ORIGINAL ARTICLE

Effect of Two Different Meal Compositions on 1-hour Plasma Ghrelin Levels in Young Men

Brinnell Annette Caszo[1], Sangeetha Shyam[2], Purushotham Krishnappa[1], Justin Vijay Gnanou[1]

[1] School of Medicine, International Medical University, 57000 Kuala Lumpur, Malaysia
[2] School of Health Sciences, International Medical University, 57000 Kuala Lumpur, Malaysia

ABSTRACT

Introduction: Calorie restriction is the main strategy for loosing body weight in obese individuals. However, persistence to this strategy is a major challenge. Ghrelin, a hormone that influences an individual to consume food by modulating the feelings of hunger. This effect may be influenced by the % fat composition of a meal. Materials and **Methods:** Twelve young male participants with normal BMI, were administered in random order one of 2 isocaloric meals after an overnight fast. The 2 meals contained either 31 or 52% fat. After a 7 day gap, cross-over of the participants was carried out and they consumed the other meal similarly. Ghrelin levels were measured after fasting and 1 hour after diet consumption. An appetite rating on a visual analogue scale (VAS) was used to measure perceived hunger and satiety before and after the meal. **Results:** When compared to the baseline values, an average of 34% and 20% decrease in ghrelin levels were noted after the high-fat meal ($p<0.025$), and low-fat meal respectively. Analysis of the VAS showed that feelings of hunger decreased, while feelings of satiety increased after the meal, however there was no difference between the two meals. **Conclusion:** Thus, within this study group, though the feelings of hunger and satiety was comparable, consumption of isocaloric high fat caused ghrelin levels to decrease within one hour post meal. This shows that manipulation of % fat of the diet can achieve lower post-meal ghrelin levels.
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Corresponding Author:
Justin Vijay Gnanou, MD
Email: justinvijay@imu.edu.my
Tel: +60173647342

INTRODUCTION

Obesity is a significant public health problem and poses significant risk for several non-communicable disorders (1). The "obesity epidemic" has taken a firm hold in several populations across the world. The increased incidence of obesity has been related to several epidemiologic factors including urbanization, increased income, environmental factors such as access to restaurants and fast-food outlets among others (2). As reviewed extensively, this "epidemic" has increased the burden on the cost of healthcare and productivity in communities (3). The consequences of obesity are also seen to affect nearly every organ in the body, including influencing mental and psychosocial well-being of the individuals affected, and on their families and communities (4).

As a health problem, obesity is particularly difficult to manage. Obesity can be treated by restriction of calorie intake and increasing energy expenditure (5). This approach is successful but long–term compliance is difficult, as individuals would return to unhealthy eating habits after a period of time.

One of the approaches to the management of obesity include regulation of appetite, hunger, and satiety. One of the hormones that plays a role in appetite regulation is ghrelin, a hormone that is secreted mainly by the stomach (6). Ghrelin provides signals that help regulate appetite and stimulate hunger and eating behavior, via receptors located in the hypothalamus (7). Regions of hypothalamus that secrete neuropeptide Y (NPY) and Agouti related polypeptide (AgRP) express receptors to ghrelin (8). A disturbance in the signals regulating and matching appetite, hunger and satiety to the energy requirements of the body via the "gut-brain" axis is considered a primary reason in the pathogenesis of obesity.

Ghrelin is also involved in the reward system activation and influences the expectation and suggest that feelings of hunger motivate consumption of food (9). Thus, an understanding hormonal response appetite and hunger to nutrients with differing protein or fat content is needed. Hence this study aims to explore the effects of two different meal compositions on the ghrelin levels.

MATERIALS AND METHODS

Subject Selection

Individuals (n=12) between 18 to 22 years of age were recruited for this study. Individuals who were smokers and with history of chronic diseases and on any medication were excluded from the study. Sample size was calculated based on p value 0.05, power of 80%, an average difference in the ghrelin values of approximately 30pg/ml, and a standard deviation of 23 pg/ml based on data reported in Korek et al (10). The study participants were administered written informed consents before being enrolled into the study . The research proposal and ethical approval was obtained from university research committee (Joint committee) (446/2019).

Meal Preparation

Two iso-caloric meals with similar protein content were designed to provide 453-488 kcal/serving. Meal 1 (Low Fat – LF) was composed of 16% protein, 53% carbohydrate and 31% fat, while Meal 2 (High Fat – HF) was composed of 17% protein, 31% carbohydrate and 52% fat, as shown in the table I. Meals were prepared and packed in sandwich bags and coded by subject number. The participants consumed their meals in the cafeteria and were supervised by a team member. Both participants and the supervisor were blinded to the meal composition.

Experimental Protocol

This study was designed as a cross-over experiment. Each participant took part in 2 experiments in random order during which they would be administered either one of the 2 meals.

Both experiments followed an identical protocol. The subjects reported to the lab after an 8 h overnight fast. They were instructed not to perform any other activity before arriving to the lab. After the subjects reported to the lab at 7:00 am they were asked to rest for 30 min.

Table I: Meal Composition

Meal 1 (488 kcal / serving): Low fat (LF) meal			
Per serving	**Protein**	**Carbohydrate**	**Fats**
Gram	19	64	16
Calories	77	257	153
Percentage (%)	16	53	31
Meal 2 (453 kcal /serving): High fat (HF) meal			
Per serving	**Protein**	**Carbohydrate**	**Fats**
Gram	19	35	26
Calories	77	142	238
Percentage (%)	17	31	52

Later, height, weight and % body fat (Table II) parameters was measured using body composition analyser (Seca GmBH, Hamburg, Germany).

After anthropometry measurements were made, a fasting blood sample was collected. An appetite rating on a visual analogue scale (VAS) was then administered. Later, the assigned meal was administered and after 1 hour, a blood sample was collected again, along with an appetite rating on a visual analogue scale. The second experiment was conducted after a wash out period of 7 days, during which the other meal was given. Participants followed a similar protocol as before.

Visual Analogue Scale

This instrument is based on the scale used to measure perceived hunger and satiety used by Bedard et al (11). It consists of a set of questions, which asks how full or hungry a person is feeling, and how strong their desire to eat is. Responses are marked on a line of standard length, one end of which represents for example, "not hungry at all" and the other end "as hungry as I have ever been". The participant marks a position on the line matching their feeling. The response is measured as the distance from one end expressed as a percentage of the length of the line.

Biochemical Analysis

The blood samples collected were stored at -80°C freezer until further analysis. Ghrelin levels were measured using an enzyme linked immunosorbent assay test (Elabscience, Texas, USA). The absorbance and calibration curves (four-parameter logistic regression analysis) were generated using a SpectraMax 5M (Molecular Devices, CA, USA) analyser.

Data Analysis

Based on the normality tests, a non-parametric test (Wilcoxon-Mann-Whitney test) was used to analyse the data. A p-value of <0.05 considered as significant was chosen to compare the plasma ghrelin values.

RESULTS

The subjects BMI and body fat % are 22±2 kg/m^2 and 18.5±5 respectively (Table II). Plasma ghrelin statistically significantly decreased (34%) after the HF meal when compared with baseline levels ($p<0.025$). It only decreased by 20% after the LF meal ($p>0.05$) (Table III).

The average score of the VAS to the question "How hungry do you feel?", "What is your level of satiety", and "How full do you feel" measured before and after the meal was consumed are shown in table IV. Feelings of hunger decreased while those of satiety and fullness increased. In all three cases the changes were statistically significant over the base line. However, the increase in satiety and fullness observed with the high fat meals was

Table II: Subject characteristics

Characteristics	Average	Standard Deviation
Height (cm)	167.95	6.13
Weight (kg)	62.36	9.7
BMI(Kg/m2)	21.98	2.46
Fat%	18.45	4.79

Table III: Plasma Ghrelin Levels with LF and HF diet

Plasma Ghrelin (ng/ml)	Average	Standard Deviation
Baseline	3.85	2.40
Low Fat (LF) diet	3.07	2.01
High Fat (HF) diet	2.54*	1.14

*Baseline vs high fat diet p<0.025

Table IV: VAS scores with LF and HF diet

	How hungry do you feel	Level of satiety	How full do you feel
LF - Pre	5.92 ± 3.74	3.46 ± 2.10	3.36 ± 3.23
LF-Post	2.3 ± 2.14	6.85 ± 1.89	6.99 ± 1.83
HF-Pre	6.29 ± 2.93	3.24 ± 2.16	2.77 ± 2.69
HF- Post	2.94 ± 2.78	6.23 ± 2.09	7.14 ± 1.50

comparable to that of the low-fat meal.

DISCUSSION

This study aimed A) to observe the immediate or acute effect of two different meal compositions; HF with low carbohydrate and LF with high carbohydrate on plasma ghrelin levels in healthy individuals, B) to observe the differences in perception of hunger, fullness and satiety between the two different meal compositions.

We observed a statistically significant decrease of 34% in plasma ghrelin levels with high-fat meal when compared to baseline levels. The reduction in ghrelin levels seen were greater when the high fat low carbohydrate meal was consumed. This decrease maybe explained as a counter regulatory mechanism to decrease feeding response and regulate the number of calories consumed by causing a concomitant increase in fullness and satiety (12). Long term effect of meal composition on ghrelin levels have been observed in both animal and human studies. In animal models, with high fat (HF) diet, ghrelin levels are reduced when compared to a control diet. The difference was more significant when the caloric content was increased. Ghrelin is a hormone which stimulates feeding and has adipogenic effects. Thus, as the calorie content of the diet increased (HF with high caloric diet), the secretion of ghrelin decreased leading to inhibition of feeding. Beck et al., study on the intake of HF diet in rats for 14 weeks, showed 30% decrease in ghrelin levels [12]. They also observed that as the carbohydrate content was increased, the decrease of ghrelin levels was more significant. In another study by Gomez et al., a HF diet for 60 weeks exerted a sustained inhibitory effect on total ghrelin levels in the rats (13).

Hence it is clear that high fat over a period of time causes decrease in expression and synthesis of ghrelin levels. The significance of the finding in our study is that these changes were observed within one hour after the consumption of diet. Few studies of explored the immediate effect of diet on plasma ghrelin levels. Geizannar et al. found a decrease in ghrelin levels at 60 min and at 180 min after consumption of diet containing whey protein (14). Premeal whey protein ingestion has also shown to reduce total daily energy intake (15). Similar findings were also shown with yoghurt consumption, with plasma ghrelin levels decreasing as early as 30 mins after ingestion (16). These findings are consistent with our study findings which shows that ghrelin levels are affected immediately after a meal with immediate feedback to regulating feeding.

Ghrelin levels have been shown to be sensitive to diet composition. In a study done by Parvaresh Rizi et al., high protein or high fat diet suppressed ghrelin levels when compared to high carbohydrate diet (17). However, Tannus et al. and Foster-Schubert et al. showed that a diet high in protein had a greater effect in reducing ghrelin levels when compared to both HF and high carbohydrate diet (18-19). In our study, we found a HF meal had a greater effect on decreasing the ghrelin levels when compared to high carbohydrate meal. This finding has a greater significance since as difference in diet composition can have variable effect on secretion of ghrelin and thence on satiety.

Our study also investigated the relationship between meal composition and ghrelin level to perception of hunger and satiety. The use of a VAS as a measure of perceived hunger is well established. Though VAS is affected by age, gender, and physical activity, they are not affected by BMI, diet and weight concerns (20). Thus, with a stringent inclusion and exclusion criteria with respect to age, gender and physical activity, VAS measures can be used as a good test of perception of hunger and satiety.

The impact of differences in meal composition has been studied to elicit difference in hunger and satiety perception. In a study done on pre-obese and obese women, high protein diet improved perception of satiety (21). In another randomised crossover study on healthy individuals, compared to HF diet, consuming less dense and high protein diet improved appetite control and satiety. It also decreased the subsequent food intake (22). In contrast, Heden et al. found no significant difference in appetite and satiety responses when compared between liquid high carbohydrate and high protein diet (23). Similarly, different breakfast compositions differing in fibre and fatty acid composition also did not alter satiety levels. Conversely, after a fibre supplemented juice drink, changes in ghrelin remained unchanged, but recorded changes in improved satiety. Our study findings also show no statistically significant difference between HF diet and high carbohydrate diet on perception of hunger, satiety and fullness.

The strengths of our study include a cross over study design to eliminate variability between subjects. We also presented and supervised the consumption of the two meals in a blinded manner. The findings of our study show that ghrelin levels were affected by the meal composition. Consumption of high fat diet with low carbohydrate caused a significant decrease in ghrelin levels. This however did not appear to affect the perception of hunger, satiety and fullness scores as measured by VAS. Thus, it appears that in addition to the role of gut hormones, other factors may influence perceptions of hunger, fullness and satiety.

CONCLUSION

It can be concluded that though differences in diet composition elicits different hormonal response, it is not reflected on the perception of satiety, fullness and hunger. This may have major implications for weight reduction strategies which rely on modifying diet compositions in order to induce the feeling of satiety. Though biologically, the response to the modified diet is intended induce satiety, the perception of satiety may be reduced thus defeating the purpose of the modified diet. Thus, we feel, there are many other factors other than the diet composition, such as visual, olfactory and touch sensation which could also a play a role. These factors could be considered as strategies when preparing a diet for weight reduction.

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