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Leptin, insulin and ghrelin levels in goat milk and in plasma of suckling kids☆

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ABSTRACT

Milk is not just the basic source of nourishment for the neonate, but it is also a vector of bioactive peptides that play a possible role in neonatal development.

For the increasing utilization of goat milk in infant formulas for human nutrition, it could be of interest to quantify the levels of leptin, insulin and ghrelin in goat milk and in plasma of suckling goat kids and to evaluate the relationship between ingested and circulating levels of hormones in growing animals.

For this purpose, 10 suckling kids were observed from the 3rd to the 5th week of age (before the beginning of the weaning period). During the experimental period, body weight and milk intake were recorded. Milk and blood samples were taken before the first meal of the day on week 3, 4 and 5. Plasma and milk were analysed for leptin, insulin and ghrelin.

As expected, body weight and milk intake increased during the experimental period. Although daily intake of hormones and growth factors with milk increased, plasma leptin level of suckling kids decreased ($P = 0.02$). On the contrary, plasma level of insulin increased more than four times ($P < 0.01$) and was highly correlated ($P < 0.01$) to the daily quantity of hormone ingested. This trend was probably associated to the increased milk intake, but it is even possible to hypothesize the presence of receptors that facilitate the migration of milk-borne insulin through the gastrointestinal mucosa of the suckling kids. This hypothesis is supported by the fundamental role played by insulin in the functional development of pancreas; in fact in rats plasma insulin level arises just before weaning and this is supposed to stimulate pancreatic α-amylase gene expression.

1. Introduction

Milk is the basic source of nourishment, containing specific proteins in colloidal dispersion, lipids in emulsified globules coated by membrane, minerals and lactose in true solution, and providing all the elements required for neonatal growth (Jensen et al., 1995). Milk is also a vector of bioactive peptides. The finding of hormones in milk was described as early as 1929 (Yaida, 1929) and, to date, many growth factors have been identified (Grosvenor et al., 1993), even in concentration exceeding that of maternal plasma (Rosi and Rapetti, 2004; Magistrelli et al., 2005).

Some bioactive molecules are transported (unchanged in structure and activity) into milk from the maternal blood; others are synthesized by the mammary gland and post-translationally modified to change their biological activity (Campana and Baumrucker, 1995). Interestingly, some hormones (i.e., thyroid hormones, estrogens, prolactin, etc.) and non-hormonal bioactive substances (i.e., lactoferrin, transferrin, etc.) appear both to be synthesized by the mammary tissue of various species and also transported from maternal circulation (Campana and Baumrucker, 1995).

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Milk-borne peptides can pass across the gastro-intestinal mucosa (Gonnella et al., 1987) and enter the systemic circulation via a receptor-mediated process (Xu et al., 2000; Blum and Baumrucker, 2002), playing a possible role in accomplishing the immature ability of the suckling animals to produce hormones and growth factors (Kinouchi et al., 2000).

Among milk bioactive compounds there are leptin, insulin and ghrelin. All these peptides are involved in the regulation of food intake. Leptin is the product of the *ob* gene and it is mainly secreted by adipose cells (Houseknecht et al., 1998). Circulating leptin has a 147 amino-acids structure and targets the hypothalamus to coordinate food intake. Leptin acts on peripheral tissue to promote energy expenditure and nutrient utilization (Ahima and Flier, 2000). Insulin is a 5.8-kDa protein synthesized in the pancreatic β-cells and secreted in response of elevation of plasma glucose level. Insulin is responsible for glucose uptake from the blood stream into adipose, muscular and hepatic cells (Pessin and Saltiel, 2000), but it also modulates peripheral satiety signals and directly targets the central nervous system to inhibit food intake (Gale et al., 2004). By contrast, ghrelin is a 28 amino-acids acylated peptide from stomach, which plays a role in stimulating GH release by the pituitary gland and in affecting feeding behaviour as stimulator of food intake (Kojima and Kangawa, 2005).

In order to understand the relationship between ingested and circulating levels of hormones in growing animal, the aim of this study was to quantify the level of leptin, insulin and ghrelin in goat milk and in plasma of suckling goat kids.

Moreover, it could be important to evaluate the presence of bioactive peptides in goat milk, because this product is widely consumed in Mediterranean and Middle East countries (Grant et al., 2005). In particular, even if data on the hypo-allergenicity, tolerability and safety of goat milk are conflicting (Wilson and Hamburger, 1988; Park, 1994; Biggart, 1996; Bellioni Businco et al., 1999), goat milk – modified and fortified to meet infant formula regulations – is used as an appropriate alternative for infants who cannot be breast-fed (Grant et al., 2005).

2. Materials and methods

Immediately after birth, 10 Saanen goat kids were separated from their mother and housed in a steel box measuring 3.0 m × 4.5 m ($l \times d$) with straw bedstead. All kids were manually fed colostrum of the respective mother and then goat milk, until the 5th week of age, when kids started a weaning protocol. The milk administered to the kids derived from the massive milk obtained from all the mothers. During the entire experimental period, goat kids were fed twice a day, at 9:00 and 19:00.

From day 4 to week 5 of age, milk intake was recorded daily and body weight was recorded weekly.

On week 3, 4 and 5 of age, jugular vein blood samples were taken before the first meal of the day. After the withdrawal, blood was immediately centrifuged at 2000 × g for 15 min and then stored at −20 °C until analysis.

On the same days, goat milk samples were also taken and stored at −20 °C. At the end of the experimental period, morning and evening milk (collected during the same day) were mixed and an aliquot was analysed for dry matter by drying at 102 °C (IDF, 1987), crude protein by Kjeldahl method (IDF, 1986), fat by Röse-Gottlieb method (IDF, 1996) and lactose content by mid-IR spectrophotometric method (IDF, 1990). Another aliquot was sonicated and centrifuged at 2150 × g for 30 min at 4 °C. Plasma and sonicated-centrifuged milk were tested for leptin (Multispecies Leptin RIA Kit, Linco Research Inc, St. Charles, MO, USA) and insulin (Insik-5, Dia Sorin Spa, Saluggia, VC, Italy) by radio-immuno assays and ghrelin (Total Ghrelin, Diagnostics System Laboratories Inc., Webster, TX, USA) by ELISA method.

Data were analysed by the ANOVA procedure of (SAS, 1999) and correlations expressed by Pearson coefficient.

Table 1
Goat milk composition between 3 and 5 weeks of lactation

	% as fed	S.D.	% Dry matter	S.D.
Dry matter	12.2	0.75		
Protein	3.25	0.09	26.6	0.72
Fat	3.61	0.57	29.5	4.67
Lactose	4.72	0.12	38.6	0.96

3. Results and discussion

Goat milk composition and milk insulin, leptin and ghrelin levels are reported in Tables 1 and 2, respectively.

As expressed in Figs. 1 and 2, body weight and milk intake increased ($P < 0.01$) during the experimental period. Although, during the experimental period, daily intake of hormones and growth factors with milk increased, plasma leptin level of suckling kids decreased ($P = 0.02$) (Fig. 3). This result is in accordance with findings in other species. For example, in rats plasma leptin is observed to be at the highest level during the first hours after birth and associated to the high expression of the *ob* gene in brown adipose tissue (Dessolin et al., 1997). In these animals, the high concentration of leptin at birth may be favourable for increasing heat production in brown adipose tissue during non-shivering thermogenesis for reaction to cold stress (Dessolin et al., 1997). In humans too, plasma leptin concentration rapidly declines during the neonatal period (Yokota, 2003) and until to 4 weeks of age (Helland et al., 1998). After that, Helland et al. (1998) observed an increase in plasma leptin concentration until to 14 weeks after birth. The experimental period of the present study was too shirt to detect an eventual later arise in plasma leptin level. Anyway, in post-weaning goats plasma leptin peaks at the onset of puberty (Vitali et al., 2005).

As shown in Fig. 3, plasma ghrelin did not vary, during our experimental period. In infancy, ghrelin may exert a strong growth hormone releasing action primarily promoting body growth (Savino et al., 2006). In humans, ghrelin level increases after birth peaking during the first 2 years of life, then decreases until the end of puberty to reach the adult level (Soriano Guillen et al., 2004). In rats gastric expression of ghrelin increases in an age-dependent manner from neonatal to adult stages (Hayashida et al., 2002). The discrepancy with our data suggests that it has to be clarified whether the regulation of ghrelin secretion in suckling goat kids is similar to that of other species. This assumption is supported by Kobayashi et al. (2006),

Table 2
Growth factors in milk between 3 and 5 weeks of lactation

	mmol/l	S.D.
Leptin	0.53	0.04
Insulin	0.30	0.06
Ghrelin	1.01	0.04