

Plasma amylin concentration in suckling goat neonates and its relationship with C-reactive protein, selected biochemical and hormonal indicators

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Abstract

Amylin is a recently discovered neuropeptide hormone that belongs to the calcitonin gene-related peptide family. It is co-secreted with insulin in response to feed intake. In goat kids, neonatal mortality and morbidity seems to be relatively higher than in other farm species. This high mortality and morbidity in goat kids may be associated with underdeveloped metabolism and immune system during the first week of life. The main objectives of this study were to determine amylin concentration and its relationship with some hormones, biochemical indicators and with a general inflammatory marker, CRP (C-reactive protein) in goat neonates. Blood samples were collected from 30 Saanen goat neonates at 20–35 days of age. Plasma amylin and other hormone concentrations were measured by ELISA, whereas serum biochemical indices were analysed by spectrophotometry. The mean values of plasma amylin concentrations were 9.07 ± 0.25 pmol/l. Plasma amylin concentrations were positively correlated with plasma non-esterified fatty acids, CRP, prolactin, cortisol, insulin; however, a negative correlation was determined between plasma amylin and serum triglyceride concentrations. The current study suggests that amylin contents are strongly associated with circulating concentrations of some hormones and with those of CRP in Saanen goat kids.

Goat kid, prolactin, inflammatory marker, NEFA

Amylin, a 37 amino acid polypeptide hormone with a molecular weight of 3.9 kDa, was firstly isolated from amyloid deposit in pancreatic islets of type 2 diabetes patients (Hou et al. 2011). Amylin is mainly co-secreted with insulin by pancreatic beta-cells in response to stimulation of glucose, free fatty acids and nutrient intake (Kairamkonda et al. 2005). Amylin is a major player in the control of long-term energy homeostasis. Amylin inhibits food intake by direct activation of its receptor in the area postrema. The lateral parabrachial nucleus and nucleus of the solitary tract are also involved in the inhibitory effect of amylin on food intake (Buttler 2005; Lutz 2005). Amylin has been found to inhibit basal and insulin induced glycogenesis in rat skeletal muscle. Furthermore, another study showed that amylin stimulated beta cell apoptosis could suppress insulin secretion which consequently led to hepatic and extrahepatic insulin resistance and glucose abnormalities in the human amylin transgenic rats (Leighton and Cooper 1988; Ciaraldi 1992; Matveyenko and Butler 2006). Additionally, amylin infusion in dogs stimulated peripheral insulin resistance (Sowa et al. 1990). Increased amylin concentrations were demonstrated in preterm neonates with feeding intolerance, obese children, and obese adults with type 2 diabetes, infants of diabetic mothers and women with polycystic ovary syndrome (Altman 1991; Percy et al. 1996; Hoppener et al. 2000; Mayer et al. 2002). Amylin also acts as a growth factor on bone cells, renal proximal tubular cells, and islet beta-cells. Amylin was shown to be expressed in the second half of gestation starting at embryonic day 11–12 in the rat pancreatic diverticulum. These findings about the early foetal expression of this peptide may indicate a role for amylin in growth factor signalling (Mulder et al. 1997;

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Wilson et al. 2002). Despite the suggested important roles of amylin in postnatal and embryonic development (Zaidi et al. 1993; Wookey et al. 1998; Horcajada-Molteni et al. 2001; Karlsson and Sandler 2001; Hegazy et al. 2009), there are as yet no data available on the concentrations of amylin in the circulation of the early postnatal Saanen goat kids. Therefore, the present study was designed, firstly, to determine circulatory concentrations of amylin in blood samples obtained from goat kids at 20–35 days of age. Secondly, we sought to determine the relationship between the concentrations of serum amylin and CRP (C-reactive protein), a high sensitive serum biomarker for long standing low grade inflammation, in Saanen neonates (Marnell et al. 2005; Francisco et al. 2006). Amylin has anti-inflammatory effects in humans (Clementi et al. 1995), and serum amylin concentrations are associated with circulating inflammatory markers (Gitter et al. 2000; Soliman et al. 2011) including CRP (Hou et al. 2011). Since amylin has been implicated in fatty acid, lipid metabolism and insulin resistance (Min et al. 1999; Hou et al. 2011), we also studied the relationships between plasma amylin and non-esterified fatty acids (NEFA) concentrations, lipid profile and some metabolic hormones.

Materials and Methods

Animals and experimental design

The study was conducted at a farm in Karacabey, Bursa, situated in the North West Turkey, 408 north latitude, 298 east longitude and at an altitude of 149 m above sea level in March (environmental temperature varying from 8 to 20 °C). All animals were handled in accordance with the European Union Directive number 86/609/EEC regarding the protection of animals used for experimental and other scientific aims. The experimental procedures were approved by the Animal Care Committee of University of Uludag, Bursa, Turkey (HADYEK 2013-11/02). A total of 30 Saanen female goat neonates aged 20–35 days were used, with a mean birth weight of 3.07 ± 0.3 kg and body weight of 11.15 ± 1.02 kg. The kids were housed with their dams in a sheltered outdoor pen with straw bedding. All dams grazed on pasture between 09:00 h and 17:00 h while all kids were offered their dams' milk only. The kids and their dams had *ad libitum* access to water. Dams were also given *ad libitum* alfalfa hay in the morning and evening.

Blood samples were collected via jugular puncture into sterile Serum clot activator and EDTA tubes at 09:00–10:00 h; about 4 h after the morning suckling. Blood samples were centrifuged at $2000 \times g$ for 10 min at 4 °C. Serum and plasma were divided into 500 μ l aliquots and stored at -20 °C until analysis day.

Assays

Plasma amylin, NEFA, CRP, prolactin, cortisol, and insulin concentrations were measured using commercially available enzyme-linked immunosorbent assay (ELISA) kits (Hangzhou Eastbiopharm CO, Ltd., Yile Road cat No: CK-E90753, CK-E90766, CK-E91040, CK-E90755, CK-E91038, CK-E90833, respectively) on an automated microplate reader (Biotek EL 808). Blood was centrifuged immediately. Serum glucose concentration was measured in 1–2 h with a commercial enzymatic colorimetric kit (Biolabo SA, Maizy, France, Cat No:REF 80009), following the manufacturer's instructions using a spectrophotometer (Schimadzu UV 1601, Kyoto, Japan). Serum triglycerides, cholesterol, and total lipid concentrations were also determined with commercial kits (REF 80019 Biolabo, Maizy, France, Teco Diagnostics REF T528-480; REF C507-480, T526-480, respectively) using spectrophotometer.

Statistics

Statistical analyses were performed using the IBM[®]SPSS[®] version 22 for Windows. Data were expressed as mean \pm standard error (SEM). The relationships between plasma amylin hormone and NEFA, CRP, prolactin, cortisol, insulin, serum glucose, triglycerides, cholesterol, total lipids and body weight were analysed using Pearson's correlation coefficient and were considered significant at $P < 0.05$.

Results

The mean values of plasma amylin, NEFA, CRP, prolactin, cortisol, insulin and serum glucose, triglycerides, cholesterol, total lipid concentrations and body weight in 20–35 days Saanen goat kids ($n = 30$) are shown in Table 1. Positive correlations were determined between plasma amylin and NEFA, CRP, prolactin, cortisol, and insulin concentrations (Table 1). There was no correlation between plasma amylin concentration

Table 1. Relationships between plasma amylin concentrations and some reproductive, metabolic hormones and C-reactive protein concentrations in goat kids.

Indicators	Amylin		$\bar{x} \pm \text{SEM}$
	r	P	
Plasma amylin (pmol/l)	N/A	N/A	9.07 \pm 0.25
Plasma NEFA (mmol/l)	0.701	0.001***	0.31 \pm 0.008
Plasma CRP (mg/l)	0.53	0.003**	6.4 \pm 0.38
Plasma prolactin (pmol)	0.70	0.001***	391.20 \pm 17.39
Plasma cortisol (nmol/l)	0.69	0.001***	26.21 \pm 0.66
Plasma insulin (pmol/l)	0.65	0.001***	40.28 \pm 2.6
Serum glucose (mmol/l)	-0.32	0.090	4.94 \pm 0.16
Serum triglyceride(mmol/l)	-0.412	0.05*	0.53 \pm 0.04
Serum cholesterol (mmol/l)	0.283	0.129	3.05 \pm 0.13
Serum total lipid (g/l)	0.265	0.158	2.15 \pm 0.12
Body weight (kg)	0.470	0.077	11.15 \pm 1.02

r – correlation coefficient, P – significance, SEM -standard error of the mean; \bar{x} - mean, N/A= not applicable
* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

and serum glucose concentration (Table 1). The Pearson's correlation analysis revealed negative relationship between amylin hormone and serum triglycerides but not serum cholesterol, total lipid concentrations, and body weight.

Discussion

In this study, we firstly determined circulatory concentrations of amylin in blood samples obtained from goat kids aged 20–35 days. These data show that elevated amylin concentrations were significantly correlated with NEFA, inflammatory status and some reproductive, metabolic hormones including prolactin, cortisol, and insulin in Saanen neonates.

Serum amylin concentrations have been established in healthy human neonates at birth and at the fifth postnatal day (Hou et al. 2011) and in lactating adult goats (Min et al. 1999). This is the first study to establish the normal range of serum amylin in healthy goat neonates. The serum amylin levels in healthy goat neonates in our study (9.07 \pm 0.25 pmol/l) correspond to those observed in healthy human neonates (5.7 pmol/l) and lactating adult goat (10.9 \pm 1.6 pmol/l) populations.

Another important finding in our study was a strong positive correlation between amylin and CRP, an acute phase protein, which is dramatically increased in blood during inflammation and has been used as a biomarker for neonatal sepsis. Ambalavanan et al. (2009) demonstrated that CRP modulated inflammatory and immune responses. Literature about the plasma CRP concentration in goats is limited. It was found to be 1.13 mg/dl in newborn goats and 0.80 mg/dl in goat kids at 6 weeks of age by He et al. (2014). Sameer et al. (2013) showed that CRP level was 3.7 in healthy goat and 7.3 in subclinical mastitic goat. In the present study, the mean plasma concentration of CRP in goat neonates was 6.4 \pm 0.38 mg/l. Previous studies have shown that circulating amylin concentrations were significantly associated with proinflammatory cytokines and inflammatory markers (Hou et al. 2011; Soliman et al. 2011), including CRP (Hou et al. 2011) in humans and rats. In accordance with these previous observations (Hou et al. 2011; Soliman et al. 2011) and with the view that circulating amylin itself may be an inflammatory marker we observed

that amylin concentrations were correlated significantly with serum CRP concentrations in Saanen goat neonates, which are susceptible to bacterial infections, especially sepsis.

We found that amylin concentrations in plasma were positively correlated with prolactin, cortisol, and insulin. Earlier studies suggested that amylin was involved in regulating food intake, glucose and lipid metabolisms, insulin action and energy homeostasis in humans and rats. Amylin inhibits insulin action and has the ability to cause insulin resistance in skeletal muscle and liver (Reinehr et al. 2007). It was also shown that amylin could inhibit insulin secretion by increasing β -cell apoptosis leading to insulin resistance and glucose dysregulation in human IAPP transgenic rats (Matveyenko and Butler 2006). Sowa et al. (1990) demonstrated that infusion of amylin also caused peripheral insulin resistance in dogs. The present results provide the first evidence that amylin concentrations correlate with insulin concentrations in female goat neonates. Lemay et al. (2013) reported that insulin has an important role in breast milk synthesis and that there is a link between insulin resistance and insufficient milk supply through the tyrosine phosphatase, receptor type, F (PTPRF) gene. The relationship between amylin and insulin concentrations in female goat neonates suggests that amylin may also be related to milk yield in later years.

In the present study we observed that plasma cortisol and prolactin concentrations were positively correlated with plasma amylin. Nyholm et al. (1996) showed that amylin analogue AC 137 caused an increase in circulating cortisol during hypoglycaemia in patients with insulin-dependent diabetes mellitus. Our data suggest that the interactions between plasma amylin and cortisol, a metabolic hormone, in goat neonates are consistent with those observed with the amylin analogue in humans. Chmielowska et al. (2005) demonstrated that amylin administered centrally and peripherally produced a significant decrease in prolactin release in rats. Together, our results show that the interactions between plasma amylin and prolactin, a reproductive hormone, in goat neonates are not consistent with the findings in rats (Nyholm et al. 1996). The observed differences between rats and goat neonates may be due to distinct receptor subtypes of amylin in each species.

Shortly after birth and before lactation, the neonates use the energy sources that develop during gestation. Since carbohydrate reserves are used up rapidly, lipids mobilized via lipolysis in fat cells serve as the major energy substrates during this first phase of life (Hegazy et al. 2009). Some data are available about the regulation of lipolysis in the neonatal period. Hormonal regulation of lipolysis during the neonatal period is important (Marcus et al. 1988; Gustafsson 2009). Based on these reports we examined the relationship between amylin hormone, which is expected to enhance lipolysis by antagonizing insulin action in adipose tissue (Min et al. 1999; Ye et al. 2001), and glucose, triglycerides, cholesterol, total lipid concentrations and body weight in goat neonates. It has been well-established that amylin has important roles in fatty acid and lipid metabolisms (Smith and Mamo 2000; Ye et al. 2001). It is reported that acute treatment with dietary fatty acids can enhance amylin expression and secretion in pancreatic β cells (Hou et al. 2011). Plasma NEFA, major components of triglycerides, are primarily important energy substrates and play a key role in the induction of insulin resistance; they are considered biomarkers of negative energy balance in the body (Marcus et al. 1988; Gustafsson 2009). Min et al. (1999) showed that a 6-fold increase in plasma amylin concentrations led to a 168% increase in plasma NEFA in lactating goats. In the present study, we observed that plasma NEFA concentration was positively correlated with plasma amylin concentrations and inversely correlated with serum triglycerides in goat neonates. There was no association between plasma amylin and glucose, cholesterol, total lipid concentrations and body weight in goat neonates, but significant associations between plasma amylin and glucose and some lipid indicators were observed in previous human and rat studies (Ye et al. 2001; Hou et al. 2011). In goat neonates, amylin does not appear to be associated with serum glucose, cholesterol, total lipid and body weight. This may be due to unique features of their metabolism.

In conclusion, our study is the first to determine plasma amylin concentrations in goat neonates. The data from the present study show that plasma amylin concentrations are correlated with plasma concentrations of some reproductive and metabolic hormones and with those of CRP. Furthermore, the results also show that plasma amylin concentration is positively related to plasma NEFA, but inversely related to serum triglyceride concentrations. Further studies will be required to elucidate the roles of these relations in the metabolism and development of neonates. The results from this pilot study will facilitate these studies by providing basic information on the circulating concentrations of amylin in goat neonates.

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