

SHORT NOTE [NOTA CORTA]

**PLASMA INSULIN AND IGF-1 AND HEPATIC ACTIVITY IN SAANEN
GOAT KIDS, AROUND WEANING**

**[INSULINA Y IGF-1 EN PLASMA Y LA ACTIVIDAD HEPÁTICA EN
CABRITOS SAANEN ALREDEDOR DEL DESTETE]**

Damiano Magistrelli* and Fabia Rosi

*Department of Animal Science, University of Milan – Via G. Celoria 2, 20133
Milan, Italy*

E-mail: damiano.magistrelli@unimi.it

Department of Animal Science, University of Milan

Tel.: +39 02 50316443

**Corresponding author*

SUMMARY

Weaning is a crucial event in the life of young ruminants. At weaning ruminal and digestive activity are still incomplete, so weaning may coincide with a period of growth stasis. Since insulin and insulin-like growth factor 1 (IGF-1) can play a fundamental role in post-natal development, the aim of the present study was to evaluate plasma variations of insulin and IGF-1 levels and their relationships with the hepatic activity, around weaning. For this purpose, eleven 3-days-old Saanen goat kids were randomly divided into MILK (6 animals) and WMIX (5 animals) groups. All kids were fed goat milk to age 29 days. After that, MILK kids continued to receive milk, while WMIX ones underwent weaning, based on the progressive replacement of milk with solid feed. WMIX kids were completely weaned on day 48. Blood samples were weekly analyzed for metabolic traits, insulin and IGF-1 levels, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities. On day 50, all animals were slaughtered, liver weight was recorded and liver samples were analyzed for DNA, RNA, phospholipids, glycogen and soluble protein content, ALT and AST activity. On day 50, plasma insulin and IGF-1 were lower in WMIX group, as possible consequence of the lower plasma glucose and amino acids levels. Liver weight was not different between groups, but liver weight expressed as percentage of body weight was lower in WMIX kids and highly correlated to plasma IGF-1. Liver glycogen was also lower in WMIX kids, as possible consequence of the lower plasma glucose. Hepatic ALT and AST activities were not different between groups and both were strongly correlated to plasma insulin. Moreover, insulin was positively correlated to the proteosynthetic capability per cell (RNA/DNA) of the liver. Our results indicate that the adopted livestock practice permitted the normal development of the animal used, avoiding growth stasis. Anyway, weaning altered plasma insulin and IGF-1, without affecting neither hepatic activity of aminotransferases, nor hepatic DNA and RNA content. Interestingly, plasma insulin was positively

correlated to hepatic ALT and AST activity and proteosynthetic capability per cell, suggesting a role for insulin as indicator of hepatic aminotransferase and proteosynthetic activity.

Key words: insulin, IGF-1, hepatic activity, goat kids, weaning.

INTRODUCTION

Weaning is the most dramatic event in the life of young mammals (Kelly and Coutts, 2000).

Weaning is considered to be the process of switching animals from milk to solid feed. Usually, in ruminants weaning is not just an event, but a period in which milk is progressively substituted by forage and concentrate or grain-based diets. The weaning program is generally chosen according to economic and practical criteria, since it can influence growth (Owens et al., 1993), maturation of the reproductive tract and even the quality of the end products (Magistrelli et al., 2007).

During the weaning period, diet shifts from a mixture of casein, lactose and triglyceride to a more complex source of nutrients, which requires the adaptation of rumen functions (Baldwin et al., 2004) and digestive activity (Kinouchi et al., 2000). At weaning, the maturation of the digestive system is still incomplete (Kelly and Coutts, 2000), so weaning often coincides to a period of growth stasis (McCracken et al., 1995).

Insulin and insulin-like growth factor 1 (IGF-1) have both growth-related and anabolic actions (Magistrelli et al., 2005) and play an important role in post-natal development (Liu and LeRoith, 1999).

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 and amino acids uptake from the blood stream into adipose, muscular and hepatic cells (Pessin and Saltiel, 2000). IGF-1 is a 7.6 kDa peptide from liver whose major role is considered to be the regulation of tissue growth and differentiation

(Oldham et al., 1999). The anabolic role of plasma IGF-1 consists of stimulating the uptake of amino acids and glucose by the cells, with an action similar to that of insulin (Magistrelli et al., 2005).

As the main regulation of plasma insulin and IGF-1 is associated with food intake, especially with amino acids and energy, respectively (Noguchi, 2000; Gale et al., 2004), the aim of the present study was to evaluate plasma insulin and IGF-1 levels, during the transition from milk to solid diet. Moreover, as liver represents a hot-point in both insulin and IGF-1 pathways, it was considered worth of interest to determine the influence of the two hormones on hepatic activity.

MATERIAL AND METHODS

Immediately after birth, 11 Saanen goat kids were separated from their mothers and randomly assigned to one of two groups: MILK (6 animals) and WMIX (5 animals).

All animals were fed colostrum for the first three days. The MILK group then received *ad libitum* goat milk for the rest of the study period (to age 50 days). The WMIX group received *ad libitum* goat milk to age 29 days and then underwent weaning in which milk was progressively replaced by solid feed. Specifically, from age 30 to 36 days the WMIX group received 2.5 l/d per head of milk plus *ad libitum* weaning mixture. From age 37 to 47 days the milk was gradually reduced to 1.0 l/d per head. On the 48th day milk was completely withdrawn. All animals were fed twice a day (9:00 am and 7:00 pm).

Goat milk was constituted by 26.3% of crude protein, 29.5% of lipids, and 38.6% of lactose. The weaning feed mixture was constituted by 15.9% of crude protein, 4.43% of lipids and 18.9% of starch. All the percentages are expressed on dry matter.

During the study period, mean dry matter intake (DMI) per group was recorded daily. Individual body weights (BWs) were recorded weekly.

At 23, 30, 37, 44 and 50 days of age, jugular vein blood samples were taken from each animal, before the first meal of the day. The blood was collected into vacuum tubes containing K₃EDTA as anti-caking agent. Plasma was analyzed for glucose (Wako Chemicals, Neuss, Germany), free amino acids (Goodwin, 1968), total protein, urea, creatinine (Giesse Diagnostics, Colle Prenestino, Rome, Italy) and albumin (Boehringer Mannheim, Mannheim, Germany) by spectrophotometric methods. Insulin (Insik-5, Dia Sorin, Saluggia, Italy) and IGF-1 (Total IGF-1 With Extraction, Diagnostics System Laboratories - Webster, TX, USA) were determined by radio-immune assays (RIA). Plasma activity of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were analyzed by

enzymatic-spectrophotometric methods (Boehringer Mannheim - Mannheim, Germany).

At age 50 days all animals were slaughtered five hours after the first meal of the day. Liver weight was recorded and liver samples were taken and analyzed for DNA and RNA, according to Munro and Fleck (1966). Liver samples were also tested for glycogen content, following Kemp and Kits van Heijningen's method (1954). For the analysis of soluble protein and phospholipids content and ALT and AST activity in liver, 250 mg of fresh tissue were homogenized in 1 ml of water with an Ultra Turrax homogenizer. 0.5 ml of the suspension were transferred in a centrifuge tube and then centrifuged for 5 minutes at 800 x g. The supernatant was analyzed for soluble protein content (Pierce Biotechnology Inc. - Rockford, IL, USA) and ALT and AST activity (Boehringer Mannheim - Mannheim, Germany) with spectrophotometric methods. Phospholipids were extracted from the homogenized suspension with Folch's method (1957) and phospholipids content of liver was determined with Allen's procedure (1940).

Data were evaluated by ANOVA (analysis of variance). The animals were cared for and slaughtered in accordance with the guidelines established by the European Union and Italian Ministry of Health.

RESULTS AND DISCUSSION

As long as all the kids were fed milk only, DMI was similar in both groups (ranging from 101 g/d/head at the beginning of the study period to 367 g/d/head on day 29). Seven days after the addition of solid feed, DMI in the WMIX group began to decrease compared to the MILK one. From 37 to 50 days of age, DMI was 407 ± 9 g/d (mean \pm SD) in the MILK group and 248 ± 73 g/d the WMIX group ($P < 0.01$). The initial decrease could be due to refusal of the new diet by the WMIX animals. Subsequently, lower DMI in this group could be because products from ruminal activity were making an increasing contribution to the nutritional requirements of the animals. In fact, despite the difference observed in DMI, body weight did not differ between the experimental groups, throughout the entire period (15.3 kg vs. 16.2 kg, for WMIX and MILK group, respectively, SEM=1.56).

During the last four weeks of life, the WMIX group had significantly lower plasma levels (overall means) of glucose (6.55 mM vs. 6.90 mM; SEM=0.27, $P < 0.05$) and free amino acids (4.89 mM vs. 5.27 mM; SEM=0.17, $P < 0.01$) and significantly higher plasma creatinine (71.7 μ M vs. 69.3 μ M; SEM=1.44, $P < 0.05$) than the MILK group. Greater differences were found at age 50 days (two days after the completion of weaning), when glucose and free amino acids were significantly lower in the WMIX group (table 1), as possible consequence of the relative inability of the solid diet to supply sufficient protein and energy. On the same day, also

urea (table 1) was lower in WMIX kids, probably because of the recycling of urea to the rumen for microbial protein synthesis. Urea recycling could have been stimulated by the lower N intake in the WMIX group, in order to improve the efficiency of protein utilization (Ludden et al., 2003). Surprisingly, plasma creatinine was significantly higher in WMIX kids (table 1), even if no difference was observed in body weights.

During the last four weeks of life there was no difference between groups for the hormones analyzed, but, on day 50, both plasma insulin and IGF-1 were more than three times lower in the WMIX than the MILK group (table 1). Both differences may have been related to the lower plasma glucose and amino acids in the WMIX group at this time (Magistrelli et al., 2005).

The plasmatic activity of both ALT (12.4 IU/l vs. 8.65 IU/l, for WMIX and MILK group, respectively, SEM=1.33, P<0.01) and AST (55.4 IU/l vs. 45.5 IU/l, SEM=2.93, P<0.05) began to be significantly different on day 44 of life. The differences were even greater, two days after the completion of weaning (table 1). One possible reason for the

obtained results is that the higher ALT and AST activities in plasma of WMIX kids have been caused by the change of diet. In humans, in fact, the reduction of protein intake may increase the activity of transaminases in plasma (Garza et al., 1977).

Liver weight was not different between groups, but liver weight expressed as percentage of body weight (% BW) was lower in WMIX kids (table 2) and highly correlated to plasma IGF-1 ($r=0.70$, $n=11$, $P<0.05$). Liver analysis pointed out differences between weaned and unweaned animals for hepatic glycogen content, which was lower in WMIX group (table 2), as possible consequence of the lower level of plasma glucose in this group, respect the MILK one. Glycogen content of liver was correlated to plasma glucose ($r=0.55$, $n=11$, $P<0.05$).

Hepatic ALT and AST activities were not different between groups and both were strongly correlated to plasma insulin ($r=0.57$, $n=11$, $P<0.05$, for hepatic ALT; $r=0.73$, $n=11$, $P<0.05$, for hepatic AST). Moreover, insulin was positively correlated to the proteosynthetic capability per cell (RNA/DNA) of the liver ($r=0.72$, $n=11$, $P<0.05$).

Table 1. Plasma metabolites, hormones and aminotransferases activity on day 50 of age.

	WMIX	MILK	SEM	P
Glucose (mM)	4.71	5.81	0.27	<0.01
Free amino acids (mM)	4.21	5.58	0.17	<0.01
Total protein (g/l)	53.1	55.4	0.94	ns
Albumin (g/l)	38.9	39.9	0.94	ns
Urea (mM)	5.67	7.66	0.38	<0.01
Creatinine (μ M)	78.9	69.3	1.45	<0.01
Insulin (pM)	7.03	21.3	3.84	<0.05
IGF-1 (mM)	19.6	64.1	6.52	<0.01
ALT (U/l)	19.2	9.27	1.33	<0.01
AST (U/l)	60.0	42.9	2.93	<0.01

Table 2. Liver analysis.

	WMIX	MILK	SEM	P
Liver weight (g)	330	377	18.7	ns
Liver weight (% BW)	2.17	2.76	0.11	<0.05
DNA (mg/g liver)	2.96	2.54	0.18	ns
RNA (mg/g liver)	5.47	5.29	0.17	ns
RNA/DNA	1.86	2.16	0.15	ns
ALT (IU/g liver)	7.71	8.22	0.97	ns
AST (IU/ g liver)	200	229	17.9	ns
Soluble protein (mg/g liver)	223	238	8.90	ns
Glycogen (mg/g liver)	40.9	55.3	4.77	<0.05
Phospholipids (mg/g liver)	3.80	3.87	0.12	ns

CONCLUSIONS

Our results indicate that the livestock practice adopted in the present study permitted the normal development of the animal used, avoiding growth stasis.

Weaning alters plasma insulin and IGF-1, without affecting neither hepatic activity of aminotransferases, nor hepatic DNA and RNA content. Interestingly, plasma insulin was positively correlated to hepatic ALT and AST activity and proteosynthetic capability per cell. This latter result suggests a role for insulin as indicator of hepatic aminotransferase and proteosynthetic activity. Anyway, further studies are needed in order to verify this hypothesis.

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