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Physiology

The concentrations of adipokines in goat milk: relation to plasma levels, inflammatory status, milk quality and composition

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ABSTRACT. The main objectives of our study were to measure the major adipokines adiponectin, leptin and resistin in goat milk, to assess their interrelationships and to assess their relationships with the plasma and serum concentrations of total protein, cholesterol, total lipids, plasma C-reactive protein (CRP), milk somatic cell count (SCC), milk total aerobic colony and lactobacillus count, and milk components in lactating Saanen goats. The study was performed on eighteen lactating Saanen goats. Milk and blood samples were collected on days 20, 35, 50, 65 and 80 of lactation postpartum. The milk and plasma adiponectin levels on days 50, 65 and 80 postpartum were significantly higher than those on day 20. The milk and plasma leptin levels were lower on day 20 than on days 35, 50, 65 and 80. The milk concentrations of these major adipokines were positively intercorrelated. The milk and plasma concentrations of these three adipokines were also positively correlated. The plasma CRP concentrations correlated positively with milk leptin and resistin concentrations and inversely with milk adiponectin concentration. Milk adiponectin concentration was inversely related with its SCC. These data confirm that adiponectin, leptin and resistin are present in goat milk. The milk concentrations of these three adipokines were interrelated and interacted with the general inflammatory marker, CRP. The inverse relationship between milk adiponectin concentrations and its SCC suggests that variations in milk adiponectin might be involved in the udder health of lactating goats, but clinical trials are needed to support this hypothesis.

KEY WORDS: adipokine, CRP, goat, milk, SCC

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White adipose tissue, which is primarily devoted to energy storage, also produces and releases proinflammatory and anti-inflammatory factors, including adipokines, such as adiponectin, leptin and resistin [22, 27, 38]. These adipokines appear to play important roles in glucose and lipid metabolism, insulin resistance, energy homeostasis, inflammation, immunity and acute phase response [26, 34]. Adiponectin, leptin and resistin are produced by adipose tissue, but they have also been found in the placenta, where they are passed to the fetus [3, 18, 24, 36, 40]. In addition, these major adipokines have been found in the milk of humans and of some animals [12, 32]. Therefore, it is likely that adiponectin, leptin and resistin are also found in goat milk and that they are related to the milk's inflammatory status, quality and composition. Interest in goat milk and products has increased worldwide, and in recent years, the volume of goat milk produced has increased [8]. Because goat milk is often more nutritious, healthier for many gastrointestinal illnesses, more easily digested, less allergenic than cow's milk and more similar to human breast milk, it has been the preferred choice for infants [7, 17]. Because little research has been conducted involving leptin concentrations in goat milk [41] and there are no data available on the adiponectin and resistin concentrations in goat milk, the main objective of our experiment was first to determine the adiponectin and resistin concentrations in goat milk and to investigate the possible relationships among milk and plasma adiponectin, leptin and resistin in lactating goats. Because of metabolic and endocrine adaptation to lactation, early lactating goats are metabolically stressed. This stress can result in clinical outbreaks of subclinical infections. Because adiponectin, leptin and resistin are reportedly associated with the inflammatory status in humans [26, 38],

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we also sought to determine the relations between the milk concentrations of these adipokines and the plasma C-reactive protein concentration (CRP), a sensitive serum inflammatory biomarker [19], milk's somatic cell count (SCC), possible markers for monitoring the inflammatory response to an intramammary infection or another trigger of the immune system, and the total aerobic colony and lactobacillus count in early lactating goats. Finally, we also aimed to determine the relationships between the milk concentrations of these three adipokines and the milk composition in lactating goats.

MATERIALS AND METHODS

Animals and specimen collection

The study was performed at a farm in Bursa, situated in Northwest Turkey, at 408 north latitude, 298 east longitude and an altitude 149 m above sea level during an 80-day period. This study was approved by the Ethical Committee at the University of Uludag, Bursa, Turkey. Eighteen Saanen goats, ages 3–4 years, were used in this study. The live weight, body condition score and milk yields of the goats were determined as follows: mean ± standard errors; 45.13 ± 2.2 kg; BCS, 3.72 ± 0.3 arbitrary units; and MY, 1.50 kg/day. The goats were housed with their kids in a sheltered outdoor pen with straw bedding. All goats grazed on pasture between 9 am and 5 pm and had access to water *ad libitum*. The 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-non-fibrous carbohydrates (14.62%), ash (8.64%), Ca-calcium (1.35%) and P-phosphorus (0.12%)] *ad libitum* in the morning and evening. On postpartum days 20, 35, 50, 65 and 80, venous blood samples were collected from the goats via jugular punctures into Vacutainers (Venoject, Terumo, Leuven, Belgium) containing EDTA as an anticoagulant and tubes with no anticoagulant after a fasting time of 3 hr. Within 30 min, the blood samples were centrifuged at 1,500 ×*g* for 10 min at 4°C, and the serum and plasma were harvested and stored at −20°C until the day of the analysis.

Two milk samples of 60 m*l* (one at the morning milking and one at the evening milking) were also individually collected in a plastic vial on the same days as the blood samples. The first collection occurred on day 20 post-parturition to avoid a collection of colostrum. The milk samples were kept at 4°C until the milk quality and composition analyses were conducted. For hormonal analyses, the milk samples were vortexed; aliquots (500 m*l*) of whole milk were obtained (into 1.5 m*l* Eppendorf tubes, Eppendorf, Hamburg, Germany) and frozen at −20°C until analysis.

Milk composition and quality analyses

The milk samples were analyzed for their fat, lactose, protein, solids-non-fat (SNF), total solid (TS), freezing point depression (FPD), density, acidity, free fatty acids (FFA), casein with infrared reflectance spectroscopy (Rev-MilkoScan^{TM} FT1, Foss Electric, Hillerød, Denmark) and SCC using a somatic cell counter (fts/fcm combi 400, Bentley, Chaska, MN, U.S.A.) within 24 hr.

For microbial analyses, the raw milk samples were immediately diluted in sterile PBS (1 m*l* in 9 m*l*) and then homogenized in a vortex mixer, and serial 10-fold dilutions were made for colony countings. Aliquots of 0.1 m*l* of selected dilutions were plated for aerobic mesophilic colony counts using casein-peptone glucose yeast extract agar plates (PCA: plate count agar, 1.05463.0500, MERCK, Darmstadt, Germany) followed by incubation at 37°C for 24 hr. MRS agar was used to isolate lactobacilli. For each sample, a pair of plates containing De Man-Rogosa-Sharpe medium (MRS agar, 1.10660.0500, MERCK) was used. After inoculation, each plate was incubated in an anaerobic chamber using anaerogenic jars (AN0035A, Oxoid, Basingstoke, Hants, U.K.) with an anaerobic pack (CN0020C, Oxoid) at 37°C for 48 hr. Plates containing 30–300 colonies were selected, and representative colonies of each morphotype previously characterized as lactobacilli were enumerated. Non-spore-former rods, gram-positive and catalase-negative isolates were regarded as lactobacilli and calculated for each sample.

Hormonal and biochemical analyses

The milk samples were thawed overnight in the refrigerator and vortexed continuously to ensure sample uniformity. Skim milk was prepared by centrifuging 500 m*l* of whole milk at 16,000× *g* at 4°C for 5 min. The fat layer was removed, and the aqueous phase was assayed. Milk and plasma adiponectin were measured using a commercially available goat-specific sandwich enzyme-linked immunosorbent assay (ELISA) kit (Goat Adiponectin ELISA Kit; Hangzhou Eastbiopharm Co., Ltd., Yile Road, China) in an automated microplate reader ($_x$808, Biotek EL, Winooski, VT, U.S.A.). A goat resistin sandwich ELISA kit (Goat resistin ELISA Kit; Hangzhou Eastbiopharm Co., Ltd.) was used to measure the resistin in the milk and plasma samples. The milk and plasma leptin levels were measured by sandwich ELISA using a goat-specific ELISA kit (Goat leptin ELISA Kit; Hangzhou Eastbiopharm Co., Ltd.). All ELISA kits were validated for use with goat-milk samples. The plasma CRP level was also measured using a commercially available goat-specific ELISA kit (Hangzhou Eastbiopharm Co., Ltd.). Serum total protein, cholesterol and total lipid concentrations were determined with commercial kits (REF T528-480; REF C507-480 and T526-480, respectively, Teco Diagnostics, Anaheim, CA, U.S.A.) following the manufacturer's instructions and using a spectrophotometer (UV 1601, Shimadzu, Kyoto, Japan). The sensitivity, milk and serum intra-assay coefficients of the variation were 0.11 μg/m*l*, 6% and 8% for adiponectin, 0.12 *n*g/m*l*, 5% and 6% for resistin, 0.27 *n*g/m*l*, 6.7% and 8% for leptin, and 0.26 μg/m*l*, 4% for CRP.

Statistical analyses

All statistical analyses were conducted using IBM® SPSS® version 22 for Windows. The relationships between two variables were determined by Pearson's correlation coefficient.

The Shapiro-Wilk test was used for normality. Changes at different time points were determined using repeated measures test

Table 1. Milk and plasma concentrations of adiponectin, leptin, resistin, CRP, serum total protein, cholesterol and lipid concentrations, milk composition and quality parameter levels on lactating days 20–80 of Saanen goats (n=18)

	20 day	35 day	50 day	65 day	80 day
Milk parameters					
Milk adipokines					
Milk adiponectin (*μ*g/m*l*)	2.4 ± 0.2	3.2 ± 0.4	3.6 ± 0.2[a)]	3.7 ± 0.2[a)]	3.9 ± 0.2[a)]
Milk resistin (*n*g/m*l*)	5.2 ± 0.4	5.4 ± 0.5	5.9 ± 0.4	5.8 ± 0.2	6.5 ± 0.4
Milk leptin (*n*g/m*l*)	7.6 ± 0.5	11.0 ± 0.8[a)]	9.0 ± 0.3[a)]	8.6 ± 0.2[a)]	8.6 ± 0.3[a)]
Milk composition					
Fat (%)	4.4 ± 0.2	4.5 ± 0.2	4.5 ± 0.2	4.8 ± 0.3	4.1 ± 0.1
Lactose (%)	4.6 ± 0.0	4.6 ± 0.0	4.6 ± 0.0	4.5 ± 0.0	4.6 ± 0.0
Protein (%)	3.4 ± 0.0	3.3 ± 0.1	3.5 ± 0.1	3.6 ± 0.1[a)]	3.7 ± 0.1[a)]
SNF (%)	8.9 ± 0.1	8.8 ± 0.1	8.9 ± 0.0	9.0 ± 0.1	9.2 ± 0.1[a)]
TS (%)	13.3 ± 0.2	13.1 ± 0.2	13.4 ± 0.2	13.9 ± 0.4	13.2 ± 0.2
FPD (°C)	0.5 ± 0.0	0.5 ± 0.0	0.5 ± 0.0	0.5 ± 0.0	0.5 ± 0.0
Density (kg/m^3)	1,029.9 ± 0.5	1,028.2 ± 0.6	1,029.7 ± 0.4	1,028.2 ± 0.8	1,032.7 ± 0.5[a)]
Acidity (°SH)	6.1 ± 0,1	6.5 ± 0.1[a)]	6.4 ± 0.1[a)]	6.4 ± 0.2[a)]	6.4 ± 0.1[a)]
FFA (mEq/*l*)	0.4 ± 0.0	0.4 ± 0.0	0.5 ± 0.0	0.4 ± 0.0	0.3 ± 0.0[a)]
Casein (%)	2.6 ± 0.0	2.6 ± 0.0	2.7 ± 0.0[a)]	2.8 ± 0.0[a)]	2.9 ± 0.0[a)]
SCC (×10^3/m*l*)	441.0 ± 23.4	303.7 ± 16.5	543.7 ± 42.5	686.7 ± 47.7	236.1 ± 25.7
Total aerobic colony (× 10^3/m*l*)	32 ± 9	41 ± 2.4	32 ± 8.1	37 ± 1.3	20 ± 1.4
Lactobacillus (× 10^3/m*l*)	1.1 ± 0.2	1.3 ± 0.4	1.5 ± 0.8	0.22 ± 0.1	0.13 ± 0.05
Blood parameters					
Plasma adiponectin (*μ*g/m*l*)	26.2 ± 4.2	27.8 ± 4.5	30.8 ± 2.1[a)]	33.0 ± 3.0[a)]	28.8 ± 4.0[a)]
Plasma resistin (*n*g/m*l*)	32.9 ± 5.2	30.7 ± 4.9	32.7 ± 5.5	29.8 ± 5.2	33.6 ± 6.0
Plasma leptin (*n*g/m*l*)	43.7 ± 6.7	54.2 ± 6.5[a)]	53.5 ± 6.6[a)]	54.6 ± 7.3[a)]	50.3 ± 4.8[a)]
Plasma CRP (mg/*l*)	2.4 ± 0.3	2.5 ± 0.3	2.5 ± 0.2	2.6 ± 0.3	2.7 ± 0.4
Serum total protein (g/*l*)	57 ± 2.0	60 ± 2.1	65 ± 2.4	63 ± 2.2	58 ± 1.1
Serum cholesterol (mmol/*l*)	1.8 ± 0.0	1.6 ± 0.0[a)]	1.3 ± 0.1[a)]	1.4 ± 0.0[a)]	1.2 ± 0.0[a)]
Serum total lipid (g/*l*)	1.3 ± 0.0	1.6 ± 0.0[a)]	1.8 ± 0.0[a)]	2.1 ± 0.1[a)]	2.2 ± 0.0[a)]

a) Significantly (P<0.01) different from the observed values for 20 post-partum lactation days. Data are given as the mean ± standard error.

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). *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*=0.01).

RESULTS

The milk and plasma concentrations of the adipokines, milk composition and quality characteristics of the lactating goats on days 20, 35, 50, 65 and 80 postpartum are shown in Table 1. Non-significant differences in milk and plasma resistin, CRP, total protein, milk fat, lactose, TS, FPD, SCC, the total aerobic colony count and lactobacilli were observed on days 20, 35, 50, 65 and 80 postpartum (Table 1). Lactating goats had significantly higher milk adiponectin levels on days 50 (*P*<0.003), 65 (*P*<0.002) and 80 (*P*<0.002) postpartum than on day 20 (Table 1). The milk leptin levels were lower on day 20 than on days 35 (*P*<0.005), 50 (*P*<0.008), 65 (*P*<0.01) and 80 (*P*<0.01; Table 1). The plasma adiponectin levels on days 50, 65 and 80 were higher (*P*<0.002, *P*<0.001 and *P*<0.01, respectively) than those on day 20 (Table 1). The plasma leptin levels were lower on day 20 than on days 35 (*P*<0.01), 50 (*P*<0.01), 65 (*P*<0.01) and 80 (*P*<0.01; Table 1). The serum cholesterol levels on day 20 were higher than those on days 35 (*P*<0.01), 50, 65 and 80 (*P*<0.000), and the serum total lipid levels on day 20 were lower than those on days 35 (*P*<0.000), 50, 65 and 80 (*P*<0.000; Table 1). The percentage of milk protein was higher on days 65 (*P*<0.01) and 80 (*P*<0.000) than on day 20 (Table 1). Milk SNF and density levels were higher on day 80 than on day 20 (*P*<0.02 and *P*<0.000; Table 1). Milk acidity levels were lower on day 20 than on days 35 (*P*<0.001), 50 (*P*<0.005), 65 (*P*<0.000) and 80 (*P*<0.000) postpartum (Table 1). Milk FFA level was lower on day 80 than on day 20 postpartum (*P*<0.01; Table 1). The goats had a significantly lower milk casein level on day 20 than on days 50 (*P*<0.01), 65 (*P*<0.000) and 80 (*P*<0.000; Table 1).

A correlation analysis revealed a positive correlation between milk concentrations of adiponectin, leptin and resistin (*P*<0.05; Table 2). There were also positive correlations between the milk and plasma concentrations of these three adipokines (Table 2). Milk adiponectin level positively correlated with plasma adiponectin, leptin and resistin levels (*P*<0.05; Table 2). Milk resistin level positively correlated with plasma adiponectin (*P*<0.05), leptin (*P*<0.01) and resistin levels (*P*<0.05; Table 2). Milk leptin level also positively correlated with plasma adiponectin and resistin levels (*P*<0.01; Table 2). There was also a positive correlation between plasma CRP level and milk leptin and resistin (*P*<0.05) concentrations and an inverse correlation between milk adiponectin and plasma CRP concentrations (Table 2). There was no correlation between milk concentrations of these adipokines and serum total

Table 2. Interrelationships between milk adiponectin, leptin and resistin concentrations and the relationships of milk adiponectin, leptin and resistin with plasma levels and plasma CRP, serum total protein, cholesterol and lipid concentrations in lactating goats

	Milk adiponectin r	Milk resistin r	Milk leptin r	Plasma adiponectin r	Plasma resistin r	Plasma leptin r	Plasma CRP r	Serum total protein r	Serum cholesterol r	Serum total lipid r
Milk adiponectin	-	0.50[a)]	0.49[a)]	0.55[a)]	0.58[a)]	0.48[a)]	–0.48[a)]	0.28	–0.20	–0.15
Milk resistin	0.50[a)]	-	0.49[a)]	0.54[a)]	0.59[b)]	0.49[a)]	0.48[a)]	–0.37	–0.08	0.17
Milk leptin	0.49[a)]	0.49[a)]	-	0.64[b)]	0.68[b)]	0.63[b)]	0.59[a)]	–0.16	–0.23	–0.14

a) $P<0.05$, b) $P<0.01$.

Table 3. The relationships of goat milk adiponectin, leptin and resistin concentrations with milk quality and composition

	Fat r	Lactose r	Protein r	SNF r	TS r	FPD r	Density r	Acidity r	FFA r	Casein r	SCC r	Total aerobic colony r	Lactobacillus r
Milk adiponectin	–0.22	0.27	–0.33	0.02	–0.24	–0.01	0.03	0.05	–0.16	–0.36	–0.47[a)]	–0.14	0.07
Milk resistin	0.01	0.02	0.19	0.22	0.09	0.01	0.30	0.55	0.00	0.25	–0.02	–0.29	–0.02
Milk leptin	–0.21	0.10	0.17	0.38	–0.07	0.00	0.38	0.30	–0.24	0.13	–0.33	0.29	0.01

a) $P<0.05$.

protein, cholesterol and total lipid levels (Table 2). A significant negative correlation was noted between milk adiponectin level and its SCC ($P<0.05$), whereas no relationship was found between milk adiponectin level and its composition parameters (Table 3). Non-significant correlations were observed between milk resistin and leptin concentrations and milk components, SCC, total aerobic colony and lactobacillus count (Table 3).

The 20-, 35-, 50-, 65- and 35-day Body Weight and BCS of goats were 42.5 ± 1.6, 43.00 ± 2.1, 44.50 ± 2.4, 45.65 ± 2.5 and 50.00 ± 2.3 kg, and 3.31 ± 0.1, 3.57 ± 0.2, 3.62 ± 0.2, 3.94 ± 0.4 and 4.20 ± 0.4, respectively and not statistically different. Non-significant correlations were observed between milk adipokine concentrations and body weights and BCS.

DISCUSSION

This is the first report of the adiponectin and resistin concentrations in the milk of Saanen goats. Leptin gene expression in the mammary gland of alpine goats was reported by Bonnet *et al*. [2]. Leptin concentrations were also previously reported in the milk of mixed-parity Boer and Boer crossbred meat-type goats by Whitley *et al*. [41]. In this study, we measured the leptin levels in lactating Saanen goats and confirmed the presence of leptin in goat milk. Our data indicate that goat milk and plasma adiponectin and leptin concentrations increased, whereas no changes were observed in the resistin levels during a lactation period of 20–80 days. The observed increase in the milk adiponectin concentration during days 20–80 of the lactation period has also been reported in humans by Ilcol *et al*. [28], Weyermann *et al.* [40], Bronsky *et al*. [4] and Martin *et al.* [20]. In good accordance with previous reports on humans [34], the adiponectin concentration in goat milk correlated positively with plasma adiponectin levels. The significant association between milk and plasma adiponectin concentrations indicates that circulating adiponectin is likely to be the major source of milk adiponectin. The mammary gland is not a meaningful source of adiponectin, its expression being 0.05% that of adipose tissue [10]. The concentrations of leptin in goat milk and plasma were lower on day 20 than on days 35, 50, 65 and 80 days. These results are similar to those reported in mixed-parity Boer and Boer crossbred meat-type goats [41]. The presence of a significant association between milk and plasma leptin concentrations during days 20 to 80 of lactation indicates that circulating leptin may be the major source of milk leptin. The observed relationship between milk and plasma leptin concentrations during days 20 to 80 of lactation has also been reported in humans by Ucar *et al*. [37] and Ilcol *et al*. [13]. As the number of lactation days increased from 20 to 80, non-significant differences were observed in the milk and plasma resistin concentrations of Saanen goats. Another study by Ilcol *et al*. [14] demonstrated that milk resistin concentrations were highest in colostrum, decreased rapidly during postpartum days 4–14 and remained low during postpartum days 15–180. The positive correlation between these three adipokine concentrations in goat milk in the current study during days 20 to 80 of lactation is also in good accordance with a previous observation in cord blood, milk and blood [14, 18, 40]. However, an inverse relationship between the resistin concentrations and both the leptin and adiponectin concentrations in milk and plasma was observed in some previous studies on humans [28, 29]. These differences between the results of the current study and those of Ilcol *et al.* [28, 29] may be due to changes in regulatory mechanisms and factors for circulating adiponectin, leptin and resistin concentrations in goats and humans. Our results show that the circulatory concentrations of these three adipokines are interrelated in days 20 to 80 of the lactation period of Saanen goats. It appears that the changes in the milk and plasma adiponectin concentrations coincide with changes in the plasma leptin and resistin concentrations.

Adiponectin, leptin and resistin hormones are secreted biologically active forms *into* milk and may transiently regulate the activities of various tissues until the endocrine system of the neonate begins to function. These bioactive milk peptides also play

important roles, such as energy intake and systemic and local imflammatory status. [5, 15, 33]. At this point, another important focus of this study was a positive correlation between CRP and these three adipokines. CRP, a marker of systemic inflammation, was known as a stimulator of monocytes and macrophages [31, 34]. There were a positive correlation between the CRP and leptin and resistin concentrations in goat milk and an inverse correlation between CRP and milk adiponectin, consistent with previous studies in humans [14, 28, 39]. Leptin has been shown to play a proinflammatory role and to protect against infections. It not only regulates T-cell proliferation and activation but also influences cytokine production from T lymphocytes [9, 16]. Furthermore, Ble *et al*. [1] demonstrated a direct CRP-stimulatory activation of leptin, independent of IL-6 or other proinflammatory cytokines in humans. The inverse relationship between CRP and milk adiponectin in this study is in strong accordance with previous studies on humans that report that proinflammatory factors suppress the adiponectin production of adipocytes [21, 25, 26, 34, 42]. Resistin, mononuclear cells and macrophages, other major sources of circulating resistin, have also been implicated in the inflammatory response of humans [11]. In good accordance with recent studies on human milk [11, 14, 23], we observed that plasma CRP [31] correlated positively with milk resistin. SCC is recognized as a reliable indicator of animals' udder health (local inflammation) and milk quality and changes in their milk composition. The milk somatic cells include leucocytes, neutrophils, macrophages, lymphocytes, erythrocytes and epithelial cells. Moreover, a high SCC is directly related to inverse effects on human health, including poor farm hygiene, antibiotic residues and the presence of pathogenic bacteria and toxins in milk [27, 35]. The inverse relationship between adiponectin and SCC or CRP may be related to the fact that adiponectin gene expression is decreased by oxidative stress or a pro-inflammatory state. In this study, no relation was observed between these three adipokines and the total aerobic colony count, which represents the total amount of viable microorganisms that could grow aerobically on plate-count agar, and the lactobacillus count. Lactobacilli, non-pathogen microorganisms, may possess potentially therapeutic properties, including anti-inflammatory and anti-cancer activities. Protective anti-tumor and anti-cancer effects of some strains of these bacteria were demonstrated in mice [6]. A recent study reported that there may be a relationship between lactobacilli and the health of bovine udders [30]. In the current study, we did not find a relationship between lactobacilli and these milk adipokines.

We also determined whether the milk levels of leptin, adiponectin and resistin are related to the milk composition of lactating goats. However, no correlation was observed between the milk concentrations of these major adipokines and milk composition.

In conclusion, the current study is the first to show adiponectin and resistin concentrations in the milk of Saanen goats during days 20 to 80 of the lactation period. The data from the current study show interrelationships between the milk concentrations of adiponectin, leptin and resistin and significant positive relationships between the milk and plasma concentrations of these three major adipokines during days 20 to 80 of the lactation period in goats. The concentrations of leptin and resistin in goat milk are also positively related to the plasma CRP concentrations, whereas the milk adiponectin concentration is inversely related to the plasma CRP concentration and milk SSC. The findings in this study indicate a possible role of these three adipokines in the inflammatory status of goat milk, but more in-depth analyses are necessary to determine the mechanism and to better define their function.

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REFERENCES

1. Ble, A., Windham, B. G., Bandinelli, S., Taub, D. D., Volpato, S., Bartali, B., Tracy, R. P., Guralnik, J. M. and Ferrucci, L. 2005. Relation of plasma leptin to C-reactive protein in older adults (from the Invecchiare nel Chianti study). *Am. J. Cardiol.* **96**: 991–995. [Medline] [CrossRef]
2. Bonnet, M., Delavaud, C., Laud, K., Gourdou, I., Leroux, C., Djiane, J. and Chilliard, Y. 2002. Mammary leptin synthesis, milk leptin and their putative physiological roles. *Reprod. Nutr. Dev.* **42**: 399–413. [Medline] [CrossRef]
3. Briana, D. D. and Malamitsi-Puchner, A. 2010. The role of adipocytokines in fetal growth. *Ann. N. Y. Acad. Sci.* **1205**: 82–87. [Medline] [CrossRef]
4. Bronsky, J., Karpísek, M., Bronská, E., Pechová, M., Jancíková, B., Kotolová, H., Stejskal, D., Prusa, R. and Nevoral, J. 2006. Adiponectin, adipocyte fatty acid binding protein, and epidermal fatty acid binding protein: proteins newly identified in human breast milk. *Clin. Chem.* **52**: 1763–1770. [Medline] [CrossRef]
5. Çatlı, G., Olgaç Dündar, N. and Dündar, B. N. 2014. Adipokines in breast milk: an update. *J. Clin. Res. Pediatr. Endocrinol.* **6**: 192–201. [Medline] [CrossRef]
6. Chen, X., Fruehauf, J., Goldsmith, J. D., Xu, H., Katchar, K. K., Koon, H. W., Zhao, D., Kokkotou, E. G., Pothoulakis, C. and Kelly, C. P. 2009. Saccharomyces boulardii inhibits EGF receptor signaling and intestinal tumor growth in Apc(min) mice. *Gastroenterology* **137**: 914–923. [Medline] [CrossRef]
7. Coveney, J. and Darnton-Hill, I. 1985. Goat's milk and infant feeding. *Med. J. Aust.* **143**: 508–510. [Medline]
8. FAOSTAT- Food And Agriculture Organization Of The United Nations Agricultural Statistics, 2012 (online: http://www.fao.org). FAO, Rome, Italy. See comment in PubMed Commons below.
9. Farooqi, I. S., Matarese, G., Lord, G. M., Keogh, J. M., Lawrence, E., Agwu, C., Sanna, V., Jebb, S. A., Perna, F., Fontana, S., Lechler, R. I., DePaoli, A. M. and O'Rahilly, S. 2002. Beneficial effects of leptin on obesity, T cell hyporesponsiveness, and neuroendocrine/metabolic dysfunction of human congenital leptin deficiency. *J. Clin. Invest.* **110**: 1093–1103. [Medline] [CrossRef]
10. Giesy, S. L., Yoon, B., Currie, W. B., Kim, J. W. and Boisclair, Y. R. 2012. Adiponectin deficit during the precarious glucose economy of early lactation in dairy cows. *Endocrinology* **153**: 5834–5844. [Medline] [CrossRef]
11. Holcomb, I. N., Kabakoff, R. C., Chan, B., Baker, T. W., Gurney, A., Henzel, W., Nelson, C., Lowman, H. B., Wright, B. D., Skelton, N. J., Frantz,

G. D., Tumas, D. B., Peale, F. V. Jr., Shelton, D. L. and Hébert, C. C. 2000. FIZZ1, a novel cysteine-rich secreted protein associated with pulmonary inflammation, defines a new gene family. *EMBO J.* **19**: 4046–4055. [Medline] [CrossRef]
12. Houseknecht, K. L., McGuire, M. K., Portocarrero, C. P., McGuire, M. A. and Beerman, K. 1997. Leptin is present in human milk and is related to maternal plasma leptin concentration and adiposity. *Biochem. Biophys. Res. Commun.* **240**: 742–747. [Medline] [CrossRef]
13. Ilcol, Y. O., Hizli, Z. B. and Ozkan, T. 2006. Leptin concentration in breast milk and its relationship to duration of lactation and hormonal status. *Int. Breastfeed. J.* **1**: 21–29. [Medline] [CrossRef]
14. Ilcol, Y. O., Hizli, Z. B. and Eroz, E. 2008. Resistin is present in human breast milk and it correlates with maternal hormonal status and serum level of C-reactive protein. *Clin. Chem. Lab. Med.* **46**: 118–124. [Medline] [CrossRef]
15. Karatas, Z., Durmus Aydogdu, S., Dinleyici, E. C., Colak, O. and Dogruel, N. 2011. Breastmilk ghrelin, leptin, and fat levels changing foremilk to hindmilk: is that important for self-control of feeding? *Eur. J. Pediatr.* **170**: 1273–1280. [Medline] [CrossRef]
16. La Cava, A., Alviggi, C. and Matarese, G. 2004. Unraveling the multiple roles of leptin in inflammation and autoimmunity. *J. Mol. Med.* **82**: 4–11. [Medline] [CrossRef]
17. Lara-Villoslada, F., Olivares, M., Jiménez, J., Boza, J. and Xaus, J. 2004. Goat milk is less immunogenic than cow milk in a murine model of atopy. *J. Pediatr. Gastroenterol. Nutr.* **39**: 354–360. [Medline] [CrossRef]
18. Marinoni, E., Corona, G., Ciardo, F., Letizia, C., Moscarini, M. and Di Iorio, R. 2010. Changes in the interrelationship between leptin, resistin and adiponectin in early neonatal life. *Front. Biosci. (Elite Ed.)* **2**: 52–58. [Medline] [CrossRef]
19. Marnell, L., Mold, C. and Du Clos, T. W. 2005. C-reactive protein: ligands, receptors and role in inflammation. *Clin. Immunol.* **117**: 104–111. [Medline] [CrossRef]
20. Martin, L. J., Woo, J. G., Geraghty, S. R., Altaye, M., Davidson, B. S., Banach, W., Dolan, L. M., Ruiz-Palacios, G. M. and Morrow, A. L. 2006. Adiponectin is present in human milk and is associated with maternal factors. *Am. J. Clin. Nutr.* **83**: 1106–1111. [Medline]
21. Matsushita, K., Yatsuya, H., Tamakoshi, K., Wada, K., Otsuka, R., Zhang, H., Sugiura, K., Kondo, T., Murohara, T. and Toyoshima, H. 2006. Inverse association between adiponectin and C-reactive protein in substantially healthy Japanese men. *Atherosclerosis* **188**: 184–189. [Medline] [CrossRef]
22. Meier, U. and Gressner, A. M. 2004. Endocrine regulation of energy metabolism: review of pathobiochemical and clinical chemical aspects of leptin, ghrelin, adiponectin, and resistin. *Clin. Chem.* **50**: 1511–1525. [Medline] [CrossRef]
23. Nagaev, I., Bokarewa, M., Tarkowski, A. and Smith, U. 2006. Valcarcel, Juan, ed. "Human Resistin Is a Systemic Immune-Derived proinflammatory Cytokine Targeting both Leukocytes and Adipocytes". *PLoS ONE* **1**:e31.
24. Ng, P. C., Lee, C. H., Lam, C. W., Chan, I. H., Wong, E. and Fok, T. F. 2005. Resistin in preterm and term newborns: relation to anthropometry, leptin, and insulin. *Pediatr. Res.* **58**: 725–730. [Medline] [CrossRef]
25. Ouchi, N., Parker, J. L., Lugus, J. J. and Walsh, K. 2011. Adipokines in inflammation and metabolic disease. *Nat. Rev. Immunol.* **11**: 85–97. [Medline] [CrossRef]
26. Ouchi, N., Kihara, S., Funahashi, T., Nakamura, T., Nishida, M., Kumada, M., Okamoto, Y., Ohashi, K., Nagaretani, H., Kishida, K., Nishizawa, H., Maeda, N., Kobayashi, H., Hiraoka, H. and Matsuzawa, Y. 2003. Reciprocal association of C-reactive protein with adiponectin in blood stream and adipose tissue. *Circulation* **107**: 671–674. [Medline] [CrossRef]
27. Pridalova, H., Janstova, B., Cupakova, S., Drackova, M., Nauratilova, P. and Vorlova, L. 2009. Somatic cell count in goat milk. *Folia Vet.* **53**: 101–105.
28. Ozarda, Y., Gunes, Y. and Tuncer, G. O. 2012*a*. The concentration of adiponectin in breast milk is related to maternal hormonal and inflammatory status during 6 months of lactation. *Clin. Chem. Lab. Med.* **50**: 911–917. [Medline] [CrossRef]
29. Ozarda, Y., Tuncer, G. O., Gunes, Y. and Eroz, E. 2012*b*. Serum levels of leptin, adiponectin and resistin are interrelated and related to total antioxidant capacity, free fatty acids and phospholipids in early neonatal life. *Clin. Biochem.* **45**: 298–302. [Medline] [CrossRef]
30. Qiao, J., Kwok, L., Zhang, J., Gao, P., Zheng, Y., Guo, Z., Hou, Q., Huo, D., Huang, W. and Zhang, H. 2015. Reduction of Lactobacillus in the milks of cows with subclinical mastitis. *Benef. Microbes* **6**: 485–490. [Medline] [CrossRef]
31. Reilly, M. P., Lehrke, M., Wolfe, M. L., Rohatgi, A., Lazar, M. A. and Rader, D. J. 2005. Resistin is an inflammatory marker of atherosclerosis in humans. *Circulation* **111**: 932–939. [Medline] [CrossRef]
32. Rosi, F., Aufy, A. A. and Magistrelli, D. 2009. Diet influences the content of bioactive peptides in goat milk. *J. Endocrinol. Invest.* **32**: 486–490. [Medline] [CrossRef]
33. Savino, F. and Liguori, S. A. 2008. Update on breast milk hormones: leptin, ghrelin and adiponectin. *Clin. Nutr.* **27:**42–47. *Epub* **2007**: 22.
34. Savino, F., Liguori, S. A. and Lupica, M. M. 2010. Adipokines in breast milk and preterm infants. *Early Hum. Dev.* **86** Suppl 1: 77–80. [Medline] [CrossRef]
35. Sharma, N., Singh, N. K. and Bhadwal, M. S. 2011. Relationship of Somatic Cell Count and Mastitis: An Overview. *Asian-Aust. J. Anim. Sci.* **24**: 429–438.
36. Tsai, P. J., Yu, C. H., Hsu, S. P., Lee, Y. H., Chiou, C. H., Hsu, Y. W., Ho, S. C. and Chu, C. H. 2004. Cord plasma concentrations of adiponectin and leptin in healthy term neonates: positive correlation with birthweight and neonatal adiposity. *Clin. Endocrinol. (Oxf.)* **61**: 88–93. [Medline] [CrossRef]
37. Uçar, B., Kirel, B., Bör, O., Kiliç, F. S., Doğruel, N., Aydoğdu, S. D. and Tekin, N. 2000. Breast milk leptin concentrations in initial and terminal milk samples: relationships to maternal and infant plasma leptin concentrations, adiposity, serum glucose, insulin, lipid and lipoprotein levels. *J. Pediatr. Endocrinol. Metab.* **13**: 149–156. [Medline] [CrossRef]
38. Vendrell, J., Broch, M., Vilarrasa, N., Molina, A., Gómez, J. M., Gutiérrez, C., Simón, I., Soler, J. and Richart, C. 2004. Resistin, adiponectin, ghrelin, leptin, and proinflammatory cytokines: relationships in obesity. *Obes. Res.* **12**: 962–971. [Medline] [CrossRef]
39. Viikari, L. A., Huupponen, R. K., Viikari, J. S., Marniemi, J., Eklund, C., Hurme, M., Lehtimäki, T., Kivimäki, M. and Raitakari, O. T. 2007. Relationship between leptin and C-reactive protein in young Finnish adults. *J. Clin. Endocrinol. Metab.* **92**: 4753–4758. [Medline] [CrossRef]
40. Weyermann, M., Beermann, C., Brenner, H. and Rothenbacher, D. 2006. Adiponectin and leptin in maternal serum, cord blood, and breast milk. *Clin. Chem.* **52**: 2095–2102. [Medline] [CrossRef]
41. Whitley, N. C., Walker, E. L., Harley, S. A., Keisler, D. H. and Jackson, D. J. 2005. Correlation between blood and milk serum leptin in goats and growth of their offspring. *J. Anim. Sci.* **83**: 1854–1859. [Medline] [CrossRef]
42. Yoshino, T., Kusunoki, N., Tanaka, N., Kaneko, K., Kusunoki, Y., Endo, H., Hasunuma, T. and Kawai, S. 2011. Elevated serum levels of resistin, leptin, and adiponectin are associated with C-reactive protein and also other clinical conditions in rheumatoid arthritis. *Intern. Med.* **50**: 269–275. [Medline] [CrossRef]