

THE CHANGE OF GHRELIN LEVELS IN INTESTINAL PARASITIC INFECTIONS PROMENE NIVOVA GRELINA KOD CREVNIH PARAZITSKIH INFEKCIJA

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Summary: The aim of this work was to examine the relationship between active (acylated ghrelin) and inactive (desacylated ghrelin) ghrelin in the serum and other serum parameters in intestinal parasitic infections and healthy controls. Conventional microscopic methods (saline and iodine solutions, trichrome stain) were used to identify intestinal parasites in stool samples of 29 subjects attending Firat University Hospital. Serum parameters were assessed in a single measurement of serum from 29 parasite subjects, and in 18 healthy controls. Serum acylated ghrelin and desacylated ghrelin levels were measured using a commercial radioimmunoassay (RIA) kit. Paraoxonase and arylesterase were measured by using a spectrophotometer at 405 nm and 270 nm, respectively. Serum concentrations of acylated ghrelin and desacylated ghrelin were more markedly decreased in helminth bearing patients than the control group. Glucose, cholesterol and triglyceride levels were higher in intestinal parasitic infections than in controls. Furthermore, there were no correlations between ghrelin levels and BMI. These results indicate that low ghrelin and PON1/AE level may be important for appetite monitoring in intestinal parasitic infections.

Keywords: serum, active/inactive ghrelin, paraoxonase, arylesterase, parasite

Introduction

Ghrelin (Ghrelin Appetite Hormone), a 28-amino acid peptide, was discovered in the stomach as an

Kratik sadržaj: Cilj studije bio je da se ispita odnos između aktivnog (aciliranog) i neaktivnog (deaciliranog) grelina u serumu i drugih serumskih parametara kod crevnih parazitskih infekcija i zdravih kontrola. Uobičajene mikroskopske metode (slani rastvor i rastvor joda, trihromatski otisak) korišćene su za identifikaciju crevnih parazita u uzorcima stolice 29 pacijenata Univerzitetske bolnice Firat. Serumski parametri određeni su u jednokratnom merenju seruma 29 pacijenata s parazitima i 18 zdravih kontrolnih subjekata. Nivoi aciliranog grelina i deaciliranog grelina u serumu izmereni su metodom radioimunoeseja. Paraoksonaza i arilesteraza su merene spektrofotometrijski na 405 nm i 270 nm. Koncentracije aciliranog i deaciliranog grelina u serumu bile su upadljivo niže kod pacijenata sa parazitima nego u kontrolnoj grupi. Nivoi glukoze, holesterola i triglicerida bili su viši kod crevnih parazitskih infekcija nego kod kontrola. Štaviše, nije bilo korelacija između nivoa grelina i indeksa telesne mase. Rezultati ukazuju na to da bi niski nivoi grelina i PON1/AE mogli biti važni za praćenje apetita kod crevnih parazitskih infekcija.

Ključne reči: serum, aktivni/neaktivni grelin, paraoksonaza, arilesteraza, paraziti

endogenous ligand for the growth hormone secretagogue receptor (GHS-R) (1). Two major molecular forms of ghrelin are found in tissues and blood: desacylated and acylated ghrelin (2). Other minor forms of ghrelin are also present in the stomach and plasma (3). Ghrelin is predominantly synthesized in

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List of Abbreviations: RIA – radioimmunoassay; BMI – body mass index; PON – paraoxonase; AE – arylesterase; GHS-R – growth hormone secretagogue receptor; VHDL – very high-density lipoprotein; HDL – high-density lipoprotein; LDL – low-density lipoprotein; WHO – World Health Organization; BH – body height; BW – body weight.

the stomach, but it is also expressed in many other organs including bowel, pancreas, kidney, myocardium, hypothalamus and pituitary gland. This hormone is transported in the circulation bound to very high-density lipoprotein (VHDL) and high-density lipoprotein (HDL). Acylated ghrelin affects glucose metabolism by modulating insulin secretion, amino acid uptake and bone formation, appetite, increases food intake, energy balance, gastrointestinal motility, cardiac performance, and anxiety (4–8). Plasma desacylated ghrelin is more than 90% of total circulating ghrelin and can cross the blood brain barrier (2, 9).

Paraoxonase-1 (PON1), a glycoprotein with 354 amino acids, can hydrolyze paraoxon. It can also hydrolyze organophosphate compounds like pesticides, neurotoxins, and arylesters, but it has been shown to have its antioxidant property (10). Humans and mice have three different PON genes (PON1, PON2, and PON3). PON2 prevents LDL oxidation, thereby inhibiting monocyte chemotaxis induced by oxidized LDL (11). PON1 and PON3 enzymes are associated with HDL and can prevent low-density lipoprotein (LDL) oxidation. PON2 and PON3 cannot hydrolyze paraoxon as they have no lysine residue at position 105 (12). Although paraoxonase and arylesterase [AE E.C.3.1.1.2] are considered two different enzymes, previous studies have shown that a single gene product in human serum has both ARE and PON activities (12).

Intestinal parasites remain a major health problem in many developing countries. World Health Organization (WHO) estimates that around 2 billion people are currently infected with schistosomes and soil-transmitted helminths. Some 300 million of these suffer from severe and permanent impairments as a result. Intestinal parasites cause anaemia, poor physical growth, poor intellectual development and impaired cognitive function (13). Because parasite infection of the gastrointestinal tract induces detrimental effects on host tissues and host physiology different processes which tend to attenuate the effect of the loss of appetite, the intestinal malabsorption or the increased tissue losses have been assessed.

Although many studies regarding intestinal parasites focused on the loss of appetite, an appetite hormone, ghrelin, and ghrelin linked PON1 and AE levels in intestinal parasitic infections have not been examined to date. The present study was therefore undertaken to investigate: (i) whether the serum levels of desacylated ghrelin and acylated ghrelin are decreased or increased in intestinal parasitic infections; (ii) the relationship between ghrelin and other biochemical parameters such as glucose in the intestinal parasitic infections. The results may provide important information concerning the activity of serum acylated ghrelin and desacylated ghrelin in intestinal parasitic infections and its association with other biochemical parameters.

Materials and Methods

Subjects

The study was approved by the Bio-ethical Committee of Firat University, and written informed consent was obtained from all patients before enrollment. The group of patients included in this study consisted of 29 parasite bearing patients, who were referred by the Department of Parasitology of the Firat University Hospital, Elazig Turkey. Conventional microscopic methods (saline and iodine solutions, trichrome stain) were used to identify parasites in the stool samples of 29 subjects attending Firat University Hospital. The control group consisted of 18 clinically healthy humans (9 female and 9 male). Exclusion criteria for the control group were: pregnancy, alcohol consumption, tobacco products (former and current), body mass index, BMI >25 kg/m², chronic medical illness, history of drug treatment or therapy within the previous months. In all the participants, body height (BH) and body weight (BW) were measured to determine the body mass index (BMI). BMI=BW (kg)/BH (m)².

Serum collection and conservation

Intravenous blood samples were collected in plain biochemistry tubes from all patients and control subjects. The tubes were centrifuged for 10 min at 3500 rpm to separate the serum, which was treated with 1/10 vol 1 mol/L HCl and then all samples were stored at -20 °C pending measurements of ghrelin. Paraoxonase and arylesterase levels, and other biochemical parameters were analyzed in serum samples. We examined serum acylated ghrelin and desacylated ghrelin levels roughly before breakfast was eaten (14).

Ghrelin Hormone Assay

Total ghrelin (Linco Research, Missouri, USA) and acylated ghrelin (Linco Research, Missouri, USA) were measured using the human-ghrelin-RIA kit. All samples were read by radioimmunoassay (RIA) with a LKB-Wallac gamma counter (MultiGamma 1261, Turku, Finland). Ghrelin concentrations were calculated from standard curves generated in the same assay with ghrelin. The level of desacylated ghrelin was calculated by subtracting the acylated from the total ghrelin.

Assay of paraoxonase activity

Paraoxonase activity (PON1) was measured by the method of Eckerson et al. (15). This method uses the increase in absorbance at 412 nm at 25 °C. The amount of p-nitrophenol released was determined from the molar absorptivity (extinction) coefficient at pH 8 (16700 M⁻¹ cm⁻¹). The paraoxonase unit was

defined as the enzyme quantity that disintegrates 1 μmol paraoxon substrate in one minute (16, 17). One unit of paraoxonase activity is defined as 1 μmol p-nitrophenol/mL serum/min (15).

Assay of arylesterase activity

Arylesterase (AE) activity was measured by the method of Haagen and Brock (16) with phenylacetate as substrate. The absorbance was continuously monitored at 270 coefficient of phenol ($1310 \text{ M}^{-1} \text{ cm}^{-1}$). AE activity was expressed as U/L and a unit of arylesterase activity is defined as 1 μmol phenol/mL serum/ min (15).

Other biochemical parameters

Biochemical parameters were determined using an automatic analyzer (Olympus Optical Co., Japan) in the Biochemistry Laboratory of Firat University Hospital, Elazig.

Statistical analysis

Statistical analyses were done using an SPSS 12 statistical package. The data are expressed as arithmetic means \pm standard deviation (S.D.). The Mann-Whitney U test is a non-parametric statistical significance test applied for group comparisons. *p* values smaller than 0.05 were accepted as significant.

Results

Parasites percent (%) distribution in study groups is shown in Table I. There were no significant inter-group differences in the duration of parasite bearing among the subjects. BMI did not differ among the

groups (Table II). The acylated ghrelin and desacylated ghrelin serum levels were not found to be correlated with body mass index (BMI) in parasite subjects and control groups.

Serum acylated ghrelin and desacylated ghrelin levels and PON1/AE levels were lower than control in patients with parasites (Table II). This means parasites subjects are associated with a decrease in acylated ghrelin and desacylated ghrelin levels and PON1/AE levels. When serum acylated ghrelin levels were compared with serum desacylated ghrelin concentrations on an individual basis, a large variance was observed, with values ranging from 11 to 56 pg/mL for acylated ghrelin, and from 151 to 434 pg/mL for desacylated ghrelin.

Serum LDL-C levels were higher in parasite bearing subjects than in controls and serum glucose, cholesterol and triglyceride levels in parasite subjects were also higher than those of controls. Serum HDL-C levels were lower in parasite bearing subjects than in controls (Table II).

Discussion

Although ghrelin is expressed in almost all tissues, its expression is highest in the stomach, where its secretion from P/D1=ghrelin cells is up-regulated during fasting and hypoglycemia (1, 18). The role of ghrelin in regulating the long-term energy balance in intestinal parasitic infections has not been investigated. In the present study, we observed a lower concentration of ghrelin in intestinal parasitic infections.

Although the precise reason for the decreasing ghrelin concentration is not known, we believe that increased glucose concentration is one of the possible reasons. Ghrelin and glucose concentrations are inversely proportional. The most significant data

Table I Parasites percent (%) distribution in study groups.

Parasite	Sex					
	Women		Men		Total	
	n	%	n	%	n	%
<i>Giardia intestinalis</i>	3	30	10	52.6	13	44.8
<i>Blastocystis hominis</i>	2	20	0	0	2	6.9
<i>Entamoeba histolytica</i>	2	20	3	15.7	5	17.2
<i>Entamoeba coli</i>	0	0	2	10.5	2	6.9
<i>Enterobius vermicularis</i>	1	10	1	5.3	2	6.9
<i>Taenia saginata</i>	1	10	1	5.3	2	6.9
<i>Blastocystis hominis</i> + <i>Giardia intestinalis</i>	0	0	1	5.3	1	3.4
<i>Enterobius vermicularis</i> + <i>Entamoeba coli</i>	0	0	1	5.3	1	3.4
<i>Hymenolepis nana</i> + <i>Giardia intestinalis</i> + <i>Entamoeba coli</i>	1	10	0	0	1	3.4
Total	10	100	19	100	29	100

Table II Comparison of BMI, serum total, acylated, desacylated ghrelin, PON1, ARE levels, and some other biochemical parameters in intestinal parasitic infections and control groups.

	Parasite (n: 29)	Control (n: 18)	p
BMI (kg/m ²)	21.8 ±4.1	21.5±1.9	p>0.05
Ghrelin (pg/mL)			
Acylated	27.4±11.2	37.0 ±15.6	p<0.05
Desacylated	260.1±69.6	365.5±55.3	p<0.05
Paraoxonase (U/L)	321.4±80.3	432.0±88.4	p<0.05
Arylesterase (U/L)	142.7±59.5	219.3±75.3	p<0.05
LDL (mmol/L)	4.00±1.10	3.18±0.51	p<0.05
HDL (mmol/L)	1.45±0.45	1.55±0.17	p>0.05
Glucose (mmol/L)	7.45±2.14	5.31±0.96	p<0.05
Triglyceride (mmol/L)	2.16±0.90	1.35±0.42	p<0.05
T-Cholesterol (mmol/L)	6.07±1.74	3.36±0.56	p<0.05

Values are expressed as arithmetic means ± standard deviations (SD), p<0.05.

supporting this view comes from studies of diabetic subjects (19). In our study, the glucose values of the control group and parasitic group were compared and were found to be higher in the parasitic group (Table II). Thus we believe that the reason for reduced ghrelin concentrations in patients with intestinal parasitic infections is to compensate for the increased glucose concentration. The literature contains studies on the relationship between insulin and ghrelin. The decreased ghrelin concentration observed in the present study is thought to be the main reason for the loss of appetite in patients with parasitic infections. Similarly, decreased ghrelin values are observed in patients with iron-deficiency anemia. Acylated and desacylated ghrelin are linked with VLDL and HDL in the circulation (20). When the control group and the patients with parasitic infections were compared, it was found that, although not statistically significant, ghrelin was partially decreased. As mentioned above, since ghrelin is transported bound to HDL, the fact that the decrease in ghrelin is associated with a decrease in HDL supports our findings. Lipid peroxidation increases in parasitic infections (21–23). Thus, another reason for decreased ghrelin might be to decrease lipid peroxidation that increased as a result of parasitic infection, due to its antioxidant property. Some previous studies have indicated that ghrelin has an antioxidant property. This may be the reason for reduced endogenous ghrelin.

Paraoxonase-1 and AE have been shown to be functions of single enzymes (24). Both are continuously generated in physiologic conditions and have

been studied extensively in relation to cardiovascular diseases (15, 25–27). In the current study, PON1 and AE activity were found to be significantly lower in intestinal parasitic infections than the control groups. Decreased PON1/AE levels in intestinal parasitic infections may have been a result of damaged cells expressing this protein at a considerably lower rate. Supporting this putative function is the report of inhibited microsomal PON1 activity in rats that were chronically administered CCl₄ (28). Moreover, the decreased lipophilic antioxidant PON1 activity and ghrelin level observed in this study are consistent with an earlier study examining serum antioxidant levels in pre-eclampsia (29), because PON1 is believed to play a central role in the inhibitory effect of HDL on lipid peroxidation (30). Lipid oxidation is also believed to occur in response to increased oxidative stress or deficiency of endogenous antioxidants. As mentioned above, because PON1 is an endogenous antioxidant, it is thought that it decreased to eliminate oxidative stress, which is increased in patients with parasitic infections.

In summary, despite some limitations (for example, inadequate number of subjects and 29 parasite bearing patients who included 4 nonpathogen intestinal parasitic patients), this is the first study to examine how ghrelin, PON1 and arylesterase values change in intestinal parasitic infections. Further research encompassing a wide series of pathogen and nonpathogen intestinal parasitic patients, the underlying physiopathological mechanisms and intestinal symptoms including appetite is warranted.

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