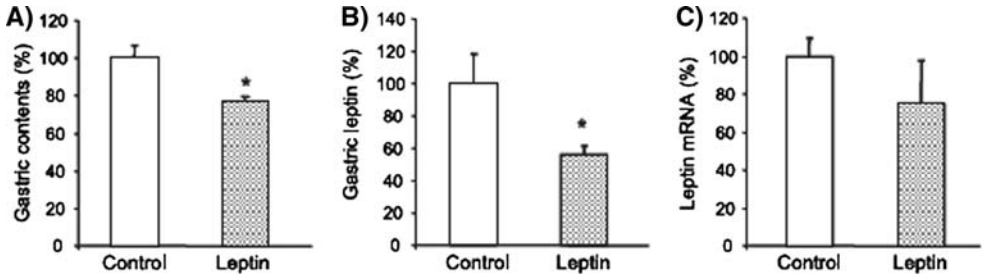

Fig. 2 Weight of gastric contents (**a**), gastric leptin concentration (**b**) and gastric leptin mRNA levels (**c**) in 21-day-old rats that received a daily oral dose of leptin or the vehicle during lactation. Leptin mRNA levels were measured by reverse transcriptase-polymerase chain reaction (RT-PCR) and values expressed relative to β-actin mRNA and as a percentage of control group. Results are expressed as mean ± SEM ($n = 5$). $^*P < 0.05$, control versus leptin-treated rats (Student's t test). Adapted from [12] (Copyright 2005, The Endocrine Society)

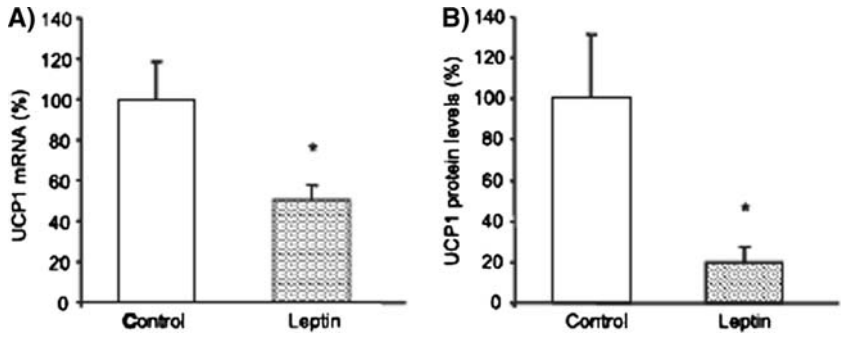

Fig. 3 **a** UCP1 mRNA expression levels (measured by northern blotting) in the interscapular BAT in 21-day-old rats that received a daily oral dose of leptin or the vehicle during lactation. UCP1 mRNA levels are expressed relative to 18S rRNA levels and as a percentage of control group. **b** UCP1 protein levels (measured by western blotting) in the interscapular BAT in 21-day-old rats that received a daily oral dose of leptin or the vehicle during lactation. Results are expressed as a percentage of control group. $^*P < 0.05$, leptin-treated versus control animals (Student's t test). Adapted from [12] (Copyright 2005, The Endocrine Society)

Pups treated with a daily oral dose of leptin during the whole lactation period showed, at the end of the suckling period compared with controls, lower gastric contents, and lower leptin production by the stomach (Fig. 2). No significant leptin staining was found at the apex of the superficial epithelial cells in either of the two groups of animals, but leptin staining was found in the basal part of the gastric glands, indicating that in 21-day-old animals the source of gastric leptin is mainly endogenous production rather than exogenous leptin absorption [12]. Neither body weight at the end of the study nor the size of the fat depots studied nor the serum leptin levels were affected by leptin treatment. However, leptin treated animals displayed a lower UCP1 expression and production in the BAT (Fig. 3), probably as a result of lower food intake during lactation.

We conclude that oral leptin is absorbed by the immature gastric epithelium of the neonate and this leptin exerts clear biological effects in the earlier stages of neonatal life, downregulating endogenous leptin production, and playing a potential role in the short-term control on food intake during this period.

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