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Research article

Low weight gain, better glycaemia and no central obesity achieved through a high-protein diet

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ABSTRACT

A compendium of metabolic diseases associated with unhealthy habits plague the modern world today. Weight gain, hyperglycemia and excess adiposity are some of the metabolic diseases plaguing our modern society. Unhealthy dietary habits coupled with a sedentary lifestyle are recognized as important factors for the development of some metabolic illnesses. Cardiovascular diseases, diabetes mellitus, hypertension, and dyslipidemia were recognized as stemming from metabolic dysregulation due to the consumption of excess calories from certain macronutrients. Our objective in this study is to find out which diet most affects circulating blood glucose levels, body weight, and visceral fat tissue deposition. 35 male Sprague - Dawley rats were separated into five groups and were given five distinct diets for the duration of 8 weeks. The five diets are normal rat feed, high-fat, high-protein, high-sugar, and high-starch. The feeding provided was ad libitum with tap water given as drinking water. Every week, each rat was weighed, and blood were sampled for glucose. Post-sacrifice, mesenteric fat was harvested, fixed, and stained for histological analysis. The results revealed a high-protein diet significantly reduces body weight gain, improved blood sugar with no development of central obesity. At the same time, a high-fat diet was shown to be a promoter of mesenteric fat tissue hypertrophy. It was concluded that the consumption of a high-protein diet was found to achieve low weight gain, better glycaemia with no central obesity.

Keywords: Blood glucose, adiposity, body weight, high-protein diet

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INTRODUCTION

Unhealthy dietary habits and excess calories leads to a cluster of clinical signs and symptoms called the metabolic syndrome [1]. A high-sugar diet was recognized to have a key role in developing metabolic diseases such as insulin resistance, central obesity and other metabolic related complications [2]. A diet rich in fat was also identified to cause many serious illnesses when consumed in a chronic manner [3], [4]. Many evidence point to the role of diet [5], a sedentary lifestyle [6] and reduced daily activity as the prime generators towards deteriorating insulin profile and cardiovascular illness [7]. Insulin resistance and central obesity are recognized as the main risk factors with several other denominators such as hypertension, triglyceride levels, High Density Lipoprotein (HDL) blood levels and hyperglycemia.

Body Mass Index (BMI) is a classic measurement adopted as an indicator to where does one's body weight is correlated to his/her own height. BMI is not a candid calculator of fat measurement nor is it a categorical indicator of one's health, but it is usually exploited as a gauge to one's weight to the ordinary value for his/her height. BMI is just a quantification of one's mass in kilograms divided by height in meters squared (BMI= kg/m2). A BMI of greater than 30 is regarded as obese [8].

Moreno-Fernández (2018) reported that the evidence of sweet, high-sugar drinks leading to poor glucose control and an obesogenic effect is formidable [9]. Prevalent, cheap and easily accessible fast foods are energy-dense while poor in micronutrient; they often contain high saturated and Trans fatty-acids, additional sugars and refined starches [10]. Constant eating of sweet foods and sugar-laden desserts consisting of high-energy boost is related to a considerable gain of weight [11]. This opinion is also thought to be true for processed red meat which is mainly consumed for its protein source [12]. A study estimated that a high sweet foodstuff consumption in toddlers was associated with an augmented BMI and increase of adipose tissue deposition some 10 years later [13].

Another comprehensive review showed that both fat and sugar play an important role in developing metabolic disorders while strongly associated excess carbohydrates with increased BMI in the last forty years [14].

Visceral obesity can be related to chronic, daily stress and high caloric food availability [15]. Evidence of cortisol hormone levels may be related to obesity and metabolic disease was stressed upon with the similarity of clinical sign and symptoms of Cushing's syndrome. The persistent hypercortisolemia in Cushing's syndrome is linked to glucose intolerance which indicates impaired glucose tolerance (IGT), and also hypertension. Removal of the adrenal glands in Cushing's syndrome subjects was proven to overturn IGT and obesity [16]. Particularly in the West, the availability of high caloric foods incorporated with a chronic, daily, high-stress lifestyle, a risk of developing visceral obesity and metabolic syndrome is ubiquitously high [17].

Almost 35% of US citizens, with 50% elderly more than 60 years old, contract metabolic syndrome [18]. In Southeast Asia, Malaysia takes the number one place regarding the prevalence of obesity and its complications for the last decade. A national statistics survey and data revealed that the Malaysian people are overweight 40% above the ASEAN consensus [19]. Arguably, this development is regarded as unhealthy with unproductive consequences for the whole nation. Recently observed, both central obesity and its correlated metabolic syndrome have developed into a worldwide health problem in these few years as to reach epidemic level [20]. This obesity and weight problem is contributed to the excessive consumption of high energy macronutrients which can be accessed with ease as they are ubiquitous and cheap [8]. Realizing this, many health conscious individuals start to exercise and practice different kinds of diets and nutritional regiments in order to ameliorate this health problem. Our objective is to find out which diet most affects circulating blood glucose levels, weight, and visceral fat tissue deposition in male Sprague-Dawley rats.

MATERIALS AND METHODS

35 male, 8-weeks old Sprague Dawley rats were caged in a controlled temperature room (22± ℃) with alternating 12 hours of day and night cycle. Male rats were particularly utilized in this study to refrain from unwanted estrous cycle effects on weight and blood sugar. Sprague-Dawley rats were selected for their docile and easy to handle behavior. After 2-weeks of acclimatization, they were divided into five different groups (n=7) [36]. Each group were given the following rat feed formula for 8 weeks and provided with tap water ad libitum.

Control group Commercial rat chow (Gold Coin). High-fat group Normal rat chow combined with palm oil (Sime Darby, Malaysia) at 50:50 ratio. Powdered rat chow mixed with palm oil and added water to form a dough. The dough was shaped into nuggets and were dried in an oven at 60 degrees Celsius overnight. All the following diets were prepared the same way. High-protein group Powdered rat chow combined with whey protein (GNC 100% Whey Protein Advanced, USA) at 50:50 ratio.

High-sugar group Powdered rat chow combined with 100% table sugar (sucrose) (Gula Prai Brand, Malaysia) at 50:50 ratio.

High-starch group Powdered rat chow combined with sticky rice flour (3 Goats Brand, Thailand) at 50:50 ratio.

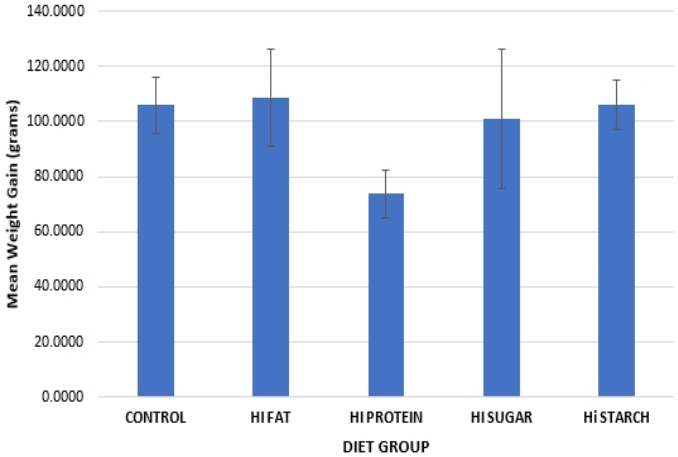
Rat feeds were fully filled into the rat's cage food container each morning of the day and will be monitored for any balance the next morning. Rat feeds were made the day before and collected from the oven the next morning, ready to be used.

Each week, the rats were weighed, and blood glucose taken from the tip of the tail using glucose lancets under aseptic technique. Using a glucometer, blood glucose level was detected and recorded. By the means of a carbon dioxide chamber, the rats were euthanized. The abdomen was dissected, small intestines with the mesenteric fat deposition was identified. The fat was then harvested and fixed in 10% formalin. Samples were then processed by a tissue processor (Leica) and staining done according to hematoxylin and eosin (H&E) protocol. Finally, samples were fixed on glass slides for viewing. An electronic light microscope (Leica) was used to examine the slides and photographs were rendered and saved with an imaging software (Leica). The mean weight gain was analyzed using one-way ANOVA with post-hoc Tukey via SPSS software version 23 and the blood glucose data was analyzed using Microsoft Excel. All animal ethics practices are in compliance with the guidelines approved by the FOM IACUC University of Malaya (Ref: 2019-21114/UNIKL/R/KAMJ).

RESULTS AND DISCUSSION

To identify the diet which affects body weight, each week the rats were weighed and recorded for eight weeks. The mean weight gain for all different groups and the results is shown in Figure1.

Figure 1. The mean weight gain for diet groups after 8 weeks of feeding

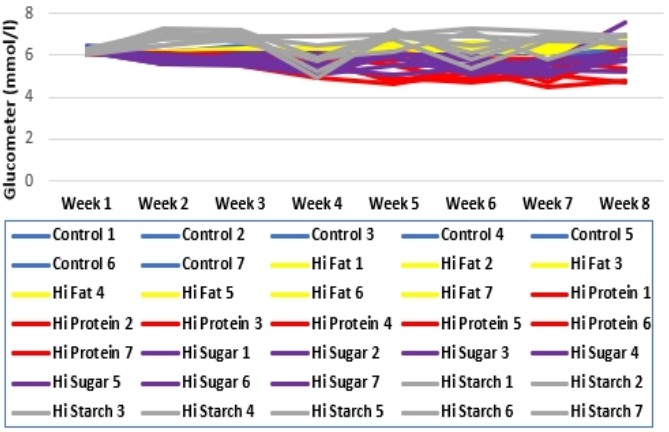


We can observe the lowest mean weight gain was achieved by the high-protein diet group. Mean weight gain for control group

were 106.08 gm, high-fat group 108.85 gm, high-protein 73.72 gm, high-sugar 101.00 gm and hi-starch 106.24 gm. There was a significant difference in the mean weight gain for high-protein group if compared to other groups with $p<0.01$. No significant difference of mean weight gain was found among the control group, high-fat group, high-sugar group and the high starch group. The high-protein group posted the least weight gain among the groups.

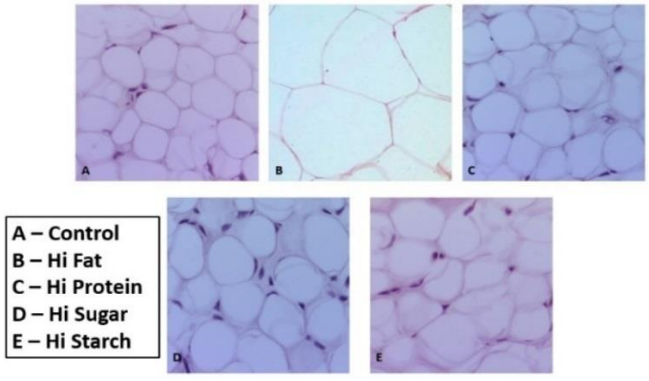
In order to determine the best diet for blood glucose control, blood glucose level was measured using a glucometer for all different groups and the results were shown as in Figure 2.

Figure 2. Blood glucose level (mmol/l) of different diet groups



In the beginning, diet groups started the blood glucose level reading from 6 to 6.3 mmol/l. By the end of 8th week, there is a difference of blood glucose levels between the diet groups. The high-protein diet group blood glucose showed 6 mmol/l or less while the high-starch group blood glucose is recorded mainly above 6 mmol/l. Blood glucose level of the other groups fluctuate between 5 mmol/l and 7 mmol/l. The red lines representing high-protein group was seen in the lower range of the graph while group grey lines representing the high-starch diet dominating the upper range. This result proposes a high-protein group gives the best blood glucose level result in comparison to the est of the group. These results proposes that ingestion of a high-protein foodstuff improves circulating blood glucose in 8 weeks.

Figure 3. Histomicrograph of the five different diet groups after feeding



To identify the outcomes of results on adipose tissue followed by different diet for different groups, the mesenteric fat was harvested, and the stained adipocytes was observed for histomicrograph at 40X magnification as shown in Figure 3.

It was noted that the high-fat group adipocytes (B) present the largest size of adipocyte when compared to the other diet groups. The surface area size and diameter of adipocytes in (B) is immediately noted to be the largest if compared to the other adipocytes indicating hypertrophy and proliferation of the mesenteric adipose tissue after eight weeks of high-fat feeding. There were no stark differences among the adipocytes of the control group (A), high-protein group (C), high-sugar group (D) and the high-starch group (E) [36].

Whey protein supplement was included in the high-protein group is utilized by bodybuilders and individuals alike who intend to lose weight without sacrificing muscle mass [21]. Whey protein supplements are popular and is widespread in usage by the average gym-goers and are frequently ingested as supplements with their main dietary consumption. A high-protein intake was promoted in several kinds of weight loss programs. In one report, 20 to 25 percent surplus in a milk-based protein macronutrient was touted to enhance lean muscle mass and lower adipose tissue deposition in rodents [22]. Another study proposes that a mix of a high-protein diet coupled with resistance training exercise markedly increase fat-free muscle [23]. In one scientific report, it was noted that a reduction in total weight resulted when protein component of the food ingested was as low as 35% [24]. Our result in Figure 1 concurs with these findings. Our current study uses 52% total protein in the high-protein diet feed.

One recent article explains that a high protein intake which far exceeds the Recommended Dietary Allowance (RDA) recommendation is the most probable macronutrient profile in promoting general fat loss [25]. An advantageous aspect of a high-protein diet is it can ameliorate the risks of contracting metabolic syndrome, especially when coupled with moderate weight training program. This study also concludes that a high-protein diet does not have any adverse effect on the kidneys of healthy individuals [26]. A clinical study reported that consumption of low-calorie diet comprising of largely whey protein for 45-days showed significant weight loss, maintenance of muscle mass and positive changes in metabolic parameters [27]. Dietary fats remain a strong factor in weight management, but the type and quantity of protein can also be important through its effects on food consumption [28].

Figure 2 suggests that a high-protein food has a distinct glucose stabilizing attribute. A study noted a high-protein diet is the potential solution to hyperglycemia [29]. This information and studies propose to diabetic patients of their ability to modulate their circulating glucose while minimizing their medication intake. A 2018 clinical study observed that a diet consisting of a low-carbohydrate and a high-protein macronutrient combination would result in an advantageous

state for diabetes mellitus type 2 patients [30]. It was also suggested that a whey supplementation brought the effect of lowered circulating glucose concentrations and suppress appetite after a meal [31]. The apparent appetite reduction linked to a heightened level of blood adiponectin in the whey-based, high-protein diet can help modulate body composition in chronic obesity induced by a rich, high-caloric foodstuff. Another study reported that switching carbohydrates with protein in a diet composition for 6 weeks was shown to lower HbA1c levels in diabetic patients [32]. While another research authored in 2019 published a high-protein diet regimen improves insulin levels in the plasma immediately after glucose was administered [33]. Future research targeting metabolic cascades would be beneficial to further clarify the mechanism of action of these particular diets of interest.

Figure 3 shows the adipocyte of the high-protein group is visibly smaller in size than the high-fat group adipocytes. One recent study reported that a high-fat diet promotes visceral adipose tissue hypertrophy exponentially, heightened adipocyte inflammation with markedly elevated biochemical markers signaling pathways of lipid proliferation [34]. Another research confirmed the outcome of high-fat diet on adipose tissue, high calories paved the way to a jump in adipocyte size measurement and enhanced adipocyte precursor generation, thereby expanding adipocyte count chiefly in the visceral fat tissue [35]. Thus, a simplified notion of a high-fat diet coupled with a high-carbohydrate diet would lead towards the formation of metabolic disturbance even as early as eight weeks of feeding is convincing.

CONCLUSION

Our study found that the consumption of a high-protein diet was able to achieve low weight gain, better glycaemia with no central obesity. Supplementation of whey should be considered for those who intend to lose weight, reduce adiposity, and improve their glucose profiles. Further in-depth study should be pursued for the elucidation of mechanisms concerning the right type and amount of macronutrient needed for physiological homeostasis and longevity.

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