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Araştırma Makalesi ***Research Article***

The Effect of Long Term Starvation on Galanin, Leptin, Thyroid Hormones, Insulin, Prolactin, Growth Hormone, Ghrelin and Factors Involved in Energy Metabolism in Adult Goats

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Abstract

Some hormonal disturbances have been demonstrated in starvation, but in ruminants such as goats, the role of galanin in adaptation to starvation or endocrine functions is not well studied. The present study was conducted to assess the effect of long term starvation on galanin, leptin, thyroid hormones, insulin, prolactin, growth hormone, ghrelin and factors involved in energy metabolism including HDL, Cholesterol, β-hydroxybutyrate, glucose, NEFA, TG and VLDL concentrations in adult goats. Eight non-lactating non-pregnant goats aged 4-5 years and BCS 3 were randomly divided to control and test groups. The animals were trained to eat their daily forage ration during a 10 day period. The experimental procedure was applied for 20 days, during which control group received 120% of maintenance energy, while the test group was supplied with 80% of maintenance energy for the first 10 days and with 40% of maintenance energy for another 10 days. Blood samples were collected at day 10 of training and 2, 4, 10, 12, 14 and 20 days after beginning of starvation. Blood parameters were measured according to standard procedures. No significant difference was observed in the concentrations of cholesterol, fT_3, T_4, T_3, growth hormone, NEFA, insulin and ghrelin between control and test groups (P=0.05). There was significant difference in galanin, leptin, fT_4, HDL, glucose, TG, VLDL and prolactin concentrations between control and test groups (P=0.05). Control of energy balance and the role of galanin in adaptation to long starvation or endocrine functions in goat are different from other species.

Özet

Erişkin Keçilerde Uzun Süreli Açlığın Galanin, Leptin, Tiroid Hormonları, İnsulin, Prolaktin, Büyüme Hormonu, Grelin ve Enerji Metabolizması ile İlgili Faktörler Üzerine Etkisi

Açlık durumunda bazı hormonal bozukluklar gösterilmiş, ancak keçiler gibi geviş getiren hayvanlarda galaninin açlığa ya da endokrin fonksiyonlara uyumu iyi araştırılmamıştır. Mevcut çalışma erişkin keçilerde uzun sureli açlığın galanin, leptin, tiroid hormonları, insulin, prolaktin, büyüme hormonu, grelin ve HDL, kolestreol, β-hidroksibutirat, glukoz, NEFA, TG ve VLDL konsatrasyonları da dahil olmak üzere enerji metabolizması ile ilgili faktörler üzerine olan etkisini değerlendirmek için yürütülmüştür. 4-5 yaşları arasında, süt emzirmeyen ve gebe olmayan vücut kondisyon skoru 3 olan sekiz keçi rastgele olarak kontrol ve test gruplarına ayrıldı. Hayvanlara 10 günlük bir süre boyunca yemleme programına alıştırıldı. Deneysel işlem 20 gün uygulandı; bu süre içinde kontrol grup idame enerjisinin %120'sini aldı, test grubuna ise ilk 10 gün içinde idame enerjisinin %80'i verildi; diğer 10 gün boyunca ise idame enerjisinin %40'ı verildi. Yemleme programına alıştırmanın 10. gününde ve açlık başlamasından sonraki 2, 4, 10, 12, 14 ve 20. günlerde kan örnekleri alındı. Kan parametreleri standart işlemlere göre ölçüldü. Kontrol ve test grupları arasında kolesterol, fT_3, T_4, T_3, büyüme hormonu, NEFA, insulin ve grelin yönünden bir farklılık görülmedi (P=0,05). Kontrol ve test grupları arasında galanin, leptin, fT_4, HDL, glukoz, TG, VLDL ve prolaktin konsantrasyonlarında anlamlı farklılıklar vardı (P=0,05). Keçilerde uzun sureli açlığa uyumda galaninin rolü ve enerji dengesi kontrolü ya da endokrin fonksiyonları diğer türlerden farklılık göstermektedir.

Introduction

Several studies have reported general physiological changes associated with food deprivation in farm and laboratory animals (Baranowska et al., 2001; Gonzalez et al., 1998). These changes have been observed in thyroid, gonadal and adrenal axis and neuropeptides concentrations such as galanin and leptin (Khazali, 2008; Leibowitz et al., 2004; Yun et al., 2005). The identification and role of neuropeptides in the control of food intake and energy balance have been extensively studied in rodents, and for more than twenty years, similar studies have been performed in sheep as small ruminant. In addition to the role of hypothalamic neuropeptides in nutrition, their hormonal regulatory systems should be considered. The involvement of insulin, leptin and ghrelin, in feeding and nutrition regulations has been elucidated in numerous species. These actions may be partly mediated by neuropeptides neuronal systems that present binding-site or receptors of these hormones.

Mean plasma concentrations of metabolic hormones such as thyroxin (T_4), triiodothyronine (T_3), growth hormone (GH), insulin, glucose and fatty acid in camels fed different energy content in diet were measured by Khazali (2008). Chaillou et al. (2003) determined the sensitivity of galanin containing neurons to long term starvation in an immunohistochemical study in 167 days fasted ewes (Chaillou et al., 2003).

In goat, the role of galanin in the regulation of food intake or endocrine functions is not well studied. Peptide-containing neuron systems, involved in the regulation of food intake and energy balance in small ruminants are generally similar to those observed in other species, but they present specific differences according to the physiological characteristics of the animal model. Small ruminants such as goat have an unusual gastric system associated with a long period of digestion. Food may be present in the rumen up to 60 hours after ingestion. Low body mass and low metabolic requirements of goats can be regarded as important characteristics since maintenance and water requirements are reduced when quality and quantity of food and water supplies are limited.

The ability to reduce metabolism allows goats to survive even after prolonged periods of severe limited food availability. A skillful grazing behavior and efficient digestive system enable goats to attain maximal food intake and maximal food utilization in a given condition (Silanikove, 2000).

To demonstrate the role of galanin in nutrition and starvation in goat, the concentration of galanin, hormones involved in energy metabolism and metabolism factors in different nutritional levels were evaluated. The present study was conducted to evaluate the effect of long term starvation on the galanin, hormones such as leptin, thyroid hormones, insulin, prolactin, growth hormone, ghrelin and factors involved in energy metabolism such as HDL, cholesterol, β-hydroxybutyrate, glucose, NEFA, TG and VLDL concentrations in adult goats.

Materials and Methods

Eight non-lactating non-pregnant goats aged 4-5 years and BCS 3 were selected for this study. Each group contained 4 goats and each animal was kept in individual pen. The animals were trained to eat their daily forage ration during a 10 day period.

This experiment was accomplished under the approval of the state committee on animal ethics, Shiraz University, Shiraz, Iran. Also, we used the recommendations of European Council Directive (86/609/EC) of November 24, 1986, regarding the standards in the protection of animals used for experimental purposes.

The experimental procedure was applied for 20 days. Control group received 120% of maintenance energy (400 g hay, 600 g straw, 30 g soybean oil, 15 g vitamins and mineral supplement and 5 g salt per animal per day) whereas the test group was supplied with 80% of maintenance energy in the first 10 days (266 g of hay, 400 g straw, 20 g soybean oil, 15 g vitamins and mineral supplement and 5 g salt per animal per day) and was supplied with 40% of maintenance energy in the second ten day period (133.3 g of hay, 200 g straw, 10 g soybean oil, 15 g vitamins and mineral supplement and 5 g salt per animal per day).

Blood samples were collected at day 10 of training and 2, 4, 10, 12, 14 and 20 days after beginning of starvation. Blood samples were collected from the jugular vein in evacuated tubes and allowed to clot for 30 min. The sera were separated following centrifugation at 750 *g* for 15 min and stored at -20˚C until used.

Galanin was measured using double-antibody sandwich enzyme-linked immunosorbent technique using commercial goat galanin (GAL) ELISA kit (Shanghai Crystal Day Biotech Co., LTD). Determination of serum T_3, T_4, fT_3 and fT_4 was carried out by microplate Enzyme immunoassay method (Monobind Inc, Lake Forest, USA). The areas of validation for T_3, T_4, fT_3 and fT_4 assays included limits of detection, and precision in standard curve following sample dilution, inter- and intra-assay coefficients of variation results were considered. Intra- and inter-assays for T_4 and T_3 were found to be below

6.2%, 8.6%, 3.3%, and 8.6%, respectively. For fT_4 and fT_3 the values were found to be below 6.5% ,7.2%, 3.5%, and 7.0%, respectively.

Leptin was measured by quantitative sandwich enzyme immunoassay technique using commercial goat Leptin (LEP) ELISA kit (Wuhan Huamei Cusabio Biotech Co., Ltd. China). Measurement of growth hormone was carried out by competitive inhibition enzyme immunoassay technique using commercial goat growth hormone (GH) ELISA kit (Wuhan Huamei Cusabio Biotech Co., Ltd. China). Prolactin was measured by competitive enzyme immunoassay technique using commercial goat prolactin (PRL) ELISA Kit (Wuhan Huamei Cusabio Biotech Co., LTD. China). Insulin was determined using quantitative sandwich enzyme immunoassay technique using commercial goat insulin (INS) ELISA Kit (Wuhan Huamei Cusabio Biotech Co., Ltd. China). Determination of ghrelin was carried out by quantitative sandwich enzyme immunoassay technique using commercial goat appetite-regulating hormone (GHRL) ELISA kit (Wuhan Huamei Cusabio Biotech Co., Ltd. China). The serum was analyzed for cholesterol using a modified Abell-Kendall/Levey-Brodie (A-K) method (Burtis and Ashwood, 1994) for triglyceride by the enzymatic procedure of McGowan et al. (1983) and for glucose using glucose oxidase method. The β-hydroxybutyrate and non-esterified fatty acids (NEFA) were measured by kinetic enzymatic and colorimetric methods, respectively using β-hydroxybutyrate and non-esterified fatty acids (NEFA) kits (Randox Laboratories, Crumlin, Antrim, UK).

Proc mixed procedure was analyzed by SAS 9.1 (SAS Institute Inc, Cary, NC, USA) software. P = 0.05 was considered as statistically significant.

Results

The results are shown as mean ± standard error (SE) in SI units. Table 1 presents the concentrations of serum galanin, leptin, thyroid hormones, insulin, glucose, prolactin, growth hormone, ghrelin, NEFA, βHBA, TG, total cholesterol and HDL in control and test groups at different times.

No significant difference was observed in the concentrations of cholesterol (2.2±0.0 versus 1.1±0.0), fT_3 (9.8±0.1 versus 9.6±0.0), T_4 (116.1±3.5 versus 107.7±3.1), T_3 (4.2±0.0 versus 4.2±0.1), growth hormone (8.3±0.1 versus 13.5±0.5), NEFA (0.15±0.0 versus 0.2±0.0), insulin (76.8±1.9 versus 63.2±3.5) and ghrelin (463±22.1 versus 637±24.8) between control and test groups (P=0.05).

There were significant differences in galanin (171±6.3 versus 413±14.7), leptin (7.9±0.1 versus 5.2±0.4), fT_4 (17.4±1.2 versus 22.9±0.2), HDL (1.77±0.2 versus 1.29±0.1), glucose (4.56±0.1 versus 6.72±0.0), TG (0.63±0.0 versus 0.19±0.0), VLDL (0.11±0.0 versus 0.04±0.0) and prolactin (28.3±0.9 versus 40.2±3.2) concentrations between control and test groups (P=0.05).

A significant difference in galanin concentration between fed and fasted goats was found at day 10 of training and the 2nd and 4th day of starvation. Significant difference in the concentration of leptin was found at day 10 of training and the 2nd, 4th, 14th and 20th day of starvation between control and test groups. A significant difference in fT_4 concentration between fed and fasted goats was found at the 10th day of starvation. A significant difference in HDL concentration was found at the 10th and 12th day of starvation between fed and fasted goats. A significant difference in glucose concentration was found between fed and fasted goats at day 10 of training and the 10th, 14th and 20th day of starvation. A significant difference in TG and VLDL concentrations between fed and fasted goats was found at day 10 of training and the 2nd and 4th day of starvation. A significant increase in prolactin concentration in test group was found at all days of starvation compared to that of control (P=0.05).

Discussion

In the central nervous system numerous neuropeptide-containing neurons play an important role in the control of food intake and energy balance. The peptide galanin (GAL) is known to stimulate eating behavior, reduce energy expenditure and affect the release of metabolic hormones. Further, the activity of this peptide in the hypothalamus is modulated, in turn, by these hormones as well as by the ingestion of nutrients (Crawley, 1999; Lopez et al., 1993; Wang and Leibowitz, 1997; Wang et al., 1998).

Khazali (2008) observed significant increase in mean plasma concentrations of GH, glucagon and fatty acid in camels fed with diet containing 25 percent energy content for thirty days. Injection of 1 and 2 µg galanin/kg BW resulted in more significant increase in concentrations of these parameters (P=0.01). Mean plasma concentrations of insulin and glucose were significantly lower in camels fed twenty five percent energy content in diet in comparison with controls (P=0.01). However, injections of 1 and 2 µg galanin/kg BW did not change the concentration of these parameters. Lower energy dietary intake and injections of 1 and 2 µg galanin/kg BW did not change the mean plasma concentrations of the T_3 and T_4 and urea in the animals of all groups. This experiment indicated that galanin positively affects mean plasma concentrations of GH, glucagon and fatty acid in camel only with negative energy balance in company with severe body weight loss (Khazali, 2008).

Table 1. The concentrations (Mean±SE) of hormones, neuropeptides and factors involved in energy metabolism in fed and fasted goats.

Tablo 1. Tok ve aç keçilerde enerji metabolizmasında yer alan faktörlerin, nöropeptidlerin ve hormonların konsantrasyonları (Ortalama±SE).

Variables		Day of Training 10	Days After Food Deprivation					
			2	4	10	12	14	20
Galanin (ng/L)	control	147±11.8	171±6.3	162±11.1	161±20.5	156±4.7	111±5.8	251±10.2
	test	279±7.3*	413±14.7*	303±62.1*	199±1.9	192±3.9	205±1.0	274±10.3
Leptin (ng/ml)	control	7.4±0.4	7.9±0.1	7.2±0.8	5.0±0.0	7.0±0.2	6.1±0.5	6.1±0.4
	test	3.96±0.0*	5.2±0.4*	4.4±0.2*	3.71±0.5	5.32±0.2	2.94±0.0*	2.11±0.4*
T_3 (nmol/L)	control	4.7±0.3	4.2±0.0	4.8±0.4	5.5±0.2	5.2±0.2	5.4±0.0	4.7±0.0
	test	4.9±0.3	4.2±0.1	4.7±0.3	3.6±0.7	5.3±0.1	5.1±0.1	4.5±0.2
T_4 (nmol/L)	control	114.3±6.3	116.1±3.5	114.6±6.1	91.0±7.6	99.1±6.2	76.9±5.8	131.5±1.0
	test	95.2±11.1	107.7±3.1	105.2±5.4	115.0±12.5	107.7±13	105.8±2.6	98.7±0.8
fT_3 (pmol/L)	control	8.7±0.4	9.8±0.1	9.6±0.3	9.3±0.6	10.6±0.8	8.1±0.6	9.6±0.1
	test	9.4±0.4	9.6±0.0	9.2±0.2	9.3±0.2	9.9±0.9	9.1±0.0	9.2±0.1
fT_4 (pmol/L)	control	22.6±0.5	24.3±0.9	23.1±1.7	17.4±1.2	19.9±1.9	18.2±1.7	20.6±0.3
	test	21.1±0.4	21.2±0.5	22.5±0.6	22.9±0.2*	18.5±0.5	19.1±0.2	21.2±0.2
Insulin (pmol/L)	control	75.8±2.5	76.8±1.9	78.0±5.0	73.6±11.9	70.8±5.6	61.1±6.7	80.0±8.5
	test	63.1±4.4	63.2±3.5	54.7±4.9	46.6±6.6	48.0±3.2	29.0±5.2	33.6±4.0
Glucose (mmol/L)	control	4.74±0.4	4.43±0.1	4.51±0.1	4.56±0.1	4.80±0.1	4.51±0.0	4.12±0.1
	test	6.41±0.3*	4.25±0.2	4.63±0.3	6.72±0.0*	4.64±0.0	5.25±0.0*	4.38±0.2*
Prolactin (μlU/ml)	control	31.5±4.0	28.9±0.3	28.3±0.9	25.0±2.0	27.6±1.1	20.6±1.2	29.6±1.5
	test	39.8±0.6	42.7±1.0*	40.2±3.2*	37.5±4.0*	40.0±1.2*	45.8±2.4*	49.1±1.8*
GH (ng/ml)	control	8.3±0.4	8.3±0.1	8.3±0.2	9.6±1.4	8.0±0.9	7.4±1.3	6.8±0.7
	test	11.5±0.9	13.5±0.5	14.0±1.0	11.4±1.8	12.7±1.6	12.6±1.8	13.0±0.7
Ghrelin (pg/ml)	control	444±71.7	463±22.1	427±42.9	405±30.2	416±25.7	457±95.5	338±18.1
	test	550±65.8	637±24.8	602±52.5	579±51.1	591±10.1	548±67.8	704±7.9
HDL (mmol/L)	control	1.74±0.0	1.43±0.1	1.62±0.1	2.14±0.1	1.77±0.2	1.56±0.1	0.83±0.1
	test	1.32±0.0	1.33±0.1	1.46±0.1	1.13±0.2*	1.29±0.1*	0.98±0.0	0.72±0.0
Cholesterol (mmol/L)	control	2.0±0.2	2.2±0.0	2.4±0.0	2.8±0.0	2.7±0.1	2.2±0.5	1.6±0.0
	test	1.0±0.0	1.1±0.0	1.6±0.2	2.0±0.0	2.2±0.0	1.86±0.2	1.76±0.3
VLDL (mmol/L)	control	0.12±0.0	0.12±0.0	0.11±0.0	0.04±0.0	0.04±0.0	0.02±0.0	0.05±0.0
	test	0.03±0.0*	0.03±0.0*	0.04±0.0*	0.05±0.0	0.06±0.0	0.05±0.0	0.06±0.0
TG (mmol/L)	control	0.61±0.0	0.63±0.0	0.57±0.0	0.22±0.0	0.23±0.0	0.10±0.0	0.29±0.0
	test	0.15±0.0*	0.19±0.0*	0.24±0.0*	0.27±0.0	0.30±0.0	0.28±0.0	0.25±0.0
β-HBA (mmol/L)	control	0.05±0.0	0.06±0.0	0.06±0.0	0.07±0.0	0.06±0.0	0.05±0.0	0.06±0.0
	test	0.07±0.0	0.08±0.0	0.07±0.0	0.07±0.0	0.08±0.0	0.08±0.0	0.06±0.0
NEFA (mmol/L)	control	0.12±0.0	0.15±0.0	0.16±0.0	0.19±0.0	0.15±0.0	0.13±0.0	0.16±0.0
	test	0.15±0.0	0.2±0.0	0.17±0.0	0.22±0.0	0.23±0.0	0.22±0.0	0.14±0.0

*****Indicates significant difference between control and test groups (P=0.05).

T_3 : triiodothyronin,T_4:thyroxin, fT_3 : free triiodothyronin,fT_4: free thyroxin, GH: growth hormone, HDL: high density lipoprotein, VLDL: very low density lipoprotein ,TG: triglyceride, β-HBA: β-hydroxybutyrate, NEFA: non-esterified fatty acids.

The implication of galanin in the regulation of feeding behavior has not been demonstrated in small ruminants. In rodents, this neuropeptide has a stimulating effect on food intake (Edwards et al., 1999; Tempel et al., 1988). The involvement of this hypothalamic neuropeptide has been partially demonstrated in studies that evaluate the effect of undernutrition on the peptide level. Galanin, quantified by optic density, was greater in the mediobasal hypothalamus of undernourished adult ewes (17 months) compared to controls with unlimited access to food supplies. However, the number of galanin-immunostained cells was unchanged (Barker-Gibb and Clarke, 1996).

In adult ewes, long-term under nutrition (167 days) leads to an increased number of galanin cells in the infundibular nucleus and the dorsal hypothalamic area (Chaillou et al., 2003). The same effect is also found in libitum refed adult ewes after a long period of under nutrition or feeding at maintenance rate in the preoptic area, the infundibular nucleus and the dorsal hypothalamic area (Chaillou et al., 2003).

In the present study, significant increase was found in galanin concentration in fasted goats at day 10 of training and the 2nd and 4th day of starvation in comparison with fed goats. However, there were no significant differences in galanin concentration in day 20 between control and test groups (P=0.05). Longer time may be needed to change galanin neuron number and galanin concentration in plasma as galanin seems to be sensitive to long term starvation (167 days) in small ruminant (Chaillou et al., 2003).

In rodents, starvation appears to act, at least in part, by suppressing thyroid releasing hormone (TRH) expression in the paraventricular nucleus (PVN). Thus, as a consequence of starvation, T4 and T3 levels fall, leading to central hypothyroidism. The dominant, and perhaps sufficient, signal to the brain that suppresses TRH expression in the PVN is a starvation-induced drop in the level of the hormone leptin (Flier et al., 2000). A fall in leptin acts through the hypothalamus to increase appetite, decrease energy expenditure, and modify neuroendocrine function in a direction that favors survival. This cytokine like protein hormone is secreted mainly by the adipose tissue and is believed to act through hypothalamic nerve centers in mediation of neuroendocrine responses to energy supply or deprivation (Zhang et al., 1994). Leptin also signals the switch from the fed to the starved state (Considine and Caro, 1997; Friedman and Halaas, 1998; Yoshida et al., 1997).

In rats, leptin decreases galanin expression in the mediobasal hypothalamus and inhibits food intake induced by galanin (Sahu, 1998b). The neurons containing galanin in infundibular nucleus possess leptin receptors (Hakansson et al., 1998). Galanin can regulate eating behavior and hormonal control via leptin (Hakansson et al., 1998; Sahu, 1998a).

The distribution of the leptin receptor (Ob-R) has been described in sheep (Williams et al., 1999), with the highest density found in the hypothalamus. As in rodents (Hakansson et al., 1998), numerous neuropeptide containing neurons are potential targets of leptin in the goat hypothalamus. Leptin injection modifies some neuropeptide expression and interacts with their action on feeding behaviour. For example, intracerebroventricular injections of leptin for 5 days decrease the expression of galanin mRNA in male rats (Sahu, 1998a). All data suggest that the variations in plasma levels of leptin that are related to nutrition act on hypothalamic neuropeptides to regulate feeding behaviour and/or pituitary hormonal secretions. No direct interacting effects of leptin and hypothalamic neuropeptides have been described for small ruminant nutritional regulations. Major differences exist between sheep and rodents, even if leptin and its receptor are sensitive to nutrition. However, in adult undernourished ewes, modifications in the number of galanin neurons have been observed, with no effect on leptin plasma level (Chaillou et al., 2002a; 2002b; 2003). In goat, the role of leptin as a key regulator in the nutritional modulation of hypothalamic neuropeptides remains to be established.

Galanin may be inhibitory to TRH neurons and contribute to the down regulation of the thyroid axis during fasting. Galanin-containing axons establish a prominent association with TRH neurons in the periventricular and medial parvocellular subdivisions of the PVN (Wittmann et al., 2004).

In the present study, significant decrease was observed in leptin concentration between fed and fasted goats at day 10 of training and the 2nd, 4th, 14th and 20th day of starvation. Also, significant difference in fT_4 concentration between fed and fasted goats was found at 10th day of starvation (P=0.05).

There was no significant difference in leptin concentration between fed and fasted goats at day 10 of training and the 20th day of starvation (P=0.05) indicating that long term starvation had no effect on leptin concentration in test group.

There was no significant difference in fT_4 concentration between fed and fasted goats at the 2nd and 10th day of starvation (P=0.05) suggesting that long term starvation had no effect on thyroid hormones concentration in test group. This finding is in agreement with the report of Khazali (2008) in which no change was observed in mean plasma concentrations of the T3 and T4 in camels fed twenty five percent energy content in diet for thirty days (P=0.01).

Galanin is a potent inhibitor of the release of a number of neurotransmitters and hormones including insulin. This may be the consequence of the hyperpolarization brought about by opening of galanin-receptor-coupled K^+-channels, or a result of the galanin-receptor-mediated closure of some Ca^{2+} channels- or of a combination of galanin effects on K^{+}-channel opening and Ca^{2+}-channel closure (De Weille et al., 1988; Nilsson et al., 1989). Interactions between insulin and hypothalamic neuropeptides have been suggested in several studies. In rats, intracerebroventricular injection of insulin leads to galanin reduction (Wang and Leibowitz, 1997). As demonstrated, no direct effect of insulin on neuropeptide expression in goats has been described.

No significant difference was found in insulin concentration between fed and fasted goats (P=0.05). However, significant difference in glucose concentration between fed and fasted goats was found at day 10 of training and the 10^{th}, 14^{th} and 20^{th} day of starvation (P=0.05). There was significant increase in glucose concentration in fasted goats at the 20^{th} day of starvation compared to fed goats (P=0.05). This finding is in agreement with the report of Khazali (2008) in which mean plasma concentration of glucose was significantly (P=0.01) lower in camels fed twenty five percent energy content in diet for thirty days (Khazali, 2008) in comparison with controls.

Lack of changes in the concentration of insulin may be due to unchanged concentration of galanin or leptin.

Ghrelin, in an antagonistic manner to leptin, regulates synthesis and secretion of several neuropeptides in the hypothalamus that regulate feeding and energy balance. The secretion of ghrelin increases under conditions of negative energy balance, such as starvation, whereas its expression decreases under conditions of positive energy balance such as feeding, hyperglycemia, and obesity. In addition to having a powerful effect on the secretion of growth hormone, ghrelin stimulates food intake and transduces signals to hypothalamic regulatory nuclei that control energy homeostasis (Hosoda et al., 2006).

Little is known about the role of ghrelin in the regulation of food intake and endocrine function in species other than humans and rodents. The ruminant presents an interesting model because the gut is not emptied between periods of feeding. Despite this, there is a well-discerned rise in plasma ghrelin levels before an expected meal in sheep, with a postprandial rise in plasma GH levels (Sugino et al., 2002a; 2004). In rams, plasma ghrelin levels increase just before feeding, tend to increase in pseudo-fed animals and decrease during feeding (Sugino et al., 2002a). These secretion profiles depend on the frequency of meal distribution (Sugino et al., 2002b). No significant difference was observed in the concentration of ghrelin between control and test groups (P=0.05).

Glucose is an important regulator of GH secretion, although GH responses to hypo- or hyperglycemia differ among animal species. In the present study, no significant difference was observed in the concentration of growth hormone between control and test groups (P=0.05). The nutritional status also markedly affects plasma insulin like growth factor I concentration. Starvation causes complete resistance to GH, and restriction of protein or calories causes a lesser degree of resistance with a consequent reduction of hepatic IGF-I production (Isley et al., 1983). Lack of changes in the concentration of growth hormone may be due to unchanged concentration of ghrelin.

Several lines of evidence indicate that galanin regulates prolactin secretion in an auto- and/or paracrine manner. These data further support the hypothesis that galanin acts as a paracrine regulator of prolactin expression and as a growth factor to the lactotrophs (Wynick et al., 1998). Galanin-like immunoreactivities have been found in the anterior lobe of rat and human pituitary glands (Hulting et al., 1989). Specifically, galanin mRNA and peptide have been detected in lactotrophs (Hyde et al., 1991) and galanin is extremely sensitive to the estrogen status of the animal (Hammond et al., 1997; Vrontakis et al., 1989). In the present study, significant increase in prolactin concentration between fed and fasted goats was found at all days of starvation (P=0.05). As estrogen concentration was not measured in this study and no information was available on estrogen status of animals, the accurate interpretation of prolactin is complicated.

The hydrolysis of TG causes the release of FA which are known to alter neuronal activity and gene expression in the brain (DeWille and Farmer, 1993; Oomura et al., 1975). Also, in studies of galanin in the PVN, mRNA levels of this peptide are found to be positively correlated with levels of circulating TG as well as the ingestion, specifically of fat, and are suppressed by an antagonist of fat metabolism (Akabayashi et al., 1994; Leibowitz, 2000; Wortley et al., 2003). The consumption of a high fat diet, which raises TG and NEFA levels, stimulates the expression of galanin in the PVN (Leibowitz et al., 2004; Wortley et al., 2003). Injection of GAL peptide directly into the PVN of rats caused a significant upregulation of lipoproteinlipase (LPL) expression in adipose tissue (Leibowitz et al., 2004; Yun et al., 2005). A significant decrease in TG and VLDL concentrations in test group was found at day 10 of training and the 2^{nd} and 4^{th} day of starvation in comparison with control group (P=0.05). However, a significant increase was found in galanin concentration between fed and fasted goats at day 10 of training and the 2^{nd} and 4^{th} day of starvation, indicating that activation of lipoprotein lipase by galanin causes decrease in TG concentration. No significant difference was observed in the concentration of NEFA between control and test groups (P=0.05).

Significant difference in HDL concentration between fed and fasted goats was found at 10th and 12th day of starvation (P=0.05). No significant difference was observed in the concentration of cholesterol between control and test groups (P=0.05).

In conclusion, this paper indicates that gradual starvation provides time for animals to adapt their metabolism to the new condition. The effect of long term starvation on hormones and biochemical parameters involved in energy metabolism in adult goats and control of energy balance and the role of galanin in adaptation to long starvation or endocrine functions in goat are different from other species. Further investigations are needed to support these hypotheses.

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