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Strength and independence of associations between ghrelin, leptin, adiponectin and insulin in stimulating basic functions to energy metabolism

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KEYWORDS

Ghrelin;
Leptin;
Insulin;
Adiponectin;
Macronutrient

Abstract *Background:* The effect of diet composition on the fat regulating hormones and its possible interaction with insulin is necessary to fully understand the mechanisms by which the body controls feed intake.
Objective: The objective of this work was to evaluate the relationship between ghrelin, leptin, adiponectin and insulin with respect to diet composition. Fasting and refeeding represent a natural challenge for stimulating basic functions to energy metabolism.
Methods: The present study was conducted on 40 male albino rats that were divided into 4 groups ($n = 10$): (I): fed ad/libitum, (II): fasted group, refed group allowed for 20 min refeeding with food stuffs divided into two groups, (III): carbohydrate meal, (IV): fat meal. The present study suggests a physiological interaction of ghrelin and leptin in the periphery. Elevated fasting ghrelin appears to modulate other hormonal systems that participate in the regulation of food intake. Ghrelin inhibition after feeding is related to different nutrients satiating capacity. In addition to feeding, several hormonal states have also been involved in the suppression of postprandial ghrelin production. Meal and insulin were found to acutely affect leptin concentration. Inverse change in ghrelin and leptin between fasting and refeeding state may indicate a role of gastric leptin in regulating ghrelin secretion. Positive correlation between adiponectin and insulin with both macronutrients suggests a role of adiponectin in enhancing insulin action. The fact that gastric leptin is stimulated by diet and ghrelin responses could be due to direct effects of multiple hormonal signals which require postgastric feed back on the stomach among the latters (glucose and insulin).

Abbreviations: GH, growth hormone; CAMP, cyclic adenosine monophosphate; ACRP30, adiponectin; PkC, protein kinase C; ARC NPY, arcuate nucleus neuropeptide; Y, neurons; AMPK, AMP activated protein kinase.

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1. Introduction

Ghrelin, a 28 amino acid peptide with an essential n-octanoyl modification on the third amino acid, was identified as the endogenous ligand for the growth hormone (GH) secretagogue receptor. It has a pleiotropic role in the modulation of energy balance.[1] It is predominantly produced by the stomach.[2] Initially, ghrelin was known for its potent stimulatory action on GH secretion,[3] but later findings showed that it was also involved in the regulation of feeding behavior and energy homeostasis.[4] Actually, ghrelin is the primary orexigenic signal from the periphery, and it has been reported that peripheral ghrelin exerts its orexigenic effects through circulation[5] or by acting in the periphery to suppress gastric vagal afferent.[6] The most important physiological state for the regulation of ghrelin synthesis is feeding.[7]

Leptin, the product of ob gene is produced by differentiated adipocytes.[8] Several ligand activated transcription factors, regulate energy metabolism such as leptin and adiponectin.[9]

Although adipose tissue is the main source of leptin, this hormone is also produced by the stomach. Leptin acts on the central nervous system suppressing food intake and stimulating energy expenditure.[10] Synthesis of leptin is modulated by several non hormonal and hormonal variables. Stimulators in both rodents and humans are overfeeding, insulin, and glucocorticoids.[11] Suppression has been shown by fasting,[12] CAMP, and B_3 adrenoceptor agonists.[13] Early studies reported no acute effect of eating on leptin concentration, but later studies demonstrated that meals and insulin affect leptin concentration.[14] The pancreatic hormone insulin was one of first adiposity signals to be described[15] and like leptin is positively correlated with long-term energy balances.[16] However unlike leptin levels, which are relatively insensitive to acute food intake, insulin secretion increases rapidly after a meal.[17] There is considerable evidence that insulin acts as an anorectic signal within the central nervous system.[18]

Adiponectin (ACRP 30) is exclusively secreted by differentiated adipocytes.[19] It circulates in serum as three distinct oligomers, trimer, hexamer, and even higher molecular weight species.[20] Adipocyte tissue secretes a large number of physiologically active polypeptides. Although leptin remains one of the best examples of an adipocyte specific secretory factor, recent reports describe potent physiological activities for another adipocyte specific secreted protein adipocyte complement-related protein, of 30 kDa (ACRP 30).[21] Early studies suggested that ACRP 30 might participate in energy homeostasis because its mRNA was decreased in obese mice[22] and humans.[23] In addition, when administered to intact mice with a fat meal, it moderately lowers plasma free fatty acid and glucose levels and when administered chronically, it causes weight loss without diminishing food intake.[24] It is not known whether the nutrients have a direct effect on modulating ghrelin levels or whether the effect is mediated by specific hormones. We aim to study the ghrelin, adiponectin, leptin and their interrelationship with insulin in response to an acute intake of two macronutrients (fats and carbohydrates given individually). In the present work we thought to characterize in detail the dynamics of these fat regulating hormones with insulin after stimulating basic functions to energy metabolism.

2. Methods

2.1. Animal care and handling

40 adult male albino rats (200–250 g) were obtained from stock at the laboratory animal unit, Medical Research Institute, Alexandria University. Animals were housed individually in an air-conditioned environment maintained at 22 °C with a 12 h light 12 h dark cycle. Rats were allowed continuous access to tap water and food. Rat groups: Rats were divided into four groups. Each group contains 10 rats, group (I) fed ad/libitum group, in which animals were provided with standard chow diet, group(II) fasted group, rats were deprived of food for 24 h, animals from the fasted group were allowed for 20 min refeeding with different food stuffs equal to 14 kcal subdivided into two groups: group(III) carbohydrate meal matched to equal amounts of 3.5 g of mixture of wheat starch and sucrose (10% fat, 81% carb. And 18% protein). group (IV): fat meal matched to 2.3 g of row bacon from loin (79% fat, 3% carbohydrate and 18% protein). ($n = 10$). The rats were killed under ether anesthesia, the stomach and retroperitoneal white adipose tissue depot were rapidly removed. The stomach was opened and rinsed with saline containing 0.1% diethylpyrocarbonate (sigma), and the whole epithelium was scraped off using a glass slide obtaining approximately 0.2 g of mucosa. Gastric mucosa and adipose tissue were immediately frozen in liquid nitrogen and stored at −70 °C until hormonal assay. Blood was also collected, stored at room temperature for 1 h and over night at 4 C, and then centrifuged at 1000*g* for 10 min. Serum glucose[25] and triglyceride[26] levels were determined enzymatically using commercial kit. Serum insulin[27] and adiponectin[28] were measured using ElISA kit.

Quantification of serum and gastric ghrelin levels was measured with rat ghrelin enzyme immunosorbent assay kit.[29] Ghrelin peptide in gastric mucosa was extracted by Lee et al. (2002)[30] with slight modifications thus, the samples of mucosa were homogenized in PBS (137 m^M Nacl, 2.7 m^M kcl, and 10 m^M phosphate buffer pH 7.4) in a Teflon/glass homogenizer. The homogenates were centrifuged at 7000*g* for 2 min at 4 °C, and the supernatant was used for ghrelin quantification. The supernatant was mixed with 10 volumes of 1 M acetic acid containing 20 m^M Hcl. Homogenates were boiled for 20 min and centrifuged at 7000*g* for 2 min at 4 °C and then resuspended in PBs. Quantification of gastric and serum leptin levels were measured with ElISA kit.[31] Gastric mucosa was homogenized at 4 °C in 1:3 (wt/vol) of PBs as explained earlier.

3. Statistical analysis

Statistical analysis was done using SPSS program "version 17" (statistical package of social science, Chicago, USA). Quantitative data (e.g. serum and gastric ghrelin, serum and gastric leptin, serum insulin, serum adiponectin, serum glucose and serum triglyceride) were described by median as a measure of central tendency & range as a measure of dispersion, as the sample size was small & hence these variables were abnormally distributed. Accordingly, non parametric tests were used. The box plots were used as a graphical presentation of the median. Kruskal Wallis test was used to compare median values for the parameters among the different studied groups. The significance level was $P \leqslant 0.05$. Mann Whitney test used for pair wise

comparison to detect significance of the parameters in the studied groups after bonferroni correction and setting of ρ – value = 0.012.

Spearman's correlation test was used to study the significance of linear relation between quantitative variables in four studied groups.

Regression equation detected the association between ghrelin, leptin, adiponectin, insulin, glucose and triglyceride in carbohydrate and fat refeeding groups.

4. Results

4.1. Ghrelin, leptin, adiponectin and insulin levels in feeding adlibitum, fasting and refeeding groups

Data are given as median and range (n = 10). A significant difference was found between the four studied groups ($p < 0.05$) (Kruskal Wallis test) (Table 1).

Food intake for 20 min after fasting produced a decrease in ghrelin concentration for the intake of both carbohydrate and fat refed diet. A marked significant decrease in serum ghrelin level in both carbohydrate and fat refed groups was obtained {0.52 (0.26–0.98), 0.85 (0.54–1.7 Pg/ml) respectively $p < 0.001$)} (Fig. 1). Lowering of gastric ghrelin level was significant after carbohydrate refed diet ($p < 0.001$) (Fig. 2). Both fat and ad/libitum groups produced a non significant decrease. As regards, comparison between the ad/libitum group and other studied groups, a significant decrease was observed in serum ghrelin in carbohydrate and fat groups as compared with the ad/libitum group ($p < 0.001$). Also there was a significant difference between carbohydrate and fat groups (Table 2).

Gastric ghrelin level in the ad/libitum group increases significantly as compared with the carbohydrate group ($p < 0.001$) (Table 2). A negative significant correlation was found between serum and gastric ghrelin in the fasting group (Table 4, Fig. 10).

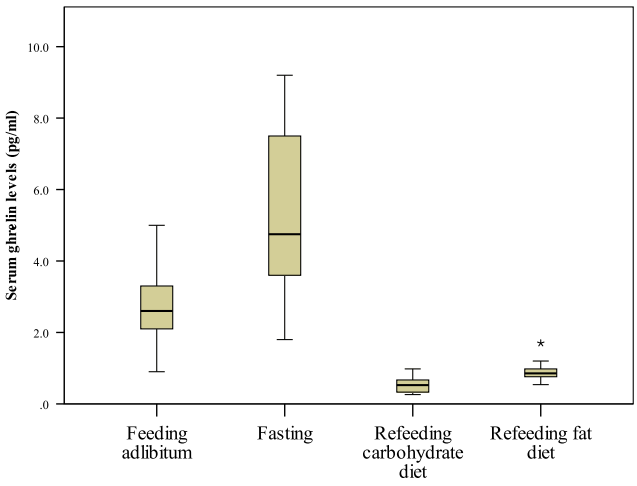

Figure 1 Serum ghrelin levels (pg/ml). • significant difference between gp II and gp III. •• significant difference between gp III and gp IV.

A marked decrease in serum and gastric leptin levels in response to 24 h fasting was obtained 3.1 (2–4.2), 2.3 (1.1–3.1)ng/ml. After refeeding carbohydrate, fat, ad/libitum a marked increase in serum and gastric leptin was obtained. Serum and gastric leptin levels increased significantly in carbohydrate refed diet [7.7 (7–9.1), 8.15 (5.6–9.8)ng/ml respectively, $p < 0.001$]. Fat diet also produced a significant increase in serum and gastric leptin levels [7.1 (6.1–8.2), 6.2 (3.3–8.4)ng/ml respectively, $p < 0.001$]. In addition a significant increase in serum and gastric leptin levels in the ad/libitum group was also obtained [4.9 (3.6–8.7), 5.75 (3.1–8.6)ng/ml, $p < 0.001$)].

As regards comparison of serum leptin in ad/libitum and other groups, a significant increase in serum leptin in the ad/libitum group as compared with the fasting group ($p < 0.001$) was obtained, while a significant decrease was

Table 1 Descriptive data of serum and gastric parameters among the studied groups.

	Feeding ad/libitum group	Fast group	Refeeding (carbohydrate diet) group	Fat diet group	Kruskal Wallis test
Serum ghrelin level (pg/ml) Md(min–max)	2.6 (.9–5)	4.75 (1.8–9.2)	.525 (.26–.98)	.85 (.54–1.7)	X^2 = 31.19 p .001*
Gastric ghrelin level (pg/ml) Md(min–max)	.72 (.41–1.2)	.89 (.53–1.6)	.42 (.23.66)	.68 (.21–.93)	X^2 = 16.78 p .001*
Serum leptin level (ng/ml)Md(min–max)	4.9 (3.6–8.7)	3.1 (2–4.2)	7.7 (7–9.1)	7.1 (6.1–8.2)	X^2 = 30.32 p .001*
Gastric leptin level (ng/ml)Md(min–max)	5.75 (3.1–8.6)	2.3 (1.1–3.1)	8.15 (5.6–9.8)	6.2 (3.3–8.4)	X^2 = 25.63 p .001*
Serum insulin level (ng/ml)Md(min–max)	29.0 (25.8–36.1)	16.7 (14–20.3)	36.25 (30.6–40.2)	34.6 (28.6–38.2)	X^2 = 27.29 p .001*
Serum adiponectin level (ng/ml)Md(min–max)	1415 (890–2600)	910 (800–1040)	2700 (1760–3900)	4800 (3400–6100)	X^2 = 32.34 p .001*
Serum glucose level (mg/ml)Md(min–max)	150.2 (135.6–180.3)	121.4 (98.7–131.1)	185 (162.3–212.3)	121.9 (129.1–131.5)	X^2 = 32.96 p .001*
Serum T.G level (mg/ml)Md(min–max)	99.5 (97–110)	97.3 (89.6–105.1)	108.7 (105–113.1)	240.4 (190.6–280)	X^2 = 35.99 p .001*

* Significant level $p < 0.05$) at Kruskal Wallis test.

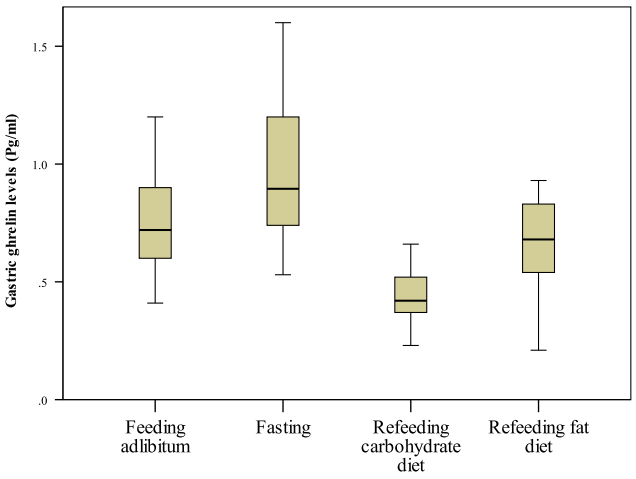

Figure 2 Gastric ghrelin levels (Pg/ml). • significant difference between gp II and gp III. •• significant difference between gp III and gp IV.

detected when compared it with carbohydrate and fat groups (Table 2).

Gastric leptin level also demonstrates a significant increase in the ad/libitum group as compared with the fasting group ($p < 0.001$) (Table 2), (Figs. 3 and 4).

A significant increase in serum insulin levels in carbohydrate, fat and ad/libitum groups as compared with fasting was obtained [36.25 (30.6–40.2), 34.6 (28.6–38.2), 29 (25.8–36.1), 16.7 (14–20.3)ng/ml, respectively $p < 0.001$)].

Serum insulin level was significantly increased in the ad/libitum group as compared with the fasting group ($p < 0.001$), but decreased significantly when compared with the carbohydrate group (Table 2) (Fig. 5).

Refeeding with carbohydrate produced a significant increase in adiponectin levels as compared with fasting [2700 (1760–3900), 910 (800–1040) ng/ml, $p < 0.001$)]. A highly elevated adiponectin level in fat refed group was obtained [4800 (3400–6100)ng/ml, $p < 0.001$)]. However, a non significant increase in the ad/libitum group was detected.

Significantly there was a decrease in the ad/libitum group as compared with carbohydrate and fat groups ($p < 0.001$). Also a significant difference was found between carbohydrate and fat groups ($p < 0.001$) (Table 2) (Fig. 6).

There was a significant increase in serum glucose level in carbohydrate refed diet as compared with the fasting group [185.6 (162.3–212.3), 121.4 (98.7–131.1) mg/ml, $p < 0.001$)]. Fat diet produced a non significant increase, a significant increase in serum glucose level was obtained in the ad/libitum group as compared with fasting and fat groups ($p < 0.001$), while a significant decrease was detected when compared with the carbohydrate group ($p < 0.001$) (Table 2) (Fig. 7).

Refeeding with both carbohydrate and fat diet produced a significant increase in triglyceride levels as compared with the fasting group. [108.7 (105–113.1), 240 (190.6–280), 97.3 (89.6–105.1) mg/ml, respectively $p < 0.001$)]. A non significant increase in the ad/libitum group was obtained.

On the other hand, a significant decrease in triglycerides level in the ad/libitum group as compared with carbohydrate and fat groups ($p < 0.001$) was obtained, a significant difference between carbohydrate and fat groups was also obtained (Table 2) (Fig. 8).

Serum ghrelin could be predicted by determining the serum leptin according to the equation. Additionally, the serum ghrelin is declined by 0.23 for each unit change in serum leptin. Moreover, 51% of the changes in the serum ghrelin could be explained by changes in serum leptin i.e., still 49% of change in serum ghrelin may be attributed to the other factors (Table 3, Fig. 9).

Serum ghrelin could be predicted by determining the serum adiponectin according to the equation. Additionally, the serum ghrelin is declined by 0.0003 for each unit change in serum adiponectin Moreover, 84% of the changes in the serum ghrelin could be explained by changes in serum adiponectin i.e., still 16% of change in serum ghrelin may be attributed to the other factors (Table 3, Fig. 11).

Table 2 Comparisons of serum and gastric parameters between different groups.

Feeding ad/libitum						
	Fast group	Refeeding carbohydrate group	Fat diet group	Fast-refeeding carbohydrate groups	Fast fat diet groups	Refeeding carbohydrate-fat diet groups
Serum ghrelin level (pg/ml)	$U = 21.5$ $p = .031$	$U = 1$ p .001*	$U = 5.5$ p .001*	$U = .000$ p .001*	$U = .000$ p .001*	$U = 12$ p .005*
Gastric ghrelin level (pg/ml)	$U = 30$ $p = .13$	$U = 12$ $p = .004$*	$U = 42$ $p = .545$	$U = 4$ $p = .001$*	$U = 23.5$ $p = .045$	$U = 17$ $p = .013$
Serum leptin level (ng/ml)	$U = 3$ $p = .001$*	$U = 7.5$ $p = .001$*	$U = 13$ $p = .005$*	$U = .000$ p .001*	$U = .000$ p .001*	$U = 16.6$ $p = .011$*
Gastric leptin level (ng/ml)	$U = 1$ p .001*	$U = 19.5$ $p = .021$	$U = 44$ $p = .650$	$U = .000$ p .001*	$U = .000$ p .001*	$U = 21.5$ $p = .031$
Serum insulin level (ng/ml)	$U = .000$ p .001*	$U = 10$ $p = .002$*	$U = 21.5$ $p = .031$	$U = .000$ p .001*	$U = .000$ p .001*	$U = 34$ $p = 22.6$
Serum adiponectin level(ng/ml)	$U = 18.5$ $p = .017$	$U = 9$ $p = .002$*	$U = .000$ p .001*	$U = .000$ p .001*	$U = .000$ p .001*	$U = 4$ p .001*
Serum glucose level (mg/ml)	$U = .000$ p .001*	$U = 6$ $p = .001$*	$U = .000$ p .001*	$U = .000$ p .001*	$U = 26$ $p = 0.069$	$U = .000$ p .001*
Serum T.G level (mg/ml)	$U = 26$ $p = .069$	$U = 16$ $p = .010$*	$U = .000$ p .001*	$U = 1$ p .001*	$U = .000$ p .001*	$U = .000$ p .001*

* Significant level ($p = 0.012$) after Bonferroni correction using Mann Whitney test.

Table 3 Correlation between serum ghrelin, leptin, insulin, adiponectin, triglycerides (TG) and glucose in the different studied groups.

		Leptin		Insulin		Adiponectin		TG		Glucose	
		rs	*p*	rs	*p*	rs	*p*	rs	*p*	rs	*p*
Group I	Serum Ghrelin	0.720*	0.019	−0.681*	0.030	−0.985*	<0.001*	−0.171	0.637	−0.581*	0.002
	Leptin			0.239	0.506	0.706*	0.023	0.215	0.550	0.485	0.156
	Insulin					0.745*	0.013	−0.097	0.789	0.661*	0.038
	Adiponectin							0.097	0.789	0.830*	0.038
	TG									0.280	0.434
Group II	Serum Ghrelin	−0.960*	<0.001	−0.903*	<0.001	−0.878*	0.001	−0.796*	0.006	−0.382	0.276
	Leptin			0.851*	0.002	0.804*	0.005	0.753*	0.012	0.225	0.532
	Insulin					0.811*	0.004	0.663*	0.037	0.236	0.511
	Adiponectin							0.618	0.057	0.445	0.197
	TG									0.280	0.434
Group III	Serum Ghrelin	−0.707*	0.022	−0.736*	0.015	−0.982*	<0.001	−0.573	0.083	−0.746*	0.013
	Leptin			0.772*	0.009	0.689*	0.028	0.774*	0.009	0.630	0.051
	Insulin					0.754*	0.012	0.407	0.243	0.738*	0.015
	Adiponectin							0.482	0.159	0.758*	0.011
	TG									0.367	0.297
Group IV	Serum Ghrelin	−0.356	0.313	−0.912*	<0.001	−0.881*	0.001	−0.933*	<0.001	−0.073	0.841
	Leptin			0.361	0.306	0.214	0.553	0.494	0.147	0.135	0.711
	Insulin					0.770*	0.009	0.869*	0.001	0.103	0.777
	Adiponectin							0.681*	0.030	0.406	0.244
	TG									−0.116	0.751

rs: Spearman's coefficient.
* Statistically significant at $p \leqslant 0.05$.

Table 4 Correlation between gastric ghrelin with both serum ghrelin and gastric leptin in different studied groups.

	Gastric ghrelin							
	Feeding ad/libitum		Fast		Refeeding Carbohydrate diet		Fat diet	
	r_s	*p*	r_s	*p*	r_s	*p*	r_s	*p*
Serum ghrelin	−0.576	0.081	−0.794*	0.006	0.091	0.802	−0.200	0.580
Gastric leptin	0.207	0.567	0.031	0.933	0.336	0.342	−0.116	0.751

r_s: Spearman's coefficient.
* Statistically significant at $p \leqslant 0.05$.

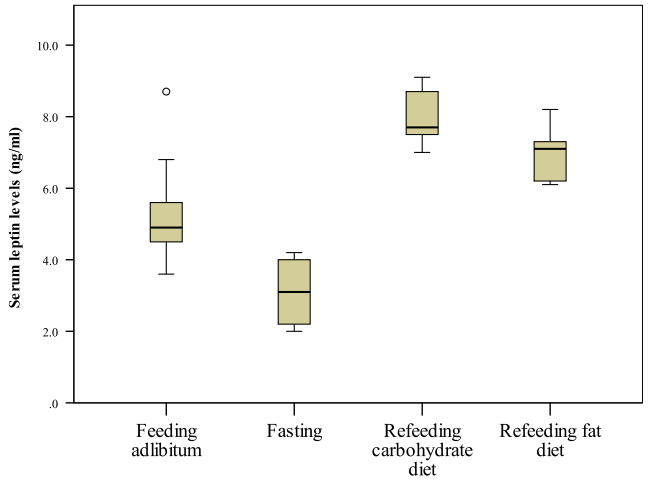

Figure 3 Serum leptin levels (ng/ml). * significant difference between gp II and gp I. • significant difference between gp II and gp III. •• significant difference between gp III and gp IV.

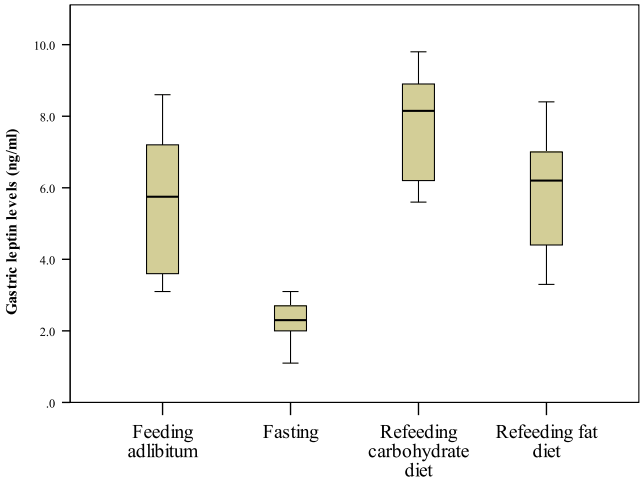

Figure 4 Gastric leptin levels (ng/ml). *significant difference between gp II and gp I. • significant difference between gp II and gp III. •• significant difference between gp III and gp IV.

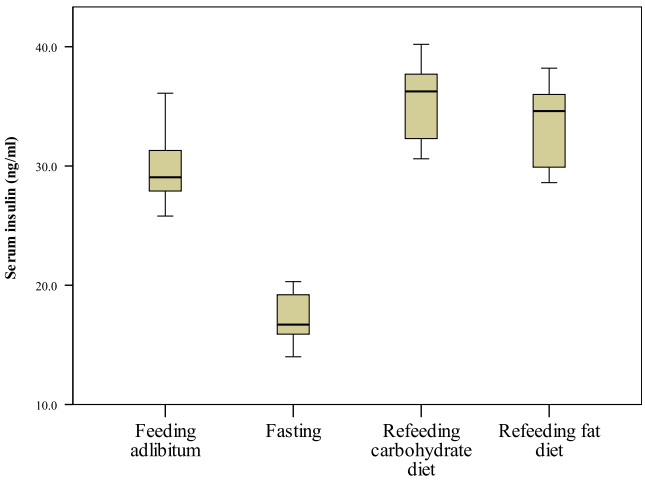

Figure 5 Serum insulin levels (ng/ml). *significant difference between gp II and gp I. • significant difference between gp II and gp III. •• significant difference between gp III and gp IV.

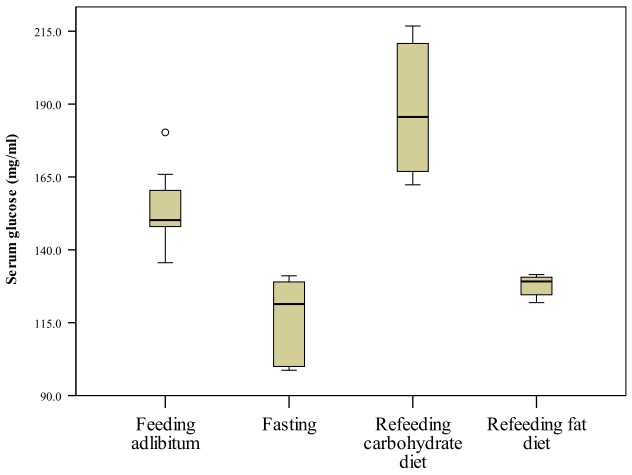

Figure 7 Serum glucose levels (mg/ml). *significant difference between gp II and gp I. • significant difference between gp II and gp III.

Serum ghrelin could be predicted by determining the serum insulin according to the equation. Additionally, the serum ghrelin is declined by 0.04 for each unit change in serum insulin. Moreover, 33% of the changes in the serum ghrelin could be explained by changes in serum insulin i.e., still 67% of change in serum ghrelin may be attributed to the other factors (Table 3, Fig. 12).

Serum ghrelin could be predicted by determining the serum glucose according to the equation. Additionally, the serum ghrelin is declined by 0.008 for each unit change in serum glucose. Moreover, 54% of the changes in the serum ghrelin could be explained by changes in serum glucose i.e., still 46% of change in serum ghrelin may be attributed to the other factors (Table 3, Fig. 13).

Serum ghrelin could be predicted by determining the serum insulin according to the equation. Additionally, the serum ghrelin is declined by 0.079 for each unit change in serum insulin. Moreover, 68% of the changes in the serum ghrelin could be explained by changes in serum insulin i.e., still 32% of

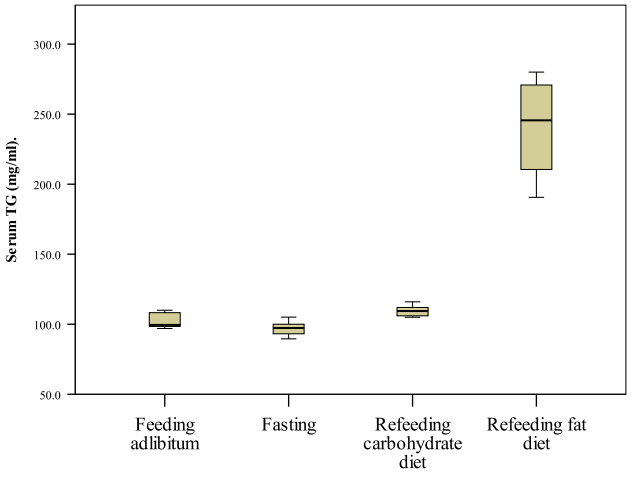

Figure 8 Serum triglyceride levels (mg/ml). • significant difference between gp II and gp III. •• significant difference between gp II and gp IV.

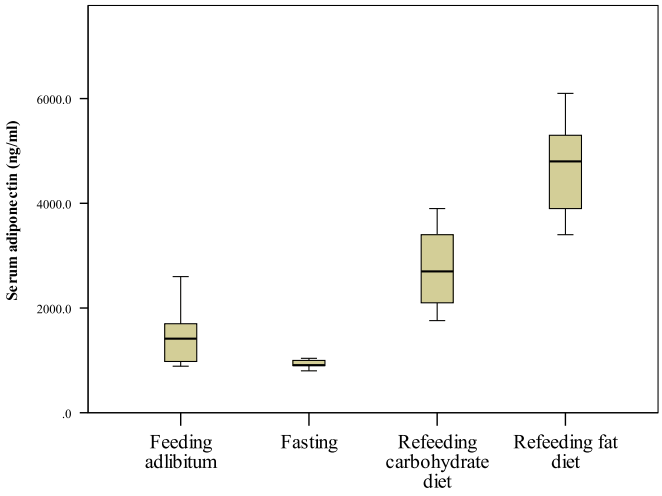

Figure 6 Serum adiponectin levels (ng/ml). • significant difference between gp II and gp III. •• significant difference between gp III and gp IV.

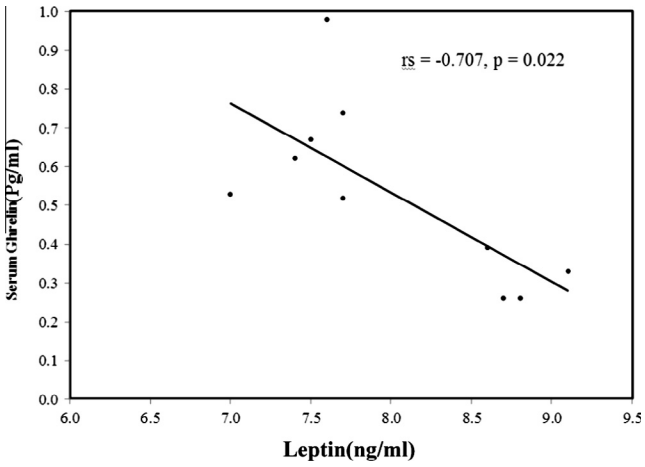

Figure 9 Scatter plot association between serum ghrelin and serum leptin. Serum ghrelin = 2.378–0.230 leptin. $R^2 = 0.516$. $t = -2.921$ $p = 0.019$.

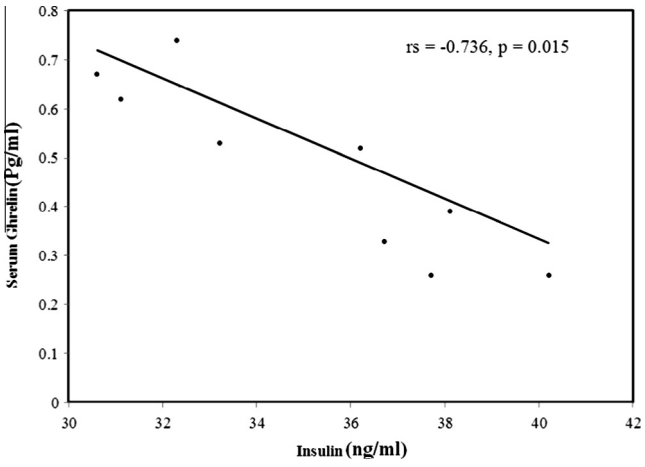

Figure 12 Scatter plot association between serum ghrelin and serum insulin. Serum ghrelin = 1.972–0.04 insulin. $R^2 = 0.33$. $t = -1.985$ $p = 0.082$.

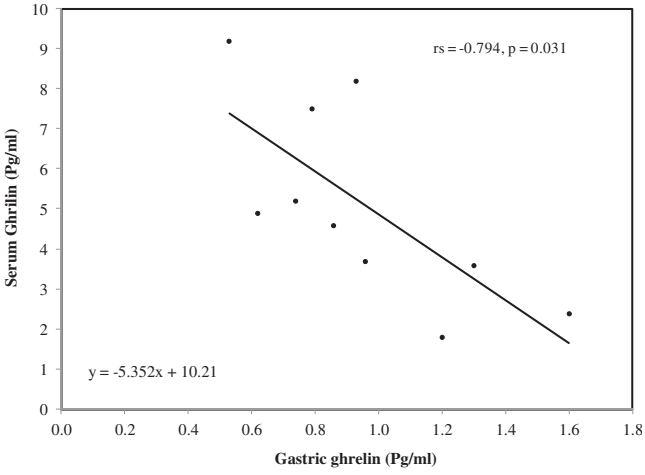

Figure 10 Correlation between serum ghrelin and gastric ghrelin in fasting group.

change in serum ghrelin may be attributed to the other factors (Table 3, Fig. 14).

Serum ghrelin could be predicted by determining the serum adiponectin according to the equation. Additionally, the serum ghrelin is declined by 0.0003 for each unit change in serum adiponectin. Moreover, 60% of the changes in the serum ghrelin could be explained by changes in serum adiponectin i.e., still 40% of change in serum ghrelin may be attributed to the other factors (Table 3, Fig. 15).

Serum ghrelin could be predicted by determining the serum triglyceride according to the equation. Additionally, the serum ghrelin is declined by 0.001 for each unit change in serum triglyceride. Moreover, 17% of the changes in the serum ghrelin could be explained by changes in serum triglyceride i.e., still 83% of change in serum ghrelin may be attributed to the other factors (Table 3, Fig. 16).

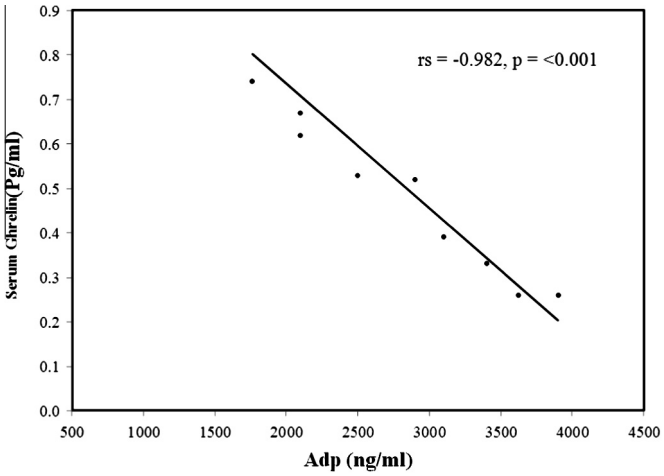

Figure 11 Scatter plot association between serum ghrelin and serum adiponectin(Adp). Serum ghrelin = 1.292–0.0003 Adp. $R^2 = 0.840$. $t = -6.469$ $p = < 0.001$.

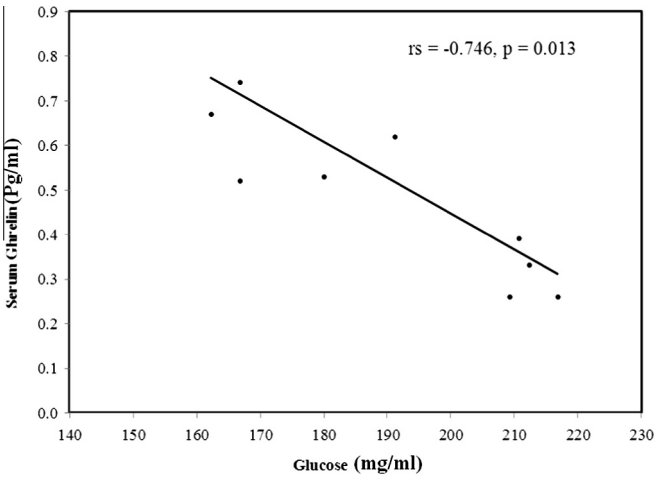

Figure 13 Scatter plot association between serum ghrelin and serum glucose. Serum ghrelin = 2.059–0.008 glucose. $R^2 = 0.549$. $t = -3.124$ $p = 0.014$.

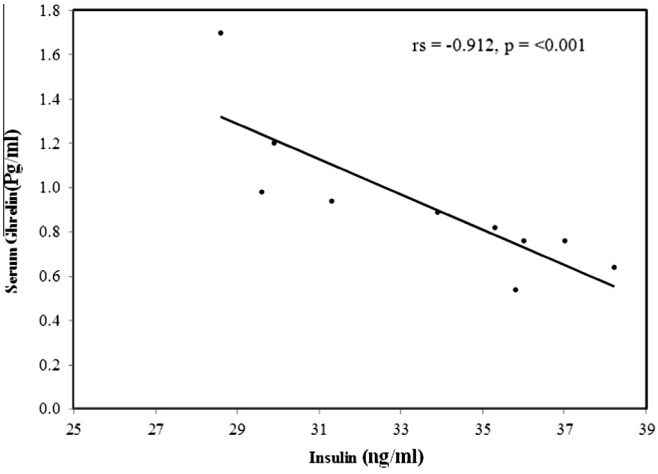

Figure 14 Scatter plot association between serum ghrelin and serum insulin in Fat refeeding group. Serum ghrelin = 3.592–0.079 insulin. $R^2 = 0.689$. $t = -4.21$ $p = 0.003$.

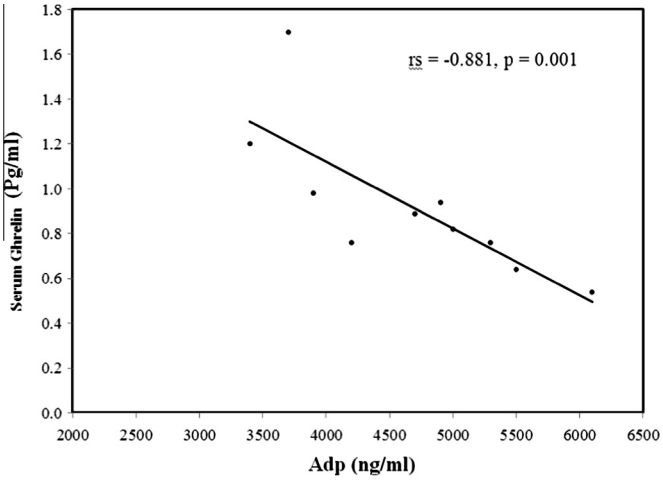

Figure 15 Scatter plot association between ghrelin and serum Adiponectin in Fat refeeding group. Serum ghrelin = 2.317–0.0003 Adiponectin. $R^2 = 0.609$. $t = -3.529$ $p = 0.008$.

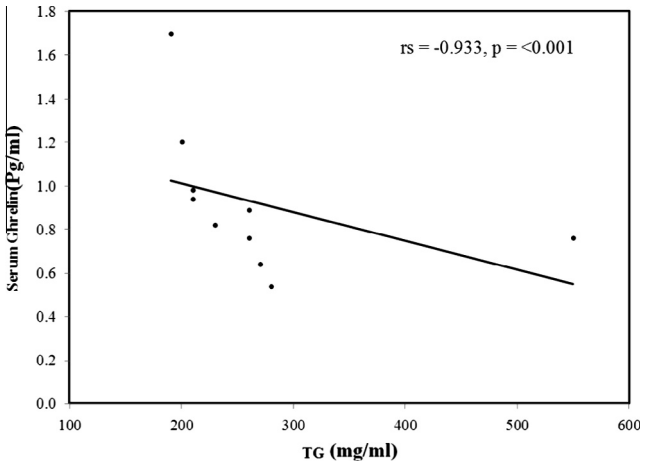

Figure 16 Scatter plot association between serum ghrelin and serum TG in Fat refeeding group. Serum ghrelin = 1.272–0.001 TG. $R^2 = 0.174$. $t = -1.297$ $p = 0.231$.

5. Discussion

In this study, we studied the details of dynamics of ghrelin, adiponectin, leptin and their possible interrelationships with insulin by stimulating basic function to energy metabolism.

In our fasting group an inverse pattern between ghrelin and leptin, insulin, adiponectin level was obtained. Elevated fasting ghrelin appears to agree with the role of ghrelin in initiating food intake. This was in agreement with Preze-Tilve.[32] who stated that ghrelin has a role in meal time hunger. The inverse pattern between ghrelin and leptin was in agreement with Leaflet.[33] Pico et al.[34] also suggested that regulation of neuroendocrine system during starvation could be the main physiological role of leptin. Decrease in gastric and circulating leptin levels was in accordance with Sanchez et al.[35] who suggested that ghrelin is an antagonist of leptin by acting upon the neuropeptide γ receptor pathway[36] explained that ghrelin increases (Ca^{+2}) mechanisms depending on phosphorylase C and adenylcyclase PKC pathways in ARC NPY neurons and that leptin counteracts ghrelin responses via phosphatidylinositol 3-kinase PDE_3 pathways. This interaction may play an important role in feeding regulation. Decreased insulin level obtained in our fasting group may also be involved in suppressing circulating leptin. Harvel.[37] stated that circulating insulin and leptin decrease during fasting ensuring that feeding is triggered before energy stores become depleted. Functional combination between leptin and adiponectin in our fasting group was in agreement with Yamauchi et al.[38] However, ghrelin and adiponectin demonstrated an inverse pattern. Ott et al.[39] also found that ghrelin administration impairs the expression of ADP in adipocyte culture. Thus fasting ghrelin appears to modulate other hormonal systems that participate in the regulation of food intake.[40]

In the present study after eating, the stomach releases less ghrelin, therefore the animal has less stimulus to eat. However ghrelin was suppressed less effectively by fat diet than carbohydrate. The weak suppression of orexigenic hormone by ingested lipid could be one of the mechanisms underlying high fat diet induced weight gain. This was in agreement with Astrup et al.[41] Ghrelin suppressive effect of carbohydrates may be explained also by the obtained high circulating glucose level which could be important for its persistent effect. Harvel.[37] also stated that changes in circulating glucose concentration elicit meal initiation and termination by regulating activity of specific hypothalamic neurons that respond to glucose. We can conclude that gastric ghrelin inhibition is related to different nutrients satiating capacity.

In the present work the stimulus for some hormonal expression and the effect of eating are tightly coupled. The inverse changes in ghrelin and leptin between fasting and refeeding state in serum and gastric ghrelin may also indicate a role of gastric leptin in regulating ghrelin secretion and its involvement in the short term control of energy metabolism. This was in agreement with Zigman and Elmpuist.[42] Zhao et al.[2] revealed that leptin producing cells were mainly located in the lower half of the gastric mucosa where most of the ghrelin cells were tightly surrounded by leptin producing cells suggesting that gastric leptin has a paracrine role in the regulation of the ghrelin cells. Also rodent study indicated that the satiating effects of leptin might include the suppression of ghrelin secretion.[43] The suppression of ghrelin level in our refeeding groups may involve in addition to ghrelin leptin interaction the absorptive mechanisms among the latters (glucose and insulin). Overduin et al.[44] explained that insulin is indirectly stimulated through the postgastric process which involves insulin secretion by glucose stimulating the incretin hormones. In the present work the inverse change between insulin and ghrelin after meals may suggest an important role of insulin in the decrease of ghrelin. This agreed with Murdolo et al.[45] Luccidi et al.[46] also demonstrated a strong positive correlation between insulin sensitivity and the percentage decrease in ghrelin after insulin infusion. We can say that postprandial ghrelin responses could be due to direct effects of multiple hormonal signals on the stomach and each macronutrient. From our results positive correlation between serum insulin and leptin may indicate that they may share common intracellular pathways allowing intracellular integration of their appetite regulations.[47] Also Lee and Fried[48] concluded that leptin synthesis and secretion are also acutely modulated in responses to hormones such as insulin and the availability of metabolic fuels. They explained that a cross-talk among rapamycin mTOR (a meal activated mammalian target) PKA and AMP activated protein kinase pathways appears to integrate hormonal and nutrient signals that regulate leptin mRNA translation.

In the present work a significant positive correlation between adiponectin and insulin with both macronutrients in our refeeding groups was obtained. The highly elevated levels in fat fed rats agree with the previous finding showing a positive association between adiponectin and total fat intake.[49] Tomas et al.[50] suggested that insulin sensitizing action of $ACRP_{30}$ could be mediated by AMPK. AMP activated protein kinase (AMPK) is a fuel enzyme which could be contributed to increase glucose transport[51] and fatty acid oxidation.[52] We can say that in our fat fed group serum insulin, adiponectin and ghrelin became disrupted. This may indicate that high fat diet may bring about signs of insulin resistance. The high serum triglyceride may also augment the risk of cardiovascular disease.

Flachs et al.[53] stated that protection against insulin resistance induced by a high fat diet is at least partially mediated by adiponectin but not leptin. The undergoing findings of the present study provide evidence that in addition to feeding several hormonal states have been shown to be involved in the

regulation of ghrelin production. The postprandial ghrelin responses could be due to direct effects of multiple hormonal signals on the stomach and each macronutrient.

The current results showed a significant association between meal induced change in serum ghrelin and other parameter studied. This explains the change in serum ghrelin may be attributed to other factors other than those investigated in this study. So further studies will be done.

Conflict of interest

None declared.

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