J. Endocrinol. Invest. *25:* 670-674, 2002

Presence of bovine leptin in edible commercial milk and infant formula

M. Lage**, R. Baldelli*, J.P. Camiña*, J. Rodriguez-Garcia***, A. Peñalva*, C. Dieguez**** and F.F. Casanueva*

*Endocrinology Service, Regina Elena Cancer Institute, Rome, Italy, **Department of Medicine, Endocrinology Section, ***Department of Biochemistry and ****Department of Physiology, Santiago de Compostela University and Complejo Hospitalario Universitario de Santiago, Santiago de Compostela, Spain

ABSTRACT. Leptin is a hormone secreted by the adipocytes that contribute to the control of energy balance, and circulating leptin levels reflect the amount of adipose tissue in the body, helping to regulate food intake and energy expenditure. Since it has been shown that human milk contains immunoreactive leptin, which is identical to intact human leptin, we decided to investigate the possible presence of immunoreactive bovine leptin in different kinds of common commercial milk. To determine the presence or absence of immunoreactive leptin in bovine milk for human consumption, 81 samples (66 commercial pasteurized milk and 15 artificial formulae for new-born babies) of the most common Spanish commercial types of milk were studied. All samples were evaluated before and after centrifugation, and leptin levels were measured by RIA. Leptin was detected in all samples and RIA standard curves were not perturbed when centrifuged and non-centrifuged milk replaced the buffer. Mean values of leptin in full-cream, semi-skimmed and skimmed samples, were: 5.7±0.3 ng/ml, 4.1±0.1 ng/ml, 3.7±0.1 ng/ml (significantly different). Leptin values were reduced after centrifugation. A significant correlation was observed between leptin levels and lipid content ($p<0.0005$, r=0.67) while no correlation was observed with respect to carbohydrate and protein levels. Interestingly, some preparations of infant formulae present very high leptin values reaching up to 18.9 ng/ml. In conclusion, leptin is present in significant and variable concentrations in edible commercial bovine milk, with higher concentrations in infant formula.

(J. Endocrinol. Invest. *25:* 670-674, 2002)

INTRODUCTION

Leptin, the *ob* gene product, is a 167 amino acid hormone that is secreted by the adipocytes, which acts as an afferent satiety hormone regulating appetite, weight gain, and body fat stores (1-12). Leptin plasma levels correlate mainly with the adipose tissue stores, but not in a simple relationship, as other factors and hormones participate in its regulation (13). The participation of leptin in new physiological and pathological processes unrelated to metabolic functions has been described (13-15). In addition, glucocorticoids and insulin stimulate leptin synthesis from the adipose tissue, whereas adrenergic activation and T induce inhibition (16-19). Our group reported that human leptin was present in milk and colostrum from nursing mothers (20), a relevant finding considering that after delivery milk replaces the placenta in providing the new-born with critical nutrients and growth factors. In rats, leptin is transferred from the mother's milk to the pup's stomach and then to the pup's serum (20-24). This suggests that during the neonatal period, leptin may be absorbed without degradation by the intestinal system and plays a role in the regulation of neonatal food intake and intestinal maturation (20, 25, 26). In the present work, the presence of leptin in edible bovine milk (commercial pasteurized samples and infant formulae) was analyzed. The targets were twofold: 1) to observe whether leptin was also present in bovine milk and 2) to observe whether different milk brands differ in leptin content.

Key words: Leptin, commercial milk, infants formula.

Correspondence: Prof. F.F. Casanueva, Department of Medicine, Endocrine Unit, San Francisco Street s.n., PO Box 563. E-15780 Santiago de Compostela, Spain.

E-mail: endocrine@usc.es

Accepted March 3, 2002.

MATERIALS AND METHODS

In order to determine the presence of immunoreactive bovine leptin levels in edible milk, samples of the most widely used Spanish commercial milk samples were collected (no.=81). In particular 66 types of milk were normal pasteurised commercial milk from different brands (1=Asturiana®, 2=Campobueno®, 3=Celta®, 4=Consumer®, 5=Dia®, 6=Feiraco®, 7=Froiz®, 8=Hypercor®, 9=La Vaquera®, 10=Lagisa®, 11=Larsa®, 12=Leader Price®, 13=Leyma®, 14=Oro del Valle®, 15=Pascual®, 16=President®, 17=Puleva®, 18=Ram®, 19=Reny Picot®, 20=Rio®, 21=Super®, 22=Vega de Oro®). All were analyzed as full-cream, semi-skimmed or skimmed samples before and after centrifugation at 3000 x g, 30 min, 4 C after which the fat layer was removed. Fifteen samples were of the kind used for artificial new-born lactation (infant formulae) (23=Adapta-1®, 24=Adapta-2®, 25=Adapta-peg®, 26=Almiron-1®, 27=Aptamil-1®, 28=Aptamil-2®, 29=Blemil-plus®, 30=Enfalac®, 31=Nativa-1®, 32=Nativa-2®, 33=Nidina-1®, 34=Nidina-2®, 35=Nutriben®, 36=O-Lac®, 37=President®). Milk lipids, proteins and carbohydrate levels data reported by manufacturers.

Leptin milk levels were measured on several occasions for each brand in duplicate by multi-species leptin RIA kit (Linco Research Inc, St. Charles, U.S.A.) The limit of sensitivity was 0.5 ng/ml, the interassay coefficient of variation was 8.7% and the intrassay coefficient of variation was 3.6%.

Data are presented as mean±SE. The Anova test, and the *t* test for paired and unpaired data were used when appropriate. The correlation study was evaluated with a simple regression test. $p<0.05$ was considered significant. Statistical analyses were carried out using a Statview 5 software for Windows (SAS Institute INC., Cary, U.S.A., 1999).

RESULTS

Leptin was detected in all milk samples analyzed and the RIA validation test indicated that milk did not interfere with the assay. RIA standard curves were not altered when 100 µl of centrifuged and non-centrifuged milk substituted the same volume of buffer (Fig. 1). With respect to the different milk subtypes, a significant difference was found in full-cream milk (5.7±0.3 ng/ml) *vs* semi-skimmed (4.1±0.1 ng/ml), and skimmed milk (3.7±0.1 ng/ml) ($p<0.0005$). All values were significantly reduced after centrifugation ($p<0.0005$) (Fig. 2). Leptin levels in the different brands of milk ranged from 2.6 to 8.5 ng/ml (Fig. 3). Levels of leptin detected in infant milk ranged from 1.9 up to strikingly high levels of 18.9 ng/ml (Fig. 3). The correlation study showed a significant correlation between leptin levels and lipid concentrations ($p<0.0001$, r=0.67) but not with proteins or carbohydrates (Fig. 4).

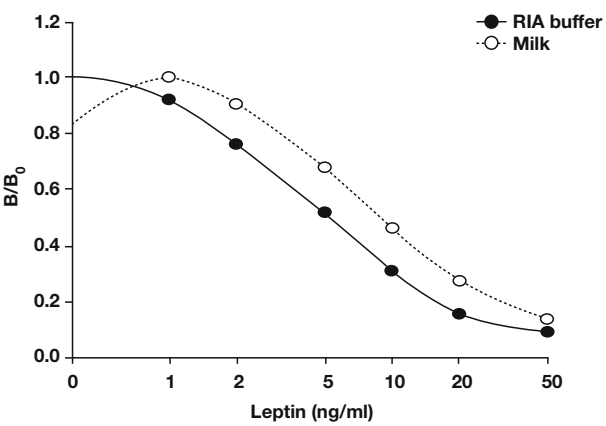

Fig. 1 - *RIA standard curves of human leptin in the absence or presence of 100 ml of commercial milk which substituted the assay buffer. Milk dilutions always paralleled standard curves.*

DISCUSSION

Human milk as well as the milk of several mammalian species contains, besides major nutrients, anti-infectious and immunocompetent substances, biologically active substances such as hormones, peptides and growth factors which can directly influence the physiology of new-born babies after intestinal absorption (27, 28). The function of these bioactive peptides and steroids in milk remains to

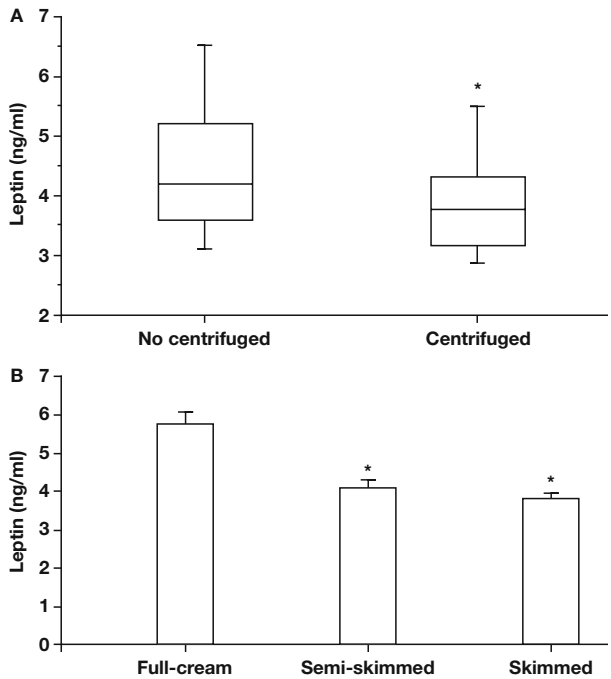

Fig. 2 - *A) Leptin concentration in non-centrifuged (no.=66) and centrifuged (no.=66) bovine milk; (p<0.0005). The error bars represent the 10-90th range, the box represents the 25-75th range and the line represents the median; B) leptin concentration in full-cream (no.=22), semi-skimmed (no.=22) and skimmed (no.=22) milk.* p<0.0005.

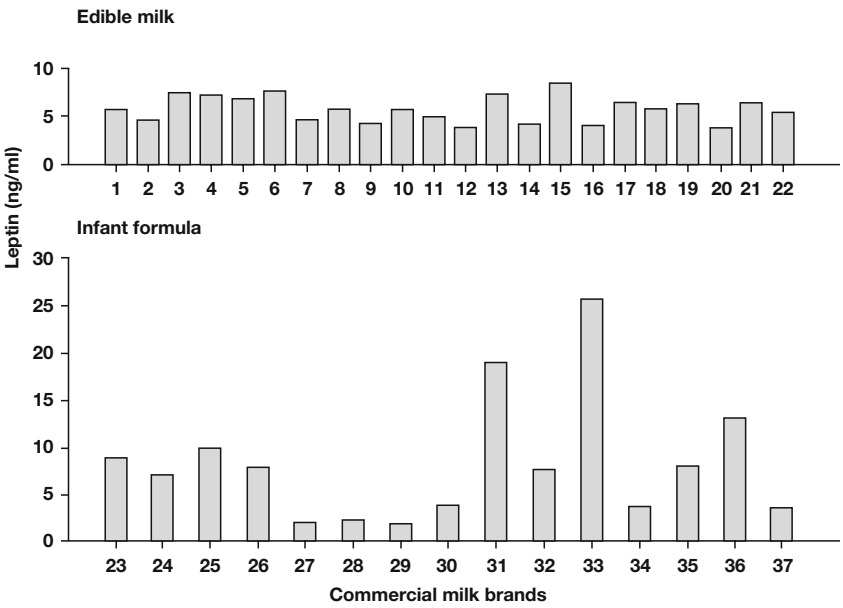

Fig. 3 - *Leptin levels in commercial samples from different brands of pasteurised full-cream edible milk (no.=22; upper panel). Leptin levels in commercial samples of infant-formulae milk brands (lower panel).*

be elucidated, although it has been proposed that these substances could be related to the development of the neonatal small intestine, or play important and different roles in immune function (29). The human neonate has a reduced immune system and needs the support of the mother with the transplacentally passage of IgG antibodies (29). In rats, the presence of leptin in milk and the transfer from milk to the pup's stomach and blood (20) raised the possibility that maternal leptin may exert biological effects on the infant at a time in which both the adipose tissue and the appetite regulatory systems are immature (30). Many bioactive compounds have been shown to be transferred from milk to the neonate blood, and although the transfer of leptin has only been demonstrated for experimental animals, it is likely that it also occurs in man (31, 32). As humans ingest a considerable amount of bovine milk and more in the neonatal period, in this work the presence of leptin in edible milk was analyzed. To the best of our knowledge, this is the first study providing evidence of variable leptin levels in normal commercial milk. Our analyses revealed that leptin concentrations in non-centrifuged milk were higher than in centrifuged milk; an explanation for this clear difference could be due to the fact that a part of leptin could be associated with the milk fat droplet, as previously shown (26). Although detectable, the leptin concentration measured in commercial milk is unlikely to be able to alter circulating leptin levels, if one considers the limited amount of milk ingested in the adult period, plus the fact that proteins are rapidly digested and destroyed in the gastrointestinal tract. Of more biological relevance, however, are the high leptin concentrations observed in infant formulae. In fact, in some infant preparations leptin levels were extremely high with respect to the previous ones found in the milk of lactating women (20) and one must be aware of the high milk intake and the repeated daily ingestion for the low plasma volume of neonates. In a recent work, leptin concentrations were determined in the circulation and milk of sows differing in body condition at farrowing and in feed consumption during lactation. The highest serum concentrations of leptin at farrowing and weaning were detected in sows exhibiting the greatest amount of backfat; leptin was detected in both skimmed and whole milk throughout lactation, but levels were not correlated with backfat thickness or circulating leptin concentration (33). As leptin from different species can be transferred from intestine to blood in the neonatal period (20, 33), biologically active leptin may be significantly increased at regular intervals in the new-born plasma due to the high levels found in artificial milk. Whilst leptin has been found to inhibit food intake in adult animals, neonatal ones need to maximize food intake and also maintain high thermo-regulatory metabolic rates to maintain body temperature. This suggests that leptin may function differently in the neonate compared with adult animals. Currently, it is not possible to ascertain whether this hypothetical bovine leptin rise may play a regulating role in the

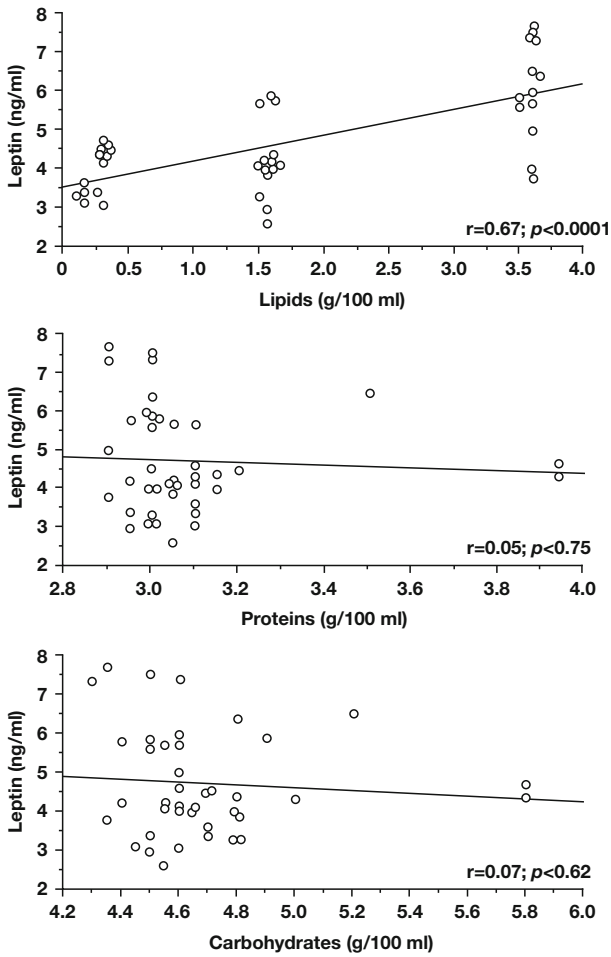

Fig. 4 - *Scatter analysis of leptin levels vs total lipids, protein and carbohydrate levels, in different brands of pasteurized edible milk.*

post-natal period; nevertheless, the point deserves consideration as this mechanism could lead to a possible role of leptin in neonatal development of the gastrointestinal tract, as suggested by the presence of leptin receptors in these tissues, and immune function, or more relevant in the regulation of food intake in neonates. This could explain the intriguing observation that children with *ob* gene mutation develop obesity only after stopping lactation while in those children with *db* gene mutation obesity starts immediately after birth (Farooqi I.S., personal communication).

The amount of leptin in a given milk brand was quite constant over time (data not shown), an observation with potential industrial applications. In fact, there is no easy explanation for the striking divergence in leptin content among the different brands of edible milk. Leptin determination in milk samples may help to control the elaboration process as well as to prevent the alterations in edible brands. The latter point deserves further examination.

In conclusion, the present work provides evidence for the presence of immunoreactive bovine leptin levels in commercial milk and infant formulae. Moreover, the presence of extremely high leptin levels in infant formulae types of milk suggests a potential link between milk–borne leptin and neonatal developing of gastrointestinal function or in the regulation of food intake. Further work is needed in order to ascertain the possible absorption of leptin in humans, and whether this milk-borne leptin exerts a regulatory role on appetite in the suckling infant.

ACKNOWLEDGMENTS

This work was supported by grants from Fondo de Investigacion Sanitaria, Spanish Ministry of Health, and Secretaria Xeral de Investigación e Desenvolvemento, Conselleria de Educacion, Xunta de Galicia and DGICYT.

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