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RESEARCH OPINIONS IN ANIMAL & VETERINARY SCIENCES

The effect of short 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

The present study was conducted to assess the effect of short term starvation on galanin, leptin, thyroid hormones, insulin, prolactin, growth hormone, ghrelin and factors involved in energy metabolism including high-density lipoprotein (HDL), cholesterol, β-hydroxybutyrate, glucose, non-esterified fatty acids (NEFA), triglyceride (TG) and very-low-density lipoprotein (VLDL) concentrations in adult goats. Eight non-lactating non-pregnant goats aged 4-5 years and body condition score (BCS) 3 were randomly assigned to control and test groups. The animals were trained to eat their daily forage ration during a period of 10 days. The experimental procedure was applied for 3 days during which control group received 120% of maintenance energy, while the test group was supplied with 60% of maintenance energy. Blood samples were collected on days 8 and 10 and at 2, 12, 24, 36 and 72 hours after beginning of starvation. Blood parameters were measured according to standard procedures. No significant differences were observed in the concentrations of cholesterol, fT_3, fT_4, glucose, growth hormone, leptin, VLDL-cholesterol, non-esterified fatty acids (NEFA), triglyceride (TG) and prolactin between control and test groups. A significant difference was found in β-hydroxybutyrate concentration between fed and fasted goats at 36th hour of starvation. Moreover, there was a significant difference in the concentration of HDL-cholesterol between control and test groups at 72nd hour ($P<0.05$). Gradual starvation had no effect on hormonal and neuropeptides concentrations probably.

Keywords: Short term starvation; hormones; biochemical parameters; energy metabolism; goat

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Introduction

Several studies have reported general physiological changes associated with food deprivation in farm and laboratory animals (Gonzalez et al., 1998; Baranowska et al., 2001). These changes have been observed in thyroid, gonadal and adrenal axis and neuropeptides concentrations such as galanin and leptin (Leibowitz et al., 2004; Yun et al., 2005; Khazali, 2008). When blood glucose concentration begins to decrease during starvation, hepatocytes use blood fatty acids as fuel instead of blood glucose. Furthermore, they use the energy derived from the catabolism ("burning") of fatty acids fuel to synthesize glucose from blood amino acid carbon skeletons. Such glucose is then exported back to the blood, thus for compensating the initial diet-imposed is observed a decrease in blood glucose concentration. The process of generating blood glucose from the liver is known as gluconeogenesis and serves to maintain a steady supply of blood glucose fuel for

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the brain. The liver therefore acts as a "thermostat" for the maintenance of a steady concentration of blood glucose. A consequence of switching from using glucose to using fatty acids as fuel is that hepatocytes release high concentrations of the ketone bodies into the bloodstream such as 3-hydroxybutyrate, a partial breakdown product of fatty acids (Berg et al., 2002).

In ruminants such as goat, the role of galanin in the regulation of food intake or endocrine functions is not well studied. Mean plasma concentrations of metabolic hormones such as thyroxin (T4), triiodothyronine (T3), growth hormone (GH), insulin, glucose and fatty acid in camels fed different energy content in diet were reported by Khazali (2008). Chailou et al. (2003) determined the sensitivity of galanin containing neurons to short term starvation in an immunohistochemical study in 24-hour fasted ewes. The effect of starvation on the relationship between neuropeptides (galanin and leptin) and hormones such as gonadal hormones and prolactin in 72-hour starved female rats was evaluated by Baranowska et al. (2001).

To demonstrate the role of galanin in nutrition and starvation, 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 short 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, non-esterified fatty acids (NEFA), triglyceride (TG) and VLDL-cholesterol concentrations in adult goats.

Materials and Methods

Animals

Eight non-lactating non-pregnant goats aged 4-5 years and BCS 3 were selected for this study and divided into treatment (n=4) and control (n=4) groups with animals being kept in individual pens. The animals were trained to eat their daily forage ration during a 10 day period.

Test procedure

The experimental procedure was applied for 3 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 60% of maintenance energy (200 g hay, 300 g straw, 15 g soybean oil, 15 g vitamins and mineral supplement and 5 g salt per animal per day).

Blood samples

Blood samples were collected on days 8 and 10 at 2, 12, 24, 36 and 72 hours after beginning of starvation. Blood samples were collected from the jugular vein in sterile test 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 assay.

Serum biochemical analysis

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 fT4 was carried out by microplate enzyme immunoassay method (Monobind Inc, Lake Forest, USA). 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 TG 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).

Statistical analysis

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-cholesterol in control and test groups at different times.

No significant differences were observed in the concentrations of cholesterol, fT_3, fT_4, glucose, growth hormone, leptin, VLDL-cholesterol, NEFA, TG and prolactin between control and test groups ($P>0.05$).

Table 1: Mean ± SE of hormones, neuropeptides and factors involved in energy metabolism in fed and fasted goats

Variables		Days of training		Hours after food deprivation				
		8	10	2	12	24	36	72
Galanin	Control	160±10.9	277±52.6	238±54.5	172±14.1	216±51.7	302±26.09	271±8.94
(ng/l)	Test	125±1.32	195±38.59	207±46.16	143±8.29	176±40.59	140±5.26	129±0.96
Leptin	control	4.48±0.42	5±0.32	5.1±0.50	4.86±0.40	4.93±0.43	5.65±0.32	4.53±0.34
ng/ml	test	4.32±0.2	5.69±0.5	4.56±0.19	5.07±0.51	4.95±0.57	6.74±0.58	5.31±1.06
T_3	control	4.26±0.50	2.97±0.00	3.10±0.20	3.25±0.20	3.2±0.20	3.3±0.00	3.00±0.00
nmol/l	test	4.5±0.4	3.1±0.1	3.8±0.3	3.7±0.4	3.8±0.4	3.5±0.00	3.3±0.00
T_4	control	79.9±11.8	68.32±5.39	63.8±5.7	70.5±6.8	69.6±6.6	64.6±0.83	63.5±0.14
nmol/l	test	56.7±4.22	63.65±1.46	63.51±5.7	63.54±5.76	63.0±5.02	66.79±4.2	72.23±6.1
fT_3	control	8.6±0.50	7.38±0.80	7.3±0.9	8.4±0.60	8.3±0.80	6.3±0.30	5.7±0.20
pmol/l	test	10.0±0.5	4.5±0.20	7.2±1.1	7.6±1.0	7.8±1.0	4.9±0.3	5.1±0.5
fT_4	control	18.4±1.4	14.29±0.80	16.00±1.6	16.6±1.7	16.4±2.0	12.0±0.1	12.1±0.1
pmol/l	test	21±0.4	11.2±1.0	17.5±1.6	16.3±2.7	17.6±2.4	11.9±0.0	11.8±0.1
Insulin	control	68.97±2.10	70.7±7.7	65.3±6.66	73.9±3.81	69.0±5.57	77.4±4.86	75.6±5.66
pmol/l	test	62.88±13.47	55.74±8.41	62.03±14.67	67.68±9.85	65.1±12.92	59.83±7.35	45.08±12.57
Glucose	control	2.34±0.09	4.91±0.31	3.34±0.38	2.89±0.28	2.69±0.14	4.52±0.15	4.50±0.11
mmol/l	test	1.97±0.13	4.21±0.16	3.12±0.64	2.2±0.27	1.95±0.08	3.01±0.20	3.50±0.24
Prolactin	control	38.42±1.51	30.4±2.18	34.3±4.39	36.72±3.71	37.52±3.73	42.22±2.83	31.77±3.32
µIU/ml	test	35.5±2.23	31.07±4.08	41.3±1.52	36.1±3.28	40.4±2.78	33.4±2.7	26.9±2.7
GH	control	5.33±0.61	6.38±1.24	5.15±0.83	6.38±0.57	6.09±0.91	8.3±0.56	7.0±0.75
ng/ml	test	5.15±1.09	6.05±0.56	6.06±0.67	6.39±0.5	6.59±0.55	10.38±0.88	8.6±1.21
Ghrelin	control	390±5.23	383±18.35	384±16.64	393±12.27	409±24.55	361±10.85	353±14.50
pg/ml	test	434±35.8	407±29.01	395±31.16	418±31.02	423±27.94	473±18.8	479±29.41
HDL	control	1.5±0.06	1.8±0.006	1.4±0.07	1.5±0.12	1.4±0.1	0.9±0.02	0.7±0.04
mmol/l	test	1.5±0.01	2.2±0.12	1.3±0.06	1.7±0.23	1.5±0.15	1.2±0.12	1.9±0.09 *
Cholesterol	control	1.99±0.06	2.08±0.12	1.89±0.03	2.00±0.10	2.00±0.12	2.38±0.11	2.39±0.15
mmol/l	test	2.16±0.00	2.91±0.18	2.81±0.36	2.58±0.21	2.43±0.11	3.29±0.03	3.12±0.01
VLDL	control	0.01±0.0024	0.016±0.0012	0.013±0.0017	0.0134±0.0014	0.0147±0.0015	0.10±0.0041	0.1±0.0132
mmol/l	test	0.018±0.0042	0.019±0.0036	0.020±0.004	0.013±0.0015	0.016±0.002	0.0838±0.0026	0.092±0.0072
TG	control	0.07±0.01	0.08±0.006	0.07±0.008	0.07±0.007	0.07±0.007	0.5±0.02	0.5±0.06
mmol/l	test	0.09±0.21	0.09±0.018	0.1±0.02	0.06±0.007	0.08±0.01	0.41±0.01	0.46±0.036
β-HBA	control	0.49±0.01	1.06±0.28	0.5±0.01	0.8±0.14	0.63±0.10	2.57±0.25	1.94±0.35
mmol/l	test	0.86±0.09	0.77±0.02	0.81±0.05	0.80±0.05	0.82±0.06	1.5±0.05 *	1.57±0.05
NEFA	control	0.11±0.00	0.26±0.07	0.11±0.00	0.18±0.04	0.13±0.02	0.51±0.02	0.42±0.05
mmol/l	test	0.19±0.03	0.155±0.00	0.157±0.00	0.157±0.01	0.167±0.01	0.385±0.01	0.385±0.01

***** Indicates significant differences between control and test groups (P<0.05)

There were significant differences in β-hydroxy-butyrate and HDL-cholesterol concentrations between control and test groups (P<0.05).

A significant difference in β-hydroxybutyrate concentration was found in goats at 36th hours of starvation. Moreover, significant difference (P<0.05) in the concentration of HDL-cholesterol between control and test groups was found at 72nd hours of starvation.

Discussion

In the present study, no significant difference was found in galanin concentration between fed and fasted goats. This finding was in agreement with the researches indicating that in sheep, as in rodents, the galanin does not seem to be sensitive to short term food deprivation (Beck et al., 1993; Wang et al., 1998). Chailou et al. (2003) reported no variation in the number of galanin neurons located in dorsal hypothalamic area and infundibular nucleus in the 24 hour food deprived ewes.

In lab 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 way that favours survival. This cytokine like protein hormone is secreted mainly by the adipose tissue and is believed to act through hypothalamic nerve centres 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; Yoshida et al., 1997; Friedman and Halaas, 1998).

In rats, leptin decreases galanin expression in the mediobasal hypothalamus and inhibits food intake induced by galanin (Sahu, 1998a). The neurons

containing galanin in infundibular nucleus possess leptin receptors (Hakansson et al., 1998). Galanin can regulate eating behaviour and hormonal control via leptin (Hakansson et al., 1998; Sahu, 1998b). Galanin is a potent inhibitor of the release of a number of neurotransmitters and hormones, including insulin, and stimulates others like growth hormone.

In the present study, no significant difference was found in the levels of leptin and thyroid hormones between fed and fasted goats. Gradual starvation provided enough time for adaptation resulting in unchanged concentrations of the mentioned hormones. Gene expression of hormones and neuropeptides may not be affected by 72-hour starvation (Baranowska et al., 2001).

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

Lack of changes in the concentrations of thyroid hormones may be due to unchanged concentration of galanin, since leptin applies changes on thyroid via galanin.

The peptide galanin (GAL) is known to stimulate eating behaviour, 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 (Lopez et al., 1993; Wang and Leibowitz, 1997; Wang et al., 1998; Crawley, 1999).

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 it could be the 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).

No significant difference was found in glucose or insulin concentrations between fed and fasted goats since glucose and insulin concentration in ruminants does not change significantly.

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 lactotroph (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 are extremely sensitive to the estrogen status of the animal (Vrontakis et al., 1989; Hammond et al., 1997). In the present study, no significant difference was found in prolactin concentration between fed and fasted goats. This result may come from the unchanged concentration of galanin. 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.

Leptin stimulates prolactin secretion from cells isolated from the anterior lobe of the pituitary gland (Yu et al., 1997). Given intracerebroventricularly, leptin stimulates prolactin (and LH) secretion in fasted adult male rats (Gonzalez et al., 1998). The concentration of leptin did not change significantly, resulting in unchanged concentration of prolactin.

In vitro, galanin stimulates growth hormone secretion from perfused pituitary and median eminence of calves (Baratta et al., 1997). In vivo, intracerebroventricular administration of galanin also stimulates GH secretion in sheep (Spencer et al., 1994). As mentioned before, no significant difference was found in galanin concentration between fed and fasted goats and as a result, no significant change occurred in GH concentration.

Glucose is an important regulator of GH secretion, although GH responses to hypo- or hyperglycemia differ among animal species. 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). No significant difference was observed in glucose concentration between fed and fasted goats. This finding may affect the concentration of growth hormone.

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).

The hydrolysis of TG causes the release of NEFA which are known to alter neuronal activity and gene expression in the brain (Oomura et al., 1975; DeWille and Farmer, 1993). Also, in the studies on galanin in the paraventricular nucleus, 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 paraventricular nucleus (Wortley et al., 2003;

Leibowitz et al., 2004). Injection of GAL peptide directly into the paraventricular nucleus of rats caused a significant upregulation of lipoprotein lipase (LPL) expression in adipose tissue (Leibowitz et al., 2004; Yun et al., 2005). Lack of changes in TG and NEFA concentrations may be due to unchanged concentration of galanin.

Peptide-containing neuron systems involved in the regulation of food intake and energy balance in sheep are generally similar to those observed in other species. However, specific differences exist according to the physiological characteristics of the animal model.

In most researches, complete starvation was applied during 24 or 72 hours. However, in the present study 40% starvation was applied which may influence the results. Also, the fact that in ruminants the rumen is not empty between meals may affect the results.

Conclusion

It was concluded that gradual starvation had no effect on hormonal and neuropeptide concentrations in adult goats. Gradual starvation provides time for animals to adapt their metabolism to the new condition.

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