Unstandardized meal does not affect plasma concentrations of leptin, acylated and unacylated ghrelin in humans

Hanna Wosik¹, Jolanta Fryczak², Gabriela Mełeń-Mucha²

¹ Mikolaj Pirogow District Hospital, Lodz, Poland ² Department of Immunoendocrinology, Medical University of Lodz, Poland

Introduction

Leptin and both forms of ghrelin (acylated and unacylated) are peptide hormones involved in appetite control and food intake regulation in humans. Ghrelin is known as the only fast-acting orexigenic hormone, playing a substantial role in meal initiation, while leptin is a mediator of long-term regulation of energy balance, suppressing food intake and thereby protecting against pathological weight gain. It has been thought that circulating ghrelin concentrations are elevated by fasting and suppressed following a meal, whereas leptin concentrations change in the opposite direction[1]. Most human clinical studies only examined these changes after a standardized meal and in carefully selected probes. Assessing total ghrelin without separate measurement of acylated and unacylated hormone together with using different assay methods may also contribute to discrepancies between the studies' results [2;3].

Method

- 10 female and 10 male volunteers between 24 and 54 years of age ($\overline{x}\pm$ SD: 36,5±9,7) were recruited for the study. Body mass index ranged from 18,1 to 37,7 kg/m² ($\overline{x}\pm$ SD: 25,30±4,55). The only exclusion criterium was any disease involving the gastrointestinal tract.
- Blood samples were obtained twice: after an overnight fast (between 6.00 and 9.00) and 2 hours after a meal with different macronutrient composition. Blood was collected into tubes with EDTA as the anticoagulating substance for plasma preparation. Samples for assaying the acylated ghrelin were also treated with *p*-hydroxymercuribenzoic acid (PHMB) and then acidified with 1M HCl to prevent the degradation of the active form of hormone. All specimens were stored at -80°C until analysis. Plasma leptin, acylated and unacylated ghrelin were measured using commercially available ELISA kits (BioVendor). A statistical analysis of the results was carried out using Student t-test and Mann Whitney rank sum test.

The aim of the present study was to investigate whether unstandardized meal influences short-term response of leptin and both biological forms of ghrelin in the group of age- and BMI-differentiated healthy volunteers.

Results

- Plasma concentrations of leptin were higher in females compared to male subjects (Me=11,80 vs 4,05 ng/ml; U=82,5, p<0,01); the analysis was performed for the whole results.
- Plasma concentrations of unacylated ghrelin were higher in females compared to male subjects (Me=777,50 vs 455,00 pg/ml; U=126,0, p<0,05); as before.
- Leptin concentrations were also higher in overweight/obese than in normal weight individuals ($\bar{x}\pm$ SD: 15,4±12,5 vs 4,03±3,78 ng/ml; p<0,001); as before.
- No significant difference has been found between fasting and short-time postprandial plasma concentrations of leptin, acylated and unacylated ghrelin, nor acylated to unacylated ghrelin ratio (Table 1, Fig. 1).

Table 1. Plasma concentrations of leptin, acylated and unacylated ghrelin and acylated/unacylated ghrelin ratio.

Women

	Me fasting	Me postprandial	U	Me fasting	Me postprandial	U
Leptin [ng/ml]	13,55	10,45	40,00*	4,50	3,80	39,50*
Acylated ghrelin [pg/ml]	59,75	68,75	47,50*	42,50	57,50	44,00*
Unacylated ghrelin [pg/ml]	782,50	765,00	45,00*	582,50	347,00	38,00*
Acylated/unacylated ghrelin ratio	0,0575	0,0967	47,50*	0,0805	0,1175	40,00

* *p*>0,05; the U Mann Whitney test showed no significant differences between fasting and postprandial concentrations measured separately for each sex.

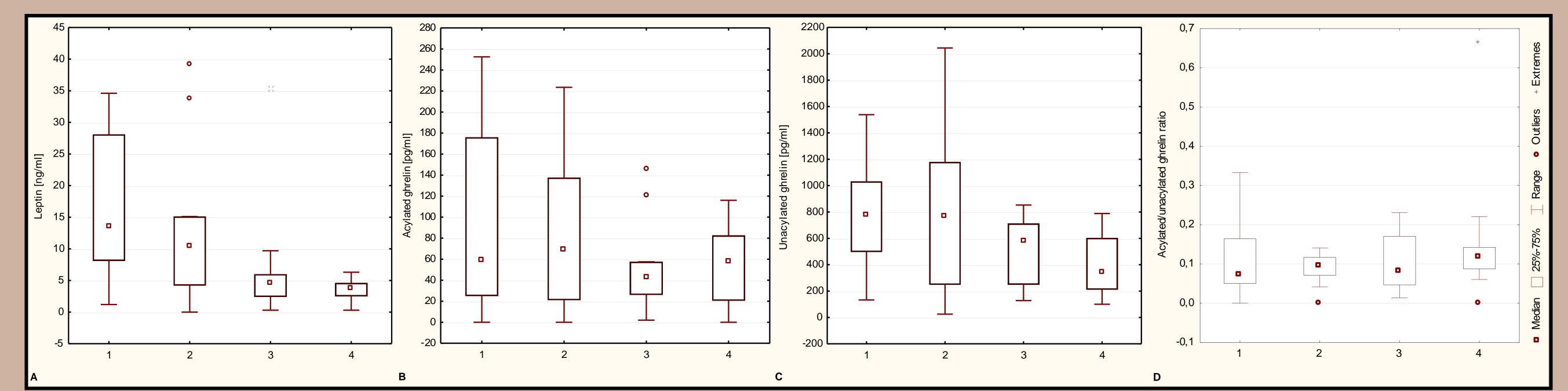


Figure 1. Plasma concentrations of leptin (**A**), acylated (**B**) and unacylated ghrelin (**C**) and acylated/unacylated ghrelin ratio (**D**). The groups are as follows: 1) females fasting, 2) females postprandial, 3) males fasting and 4) males postprandial.

Conclusions

- No *significant* short-term changes were observed in plasma concentrations of appetite hormones before and after the meal.
- It seems that the short-term regulation of postprandial satiety is only partially dependent on ghrelin and leptin response.
- The meal composition may affect leptin and ghrelin plasma concentrations to the greater degree than just food ingestion [4;5].

References

- [1] Cummings DE, Overduin J. Gastrointestinal regulation of food intake. J Clin Invest 2007;117(1):13-23.
- [2] Trivedi A, Babic S, Chanoine JP. Pitfalls in the determination of human acylated ghrelin plasma concentrations using a double antibody enzyme immunometric assay. Clin Biochem 2012;45(1-2):178-80.
- [3] Akamizu T, Shinomiya T, Irako T, Fukunaga M, Nakai Y, Nakai Y, Kangawa K. Separate measurement of plasma levels of acylated and desacyl ghrelin in healthy subjects using a new direct ELISA assay. J Clin Endocrinol Metab 2005;90(1):6-9.
- [4] Romon M, Lebel P, Velly C, Marecaux N, Fruchart JC, Dallongeville J. Leptin response to carbohydrate or fat meal and association with subsequent satiety and energy intake. Am J Physiol 1999;277:E855-61.
- [5] Al Awar R, Obeid O, Hwalla N, Azar S. Postprandial acylated ghrelin status following fat and protein manipulation of meals in healthy young women. Clin Sci 2005; 109(4):405-11.

The study was supported by Medical University of Lodz grant No 502-03/1-153-03/502-14-137.