The interrelationship between resistin and amylin plasma concentrations and their relation to the concentrations of selected reproductive and metabolic hormones and biochemical variables in the early lactation stage of Saanen goats

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SUMMARY

Resistin and amylin are metabolic hormones that improve energy homeostasis and insulin resistance in rodents but have not been studied in early lactating goats, which are known to be resistant to insulin. This study sought to determine plasma resistin and amylin concentrations and their interrelationship and correlation with select reproductive and metabolic hormones and biochemical variables in early lactating goats. Blood specimens were collected from thirty lactating goats enrolled in the 3rd, 6th and 9th postpartum weeks. Plasma hormone concentrations were measured by ELISA, and the biochemical variables were determined spectrophotometrically. There were no differences in plasma resistin and amylin concentrations at the 3rd, 6th and 9th postpartum weeks. Plasma resistin and amylin concentrations were interrelated and positively correlated with plasma insulin, cortisol, prolactin and NEFA concentrations ($p < 0.001$) in the early lactation stage of goats. Amylin and resistin may play a critical role in the decreased insulin sensitivity of peripheral tissues during the early lactation period of goats. Further studies are needed to clarify the causal relationships and secretory mechanisms of resistin and amylin in early lactating goats.

Keywords: Resistin, amylin, lactating goat, insulin

RÉSUMÉ

Interrelation entre les concentrations plasmatiques en résistine et amyline et relation aux concentrations de certaines hormones et variables biochimiques au stade précoce de lactation chez les chèvres Saanen

Resistine et amyline sont des hormones métaboliques qui améliorent l’homeostasie énergétique et la résistance à l’insuline chez les rongeurs mais dont les rôles n’ont pas été étudiés chez des chèvres allaitantes, qui sont connus pour être résistants à l’insuline. Cette étude visait à déterminer les concentrations plasmatiques en résistine et amyline, leur interrelation, leur corrélation avec certaines hormones de la reproduction et certaines variables biochimiques en début de lactation chez la chèvre. Des échantillons de sang ont été prélevés sur trente chèvres en lactation aux 3èmes, 6èmes et 9èmes semaines post-partum. Les concentrations en hormones ont été mesurées par ELISA, et les variables biochimiques ont été déterminées par spectrophotométrie. Il n’y avait pas de différences dans les concentrations plasmatiques en résistine et amyline à la 3ème, 6ème et 9ème semaines post-partum. Les concentrations plasmatiques en résistine et amyline étaient interdépendantes et en corrélation positive avec l’insuline plasmatique, le cortisol, la prolactine et les concentrations d’AGNE ($P < 0.001$). L’amyline et la résistine peuvent jouer un rôle essentiel dans la diminution de la sensibilité des tissus périphériques à l’insuline au début de lactation chez les chèvres. D’autres études sont nécessaires pour clarifier les relations causales et les mécanismes de sécrétion de la résistine et l’amyline.

Mots-clés: Résistine, Amyline, Insuline, Chèvre, Lactation

Introduction

In ruminant, early lactation is a period of increasing nutrient mobilization because of the substantial needs for milk synthesis [4]. During this period, sensitivity of peripheral tissues to insulin is reduced, allowing the mobilization of NEFAs (non esterified fatty acids) and amino acids. Diminished sensitivity of peripheral tissues to insulin facilitates the preferential use of glucose by the mammary gland [3]. During the early lactation period, goats become resistant to insulin which supports the transfer of glucose to the mammary gland [4]. The decrease in insulin sensitivity of early lactating goats and hormonal changes has not clearly understood. In particular, resistin (or “resistance to insulin”), also known as ADSF (adipocyte-specific secretory factor) or FIZZZ (found in the inflammatory zone), is known to decrease insulin sensitivity in rodents [1]. Resistin, belongs to a family of proteins named FIZZ or RELM (resistin-like molecule), is a recently discovered low molecular weight (12.5-kDa) cysteine-rich protein. Resistin is secreted primarily by adipose tissue in rodents whereas it is secreted primarily by immune and epithelial cells in primates, pigs and dogs [22]. It opposes the role of insulin in peripheral tissues and has effect on inflammation and immunity. This protein induces insulin resistance in obesity and is named for its ability to resist the action of insulin [13]. Amylin was also found to inhibit insulin secretion in vitro by increasing β-cell apoptosis, which induced hepatic and extrahepatic insulin resistance in human IAPP (islet amyloid polypeptide) transgenic rats [12]. Amylin, derived of the calcitonin gene related peptide family, is co-released with insulin from pancreatic beta cells in response to food intake and produces hypocalcemia [6,7]. It delays gastric emptying and plays an important role in controlling carbohydrate absorption by regulating efflux from the stomach to the small intestine. Increased amylin concentrations were demonstrated in obese
children, obese adults with type 2 diabetes and infants of diabetic mothers [17,18].

The decrease in insulin sensitivity that occurs in adipocytes in early lactating goats and its relationship with the hormonal mechanism remains poorly understood. Because amylin and resistin cause insulin resistance, we hypothesize that these hormones may play a role in decreasing the insulin sensitivity of peripheral tissues and in regulating the hormones associated with mammary differentiation, milk secretion and energy metabolism. Therefore, this study was undertaken to investigate the interrelationship between the resistin and amylin plasma concentrations and their relation to concentrations of select reproductive and metabolic hormones and biochemical variables, which are known to affect mammary differentiation, milk secretion and energy metabolism [15]. In addition, we sought to determine plasma resistin and amylin concentrations in the early lactation stages of goats at the 3rd, 6th and 9th postpartum weeks, as there is no data available on the concentrations of these two hormones in the circulation during the early lactation stage of the Saanen goats.

Material and methods

STUDY AREA AND ANIMALS

The study was carried out at a farm in Karacabey, Bursa, situated in North West Turkey, 408 north latitude, 298 east longitude and at an altitude of 149 m above sea concentration during a 9-week period. All animals were handled according to the European Union directive number 86/609/EEC regarding the protection of animals used for experimental and other scientific aims. In addition, this study was approved by an Animal Care and Use Committee at the University of Uludag, Bursa, Turkey.

Thirty homogeneous Turkish Saanen goats, ages 3–4 years, in the 3rd week of lactation were used in this study. The mean and standard errors of live weight, body condition score and milk yields of the goats in the 3rd week of lactation were determined as follows: BW, 47.62 ± 1.5 kg; BCS, 3.18 ± 0.5 arbitrary units; and MY, 1.40 kg/day, respectively. The goats were housed with their kids in a sheltered outdoor pen with straw bedding. All of the goats grazed on pasture between 9 am and 5 pm and had access to water ad libitum. Goats were also given alfalfa hay [dry matter (89.42%), CP-crude protein (16.50%), ether extract (1.39%), NDF-neutral detergent fiber (58.85%), ADF-acid detergent fiber (52.42%), ADL-acid detergent lignin (11.40%), NFC-nonfibrous carbohydrates (14.62%), Ash (8.64%), Ca-calcium (1.35%), P-phosphorus (0.12%)], ad libitum, in the morning and evening.

COLLECTION OF BLOOD SPECIMENS

On the 3rd, 6th and 9th weeks, venous blood specimens were collected from the vena jugularis of 30 saanen goats using 18 gauge needles (BD Vacutainer) into vacutainers (Venoject, Terumo, Leuven, Belgium) containing EDTA as an anticoagulant and tubes with no anticoagulant (serum tube) at 9 a.m. after a fasting time of 3 hours. Blood specimens were centrifuged within 30 minutes at 2000 × g for 10 min at 4 °C. The serum and plasma were stored at -20 °C for 3 weeks until analysis day.

BIOCHEMICAL AND HORMONAL ANALYSES

A goat resistin ELISA kit (Goat resistin ELISA Kit; Hangzhou Eastbiopharm CO., Ltd., Yile Road cat No: CK-E91039) was used to measure the resistin in the plasma specimens. Plasma amylin, insulin, cortisol, prolactin, and NEFA concentrations were also measured using commercially available goat ELISA (enzyme-linked immunosorbent assay) kits (Hangzhou Eastbiopharm CO., Ltd., Yile Road cat No: CK-E90753, CK-E90833, CK-E91038, CK-E90755, CK-E90766 respectively) in an automated microplate reader (Biotek EL, 808, USA). Serum glucose, calcium, HDL, LDL, and triglyceride concentrations were determined with commercial kits (Biolabo SA, Maizy, France, Cat No:REF 80009; REF 80004; Teco Diagnostics U.S.A. Cat No: REF H511-20; REF L530-100; Biolabo, Maizy, France, Cat No: REF 87319, respectively) following the manufacturer's instructions using a spectrophotometer (Schimadzu UV 1601, Kyoto, Japan). The limit of detection and intraassay coefficients of variation were 0.02 ng/mL, 5% for resistin, 9.27 ng/mL, 8% for amylin, 0.058 mIU/L, 4% for insulin and 0.22 ng/mL, % 8 for cortisol, 0.21 ng/mL 3.5% for prolactin and 0.12 mmol/L, 4% for NEFA, respectively.

STATISTICAL ANALYSES

All statistical analyses were conducted using IBM SPSS® version 22 for Windows. The relationship between plasma resistin and amylin hormone and their correlation with plasma insulin, cortisol, prolactin, NEFA, serum glucose, calcium, HDL (high density lipoprotein) and LDL (low density lipoprotein) cholesterol and triglyceride was analyzed using Pearson's correlation coefficient and relative correlations between variables were accounted by taking in to 3rd week observations. The Shapiro-Wilk test was used for normality. Changes at the 3rd, 6th and 9th week time points were determined using repeated measures test for gaussian distributed variables and Friedman test for non Gaussian distributed variables. The data are given as the means±SEM (standard error of the mean) or median with minimum and maximum values. P values less than 0.05 were considered statistically significant in all tests and P value with Bonferroni Correction was used for multiple comparisons (α*=0.016).

Results

The plasma concentrations of reproductive and metabolic hormones and serum concentrations of select biochemical variables in Saanen goats at the 3rd, 6th and 9th postpartum lactation weeks are shown in Table 1. There were no differences in plasma resistin, amylin, cortisol, calcium and...
LDL concentrations at the 3rd, 6th and 9th postpartum weeks. Lactating goats at the 9th postpartum week had significantly lower plasma insulin ($p < 0.016$) and serum triglyceride ($p < 0.01$) concentrations than at the 3rd and 6th lactation weeks and significantly lower plasma NEFA ($p < 0.016$) concentrations than at the 3rd lactation week (Table I). In contrast, goats at the 9th postpartum lactation week had significantly higher plasma prolactin ($p < 0.016$), HDL ($p < 0.01$), and serum glucose concentrations than at the 3rd lactation week.

Correlation analysis revealed a strong positive correlation between plasma concentrations of resistin and amylin (Table II). Plasma resistin and amylin concentrations were correlated with plasma insulin, cortisol, prolactin, and NEFA at the 3rd lactation weeks ($p < 0.001$) (Table II). There was no correlation between glucose, HDL, LDL, or triglyceride concentrations with plasma resistin and amylin concentrations at the 3rd, postpartum week (Table II). There was a positive relative correlation between amylin and resistin at 6th and 9th postpartum weeks by taking in to account 3rd lactation week (Table II).

**Discussion**

Resistin and amylin are newly discovered hormones, and their plasma concentrations and functions are not completely clear, particularly in lactating ruminants.

**Blood Variables**

<table>
<thead>
<tr>
<th>Blood Variable</th>
<th>Week 3</th>
<th>Week 6</th>
<th>Week 9</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma resistin (ng/ml)</td>
<td>29.8 (7.6-92.2)a</td>
<td>26.1 (8.3-78.4)a</td>
<td>23.63 (6.4-80.2)a</td>
</tr>
<tr>
<td>Plasma amylin (pmol/L)</td>
<td>20.8 (4.8-32.0)a</td>
<td>20.1 (4.0-29.8)a</td>
<td>15.8 (3.6-31.8)a</td>
</tr>
<tr>
<td>Plasma insulin (pmol/L)</td>
<td>162.9±16.6a</td>
<td>176.4±18.0a</td>
<td>138.9±13.8b</td>
</tr>
<tr>
<td>Plasma cortisol (nmol/L)</td>
<td>55.3(23.0-157.1)a</td>
<td>40.9 (20.7-230.1)a</td>
<td>46.2 (20.1-243.6)a</td>
</tr>
<tr>
<td>Plasma prolactin (pmol)</td>
<td>1980.8±173.9a</td>
<td>2247.8±169.9b</td>
<td>2347.8±195.6c</td>
</tr>
<tr>
<td>Plasma NEFA (mmol/L)</td>
<td>1.3±0.1a</td>
<td>1.1±0.1b</td>
<td>1.1±0.1b</td>
</tr>
<tr>
<td>Serum glucose (mmol/L)</td>
<td>2.2±0.1a</td>
<td>2.8±0.1b</td>
<td>2.6±0.0b</td>
</tr>
<tr>
<td>Serum calcium (mmol/L)</td>
<td>2.6 (2.3-3.5)a</td>
<td>2.4 (2.2-4.0)a</td>
<td>2.7 (2.2-3.9)a</td>
</tr>
<tr>
<td>Serum HDL (mmol/L)</td>
<td>0.5±0.0a</td>
<td>0.6±0.0b</td>
<td>0.6±0.0b</td>
</tr>
<tr>
<td>Serum LDL (mmol/L)</td>
<td>0.4 (0.2-0.6)a</td>
<td>0.4 (0.3-0.6)a</td>
<td>0.4 (0.3-0.6)a</td>
</tr>
<tr>
<td>Serum triglyceride (mmol/L)</td>
<td>0.3 (0.0-0.5)a</td>
<td>0.2 (0.0-0.3)b</td>
<td>0.1 (0.0-0.2)c</td>
</tr>
</tbody>
</table>

Data are given as mean±standard error of the mean for gaussian distributions and median (min-max) for non gaussian distributions. a,b Values within a row with different superscripts differ significantly at $P<0.016$.

**Table I:** Plasma and serum concentrations of select reproductive and metabolic hormones and biochemical variables in early lactating goats.

**Corr 1**

<table>
<thead>
<tr>
<th>Blood Variable</th>
<th>Week 3</th>
<th>Week 3-6</th>
<th>Week 3-9</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma Resistin</td>
<td>r -</td>
<td>0.804***</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>p -</td>
<td>0.000</td>
<td>-</td>
</tr>
<tr>
<td>Plasma Amylin</td>
<td>r 0.804***</td>
<td>0.564</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>p 0.000</td>
<td>0.015</td>
<td>0.604***</td>
</tr>
<tr>
<td>Plasma Insulin</td>
<td>r 0.917***</td>
<td>0.898***</td>
<td>0.030</td>
</tr>
<tr>
<td></td>
<td>p 0.000</td>
<td>0.906</td>
<td>0.932</td>
</tr>
<tr>
<td>Plasma Cortisol</td>
<td>r 0.729***</td>
<td>0.735***</td>
<td>0.032</td>
</tr>
<tr>
<td></td>
<td>p 0.000</td>
<td>0.900</td>
<td>0.404</td>
</tr>
<tr>
<td>Plasma Prolactin</td>
<td>r 0.857***</td>
<td>0.935***</td>
<td>0.003</td>
</tr>
<tr>
<td></td>
<td>p 0.000</td>
<td>0.990</td>
<td>0.773</td>
</tr>
<tr>
<td>Plasma NEFA</td>
<td>r 0.954***</td>
<td>0.875***</td>
<td>0.077</td>
</tr>
<tr>
<td></td>
<td>p 0.000</td>
<td>0.760</td>
<td>0.938</td>
</tr>
</tbody>
</table>

Correlations between parameters at 3rd lactation week and relative correlations between 3rd week and the 6th and 9th week time points were calculated, respectively.

*Corr 1: $p<0.001$, **Corr 1: *p<0.01, *p<0.05)*

**Table II:** Correlations of plasma resistin and amylin concentrations with plasma hormones and biochemical variables.
Metabolic adaptations in early lactation period are regulated by changes in the plasma concentration of key hormones [2]. Plasma resistin and amylin concentrations have never been determined in early lactation goats, and the interrelationship between plasma concentrations of resistin and amylin has never been studied. In this study, we obtained reference values for plasma resistin concentrations of 6.4 to 92.2 ng/ml in the early lactation stage of Saanen goats. Serum resistin concentrations were found to be 2 to 15 ng/ml in humans and rodents [5,24], REVERCHON et al. [18] obtained values for plasma resistin concentrations of 30 to 90 ng/ml in early lactation stage dairy cattle. In this study, the reference values for plasma amylin concentration is approximately 3.6 to 32 pmol/L during the early lactation stage of Saanen goats. MIN et al. [14] observed a metabolic effect of amylin in mid and late lactation. They found amylin concentration to be 10.8 ± 1.6 pmol/L before infusion and 66.7 ± 1.8 pmol/L during infusion. SZABO et al. [23] demonstrated an increased expression of amylin in the preoptic area, which plays a central role in the control of maternal adaptations postpartum but not prepartum in rats. The interrelationship of these hormones during early lactation is considered to be caused by an increase in insulin resistance, resulting in greater glucose supply to the mammary gland.

The changes in plasma resistin and amylin concentrations during early lactation could have resulted from changes in the hormonal status of lactating goats. In a recent study, HOU et al. [8] showed that circulating amylin was positively associated with insulin in a healthy Chinese population. The present results provided the first evidence that amylin is also correlated with insulin in early lactating goats. This is further evidence of the co-secretion of amylin and insulin. A positive influence of the insulin hormone on serum resistin concentrations has also been reported in humans. A study by SILHA et al. [21] showed that serum-resistin concentrations were correlated positively with fasting insulin concentrations in both lean and obese male and female subjects. In accordance with the report by SILHA et al. [21] we found a positive relationship between plasma resistin and insulin concentrations in early lactating goats. Conversely, REVERCHON et al. [18] found no relationship between serum resistin and insulin concentrations in early lactating dairy cows, and LEE et al. [11] and ILCOL et al. [9] found no relationship between the same hormones in non-breeding women and breastfeeding women. As for prolactin and cortisol, we observed that plasma cortisol and prolactin concentrations were related positively to plasma resistin in the early lactation period of goats, which is consistent with the findings by ILCOL et al. [9]. In addition, there was a positive relationship between plasma amylin and prolactin concentrations. Our results demonstrate that the relationship between plasma amylin and cortisol, a metabolic hormone, in lactating goats is consistent with that observed with the amylin analog AC 137 in humans, as shown by NYHOLM et al. [16]. In the present study, plasma resistin and amylin concentrations were positively correlated with plasma NEFA concentrations, which is consistent with the findings of REVERCHON et al. [18] and MIN et al. [14]. The positive relationship between NEFA, resistin and amylin concentrations may be associated with lipid mobilization and insulin resistance during early lactation in goats. It is apparent that amylin and resistin in early lactating goats has no association with serum calcium, triglyceride, HDL and LDL cholesterol concentrations.

As a conclusion, we first determined the resistin and amylin concentrations during the early lactation period of goats. Furthermore, goats became even more resistant to insulin to support the transfer of glucose to the mammary glands during early lactation, and this resulted in greater NEFA plasma concentrations. Consistent with these adaptations, this study also shows that plasma resistin and amylin concentrations, which are related to insulin resistance, are interrelated and correlated with changes in plasma concentrations of insulin, cortisol, prolactin and NEFA during the early lactation period of goats. Amylin and resistin may play an important role in decreased insulin sensitivity in adipocytes. Further studies are required to determine the causal relationships, secretory mechanism and physiological and metabolic functions of resistin and amylin to clarify the biological implications of the observed associations in lactating goats.

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References