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Dietary Factors in Fasting Blood Glucose Levels and Weight Gain in Female Sprague Dawley in Rats

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Abstract

This was a controlled intervention study with 30 female Sprague Dawley rats to determine the effect of the various classes of food in fasting blood glucose most importantly the effects of these variant feeds on their weight, also assessing the modulating effects of dietary fibre, and the relationship between weight gain and fasting blood sugar. After acclimatization for two weeks, during which the rats were fed with food and water *ad libitum* and during which they were trained to eat various types of diets. They were randomly allocated into four groups: A, B, C and D. They were given water *ad libitum* throughout the duration of the study. Fasting blood glucose was estimated in all rats on day 1 and 21 of the study using Accu Check Roche glucometer and strips. The rats were also weighed on day 1 and 21 of the study. The observed differences in the mean weight gain between the control group A and B, C and D respectively were subjected to student t-test of difference of mean at 5% (0.05) level of significance. The correlation coefficient between the mean weight gain and fasting blood sugar was calculated. The highest weight gain recorded in this study was in the control group A fed with grower's mash specially prepared for the growth of poulets that are of commercial use due to the presence of low dietary protein. Sprague Dawley rats fed with crayfish, a protein diet had the lowest fasting blood sugar after 21 days of constant administration/ feeding which is a desirable outcome in the treatment of diabetes mellitus. The Sprague Dawley rats fed with ground palm kernel also had a significantly weight loss during the study period. Though a completely lipid diet like palm kernel is expected to have a markedly increased weight gain, this was not so in this study due to the presence of dietary fibres contained in palm kernel.

Keywords: Fasting blood glucose; Obesity; Diabetes mellitus; Dietary factors; Glycemic index; Body mass; Dietary fibres; Dietary proteins

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Introduction

The amount of food consumed is a major determinant of postprandial hyperglycemia, and the concept of Glycemic Load (GL) takes account of the Glycemic Index (GI) of a food and the amount eaten (Venn and Green, 2007) [1]. GI was originally designed for people with diabetes as a guide to food selection, advice being given to select foods with a low GI [2]. Lower GI foods were considered to confer benefit as a result of the relatively low glycemic response following ingestion compared with high GI foods. GI is defined as the blood glucose response measured as Area Under the Curve (AUC) in response to a test food consumed by an individual under standard conditions expressed as a percentage of the AUC following consumption of a reference food consumed by the same person on a different day [3]. GI cannot be predicted from the fibre content of a carbohydrate containing food or from the terms whole meal and wholegrain for which there are no universally accepted definitions. For example, from the International Tables, the mean GI of whole meal bread from 13 studies is 71, while that of white wheat bread (mean of six studies) is 70 [4]. Whole grains, when largely intact, have been long found to lower GI [5-8], but whole grain products contain a variable proportion of intact grains.

Within the past two decades, substantial information has accumulated showing that long-term consumption of high glycemic load carbohydrates can adversely affect metabolism and health [9-11]. Specifically, chronic hyperglycemia and hyperinsulinemia, induced by high glycemic load carbohydrates may elicit a number of hormonal and physiological changes that promote insulin resistance [9-11]. Diseases of insulin resistance are frequently referred to as "diseases of civilization" [12] and include obesity, coronary heart disease, type 2 diabetes, hypertension, and dyslipidemia (elevated serum triacylglycerols; small-dense, low-density lipoprotein cholesterol; and reduced high-density lipoprotein cholesterol).

Glycemic load estimates the impact of carbohydrate consumption using the glycemic index while taking into account the amount of carbohydrate that is consumed. GL is a GI-weighted measure of carbohydrate content. For instance, watermelon has a high GI, but a typical serving of watermelon does not contain much carbohydrate, so the glycemic load of eating it is low. Whereas glycemic index is defined for each type of food, glycemic load can be calculated for any size serving of a food, an entire meal, or an entire day's meals.

One 2007 study has questioned the value of using glycemic load as a basis for weight-loss programmes. Das et al. conducted a study on 36 healthy, overweight adults, using a randomised test to measure the efficacy of two diets, one with a high glycemic load and one with a low GL. The study concluded that there is no statistically significant difference between the outcomes of the two diets [13].

Glycemic load appears to be beneficial in dietary programs targeting metabolic syndrome, insulin resistance, and weight loss; studies have shown that sustained spikes in blood sugar and insulin levels may lead to increased diabetes risk [10]. The Shanghai Women's Health Study concluded that women whose diets had the highest glycemic index were 21 percent more likely to develop type 2 diabetes than women whose diets had the lowest glycemic index [14]. Similar findings were reported in the Black Women's Health Study [15]. A diet program that manages the glycemic load aims to avoid sustained blood-sugar spikes and can help avoid onset of type 2 diabetes. For diabetics, glycemic load is a highly recommended tool for managing blood sugar.

Relationships between dietary GI and blood lipid fractions have been assessed in several prospective observational studies. A reasonably consistent finding has been an inverse association between fasting HDL cholesterol concentrations and dietary GI [16-18], although one study found no association [19]. Ma et al. [20] found inverse associations between dietary GI and GL in a cross-sectional analysis, but the associations were lost during follow-up. An inverse association between GI and HDL-cholesterol concentration has also been found in a nationally representative sample of US adults [21]. Findings from intervention trials differed from those of observational studies.

Kelly et al. [22] conducted a meta-analysis of intervention trials that had examined the effect of low GI diets on coronary heart disease risk factors. Results from that analysis showed limited and weak evidence of an inverse relationship between GI and total cholesterol, with no effect of dietary GI on LDL and HDL cholesterol, triglycerides, fasting glucose and fasting insulin. Opperman et al. [23] conducted a meta-analysis of 14 randomized controlled trials relating to the effects on blood lipids of altering the GI of test diets.

It has been known for some time that insulin response cannot be predicted based solely on the glycemic response to a food. Collier and O'Dea [24] found marked differences in the glycemic response to potato with or without added butter, but a very similar insulin response. The effect of GI on insulin response may also depend upon insulin sensitivity. Dietary GI has not been shown to have a marked effect on insulin sensitivity whereas dietary fibre has an important justification for the claim of an overall health benefit of low GI foods is that low GI foods may aid weight control because they promote satiety [25]. Ideally, weight loss studies comparing low and high GI diets would need to assess differences between diets based on *ad libitum* intake to show that the apparently greater satiating effect of low GI foods led to a reduced energy intake.

Aim of Study

The primary concern of this controlled intervention study was to satisfy the following; does the class of food have effect on fasting blood glucose level in female Sprague Dawley rats? Does dietary fibre modulates fasting blood glucose in female Sprague Dawley rats? Is the fasting blood glucose related to weight gain or loss? The general objective however of this controlled intervention study was to determine the effect of various classes of food in fasting blood glucose most importantly the effects of these variant feeds on their weight whilst assessing the modulating effects of dietary fibre, and the relationship between weight gain and fasting blood sugar.

Materials and Methods

Materials

During the course of this study, the following materials were used; wooden cage consisting of 24 spaced compartments for housing and accommodation of the experimental animals, sterilized lancets for piercing during specimen collection. An accu-check glucometer was also used in this study for specimen analysis alongside the cotton wool and 70% alcohol. During the course of this study and at different daily intervals, laboratory clothing, hand gloves and face masks were used by the personnel (i.e., Group members) in charge of maintaining, feeding and testing procedures. Miscellaneous materials were also used to assist the aforementioned materials listed above in the successful progression of this study. Such miscellaneous materials included the wood chips for bedding, feeding and water troughs, rubber baskets for temporary housing while bedding materials were been changed, marker pen and gelatinous blue for animal classing and differentiation into groups and subgroups.

Experimental feeds

During the course of this study the following feed materials were used; grower's mash for control, grounded Palm kernel, yam flour and crayfish, which contained considerable and varying nutrient constituents, for the daily consumption of the grouped experimental animals under this study. Each of the feeds in its own proportion represented the normal universal daily food nutrients. The yam flour contained a considerable percentage of carbohydrate, crayfish contained even more protein and the grounded palm kernel contained a significant percentage of fats from its oil extract and dietary fibres, while the Grower's mash, the control feed represented a well balanced diet consisting of significant percentage of carbohydrate, protein, fats (lipid), vitamins and minerals.

Experimental animal

Thirty (30) female Sprague-Dawley rats were used in the course of this study.

Grouping

The experimental animals were divided into 4 groups of 6 female Sprague-Dawley rats each, except group A with a total number of 12 experimental animals alone for control. These groups were promptly labelled A, B, C and D.

Acclimatization

The different groups of animals were acclimatized in their various cage compartments for 14 days. During this 14 day period they were also acclimatized to physical restraint. Dry hard wood chip was used as animal beddings in the cage and was changed every 2 days. This procedure was held up to first monitor the experimental animals, watch their adaptation to the change in feed and its effect on their initial weight and fasting blood glucose concentration and second, to acclimatize them to their new environment which served as the location of this study.

Administration of food

During the course of this study, each group of rats was placed on a specific diet. Each rat was fed with 30 g of experimental meals and 35 ml of clean water daily.

Group A: Were fed with growers mash.

Group B: Were fed with grounded palm kernel.

Group C: Were fed with dried yam flour.

Group D: Were fed with dried Cray-fish.

The feeds were served on small stainless plates and water on a small rubber plate. Both plates were washed with detergent and rinsed with clean water every day before fresh feeds and water was served again.

Experimental weighing

First of the imperative procedures of this study is the weight acquisition or weight losses of the experimental animals. In order to determine this, experimental animals were subjected to a fairly amount of feed (30 g) daily and weighed severally at fixed occasions. The experimental animals were weighed using a weighing balance and the weight of each experimental animal was noted even before acclimatization and after acclimatization. This was however done to obtain the initial weight of these experimental animals before they were subjected to the experimental feeds. The weight results collected before acclimatization served as the initial weights of the experimental animals, while the weight results collected after acclimatization helped determine the experimental animal's adjustment to the change in feed. The weight results of the experimental animals were also collected for 21 days, beginning 7 days after acclimatization and on the last day of the study. However, the weight results were also collected alongside Blood testing results.

Sample (blood) testing

Testing which strappingly is another imperative procedure of this study was carried out at subsequent day(s) interval in a serene environment. The experimental animals (Sprague Dawley rats) were fasted over a 12 hour period before a fasting blood glucose test was done. That is their feeds were immediately removed before the morrow of the testing. Blood samples were collected after a sharp prick through the straight vein supplying the tail with sterilized lancets. Preceding the above mentioned steps were, physical restraints of the experimental animals, cotton wool swabs in 70% alcohol to first sterilize the surface of the tail. Shortly after pricking, the first blood run-out was swiftly wiped off to allow for succeeding and uncontaminated blood (alcohol free blood). The purpose of this first clean up is to avoid to the barest minimum, the use of blood that had made contact with the Alcohol on the surface of the tail which would influence the results immensely because of the high amount of glucose in the Alcohol. In such cases, dried and clean cotton wool were used. Proceeding immediately the pricking and first blood run-off, a mechanical device used in the determination of blood glucose level is used to test and collect results from its output reader; the input however being the blood sample and the test strips serving as the input-reader modulator.

This device (the accu-check glucometer) is a generally used mechanical device for this peculiar purpose. It is however reliable to note that the accu-check glucometer is no discriminator of animal species' blood samples. In other words, it can be used in testing the blood glucose levels in animals provided the experimental animal(s) under study proffers the required criteria. The testing period for this study lasted 21 days with blood sampling and testing carried out on the 7th and 21st day of the study beginning from the 7th day after acclimatization. Finally, the results obtained from the accu-check glucometer was collated, recorded and stored out in the group's inventory.

Result

In this study, the mean weight of the experimental animals were recorded as with group A (fed with control diet; grower's mash) gaining the highest weight of about 30.67 ± 9.95 g, beginning with an initial weight size of 160 ± 11.16 g, before acclimatization and at the end of the experiment, its final weight weight rose to 191.25 ± 16.78 g, while group C (constantly fed carbohydrate diet; yam flour) gained the least weight of 1.83 ± 12.5 g, after an initial weight size of 150.17 ± 7.19 g. However, group B (fed on fat diet; grounded palm kernel) and D (fed on protein diet; crayfish) also had an increase in the weight gain at the completion of the experimental study, with about 11.67 ± 5.75 g and 4.67 ± 10.56 g respectively **(Table 1)**. In comparison with the Experimental animals fed with grower's mash, grounded palm kernel seed, yam flour and crayfish, the statistical deductions for P value ($p<0.05$) showed that there was a statistical significance which indicated that there was actually a significant decrease in the weight gained by experimental animals when compared to those administered control diet **(Table 2 and Figure 1)**. It showed that there were lapses and decreases in the weight parameters of these animals either due to change in diet, adaptation or acclimatization to a newly introduced diet or, even, slow acclimatization to change in the environment which was a necessary phase in the experimental procedures. **Table 3 and Figure 2** shows the mean fasting blood glucose concentration distribution between Sprague Dawley rats fed with experimental dietary feeds. Here, the serum fasting blood glucose concentration was highest in the experimental animals of the group B category fed with grounded palm kernel seeds with a mean value of 6.04 ± 1.27 mmol/L while the serum fasting blood glucose concentration was least in experimental animals of the group 4 fed with crayfish with a mean value of 5.54 ± 1.16 mmol/L. It could also be clearly stated that the fasting blood glucose concentration in group B, C and D were not significantly different from the fasting blood glucose in the control group ($P>0.05$; **Table 3 and Figure 2**). In this study, a simple analysis was run to correlate the relationship between the mean weight gain and the mean fasting blood glucose levels considered in this study to determine the relationship between these two parameters. Was there any relationship between the weight gained and the fasting blood glucose concentration of the experimental animal and was this relationship positive or negative? What would this imply?

However, after a series of analytical statistics were carried out and a correlation figure derived **(Tables 4 and 5 and Figure 3)**, there was a negative correlation between the two parameters which indicated that an increase in weight gain will not necessarily mean that there will be an increase in fasting blood glucose concentration as can be found that not all obese persons are diabetic or with high levels of glucose concentration (hyperglycemia), and even lean personalities can be diabetic regardless their sizes or weight. In other words, fasting blood glucose concentration is not directly affected by the weight gain of an individual or vice versa.

Discussion

Obesity which simply implies excessive weight gain is a positive risk factor in the development of hypertension, diabetes mellitus, gall bladder diseases, coronary heart diseases and certain types of cancers. Type 2 Diabetes mellitus or Non-Insulin Dependent Diabetes Mellitus (NIDDM) is closely associated with obesity which is closely associated with sedentary life style, and diet. Appropriate lifestyle and behaviour interventions are still the crucial cornerstone to weight loss success, but maintaining such a healthy lifestyle is extremely challenging. Abundant natural materials have been explored for their obesity treatment potential and widely used to promote the development of anti-obesity products [25]. The weight loss segment is one of the major contributors to the overall revenue of the dietary supplements market. In this study, equal weight of predominantly protein, carbohydrates and lipids diets were matched against a balanced diet of Grower's mash. Crayfish, yam, palm kernel represented protein, carbohydrate and lipid respectively. Women generally have higher rate of obesity than men hence the use of female Sprague Dawley rats for this study. The distribution of fat induced weight gain affects the risks associated with obesity and the kind of disease that results. Android obesity (abdominal obesity) predisposes to coronary artery thromboses are found more frequently than gynoid fat distribution common in females. Though the 75 g oral glucose tolerance test alone or in combination with fasting blood sugar remains the best way of screening for diabetes mellitus; it is rather cumbersome for screening large population. Since fasting blood sugar offers a quick and relatively reliable method of estimating glucose tolerance, it was used in estimating blood glucose in this study. The highest weight gain recorded in this study was in the control group A fed with

Table 1: Showing the mean distribution for weight of experimental animals before and after study.

Diet Administered	Grower's mash (Control)	Ground Palm kernel	Yam flour	Crayfish
No of SD Rats	12	6	6	6
Initial Mean weight (g)	160.58 ± 11.16	145.00 ± 3.79	150.17 ± 7.19	155.17 ± 7.36
Final Mean weight (g)	191.25 ± 16.78	156.67 ± 4.55	152.00 ± 9.47	159.83 ± 4.07
Mean weight gain (g)	30.67 ± 9.95	11.67 ± 5.75	1.83 ± 12.54	4.67 ± 10.56

Table 2: Showing comparison between the mean weight gain in group A in relation to the mean weight gain obtained from group B, C, and D representing significant decrease in the mean weight gained by the experimental animals ($P<0.05$).

Group	Diet Administered	No of SD Rats	Mean weight gain (g)	Comparison	P vale	Remark
A	Grower's mash (Control)	12	30.67 ± 9.95	-	-	-
B	Ground Palm kernel	6	11.67 ± 5.75	A vs. B	0.000	P<0.05
C	Yam flour	6	1.83 ± 12.54	A vs. C	0.000	P<0.05
D	Crayfish	6	4.67 ± 10.56	A vs. D	0.000	P<0.05

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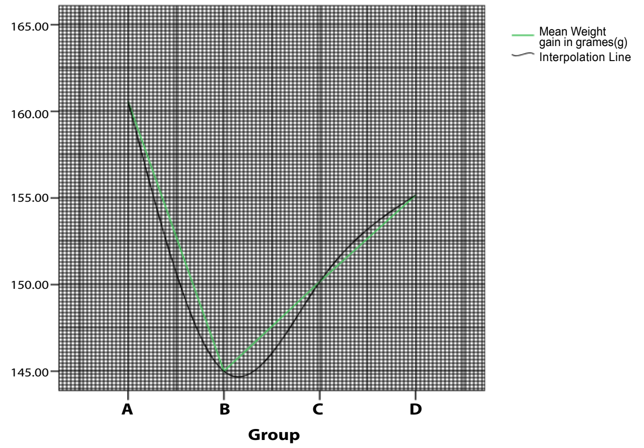

Figure 1 Showing the mean of the weight derived from experimental animals in this study administered different diets.

Table 3: Showing comparison between the mean fasting blood glucose (FBG) concentration. P>0.05: Not significant.

Group	Diet Administered	No of SD Rats	Mean Final FBG (mmol/l)	Comparison	P vale	Remark
A	Grower's mash (Control)	12	5.63 ± 0.58	-	-	-
B	Ground Palm kernel	6	6.04 ± 1.27	A vs. B	0.353	P>0.05
C	Yam flour	6	5.75 ± 0.95	A vs. C	0.742	P>0.05
D	Crayfish	6	5.54 ± 1.16	A vs. D	0.826	P>0.05

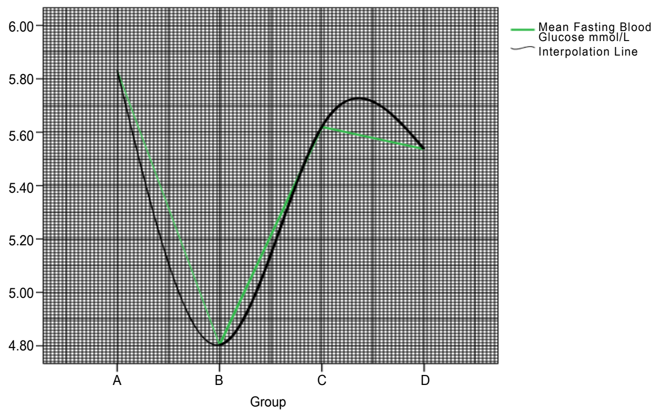

Figure 2 Showing the mean of the final fasting blood glucose (FBG) concentration derived from experimental animals in this study administered different diets. Group A: Administered Grower's mash; Group B: Administered Palm kernel; Group C: Administered Yam flour; Group D: Administered Crayfish.

grower's mash specially prepared for the growth of poulets that are of commercial use. Diets deficient in protein often increase food consumption, body weight and fat mass [26-30]; however, the underlying mechanisms remain poorly understood of the underlying mechanisms by which moderate protein deficiency elicits such behavioural and metabolic adaptations and promotes positive energy balance with consequent predisposition to obesity and other metabolic disorders. Besides, an almost completely protein diet is usually recommended for diabetic patients. In this study, Sprague Dawley rats fed with crayfish, a protein diet had the lowest fasting blood sugar after 21 days of constant administration/feeding. This is a desirable outcome in the treatment of diabetes mellitus. In addition, the weight gain during the study was significantly lower than the weight gain found in the control group. The Sprague Dawley rats in fed with ground palm kernel also had a significantly lower weight (weight loss) during the study period. Though a completely lipid diet like palm kernel is expected to have a markedly increased weight gain, this was not so in this study. The weight gain in the palm kernel fed rats were 11.67 ± 9.95 g when compared to 30.67 ± 9.95 g in the control group. A high saturated fat intake has been associated with impaired glucose intolerance which was not the case in this study. This may be explained by the high quantity of dietary fibre in palm kernel. However there are contradictory results in previous studies. In a study where more than 5% palm kernel was included in broiler diet, there was reduced growth. In another study in Costa Rica that included 20% of palm kernel in broiler diet, there was an increased weight gain. An interesting conclusion was drawn in a study with more than 37,000 participants, of which 915 incidences of diabetes reported over 10 years [31]. Their study has confirmed that a positive correlation exists between higher GI food and diabetes and that fibre intake inversely correlated with diabetes indicating that fiber is an important instrument in starch and cholesterol control. They concluded that diet constituents play a major part in controlling diabetes [32-34].

The Sprague Dawley rats fed with yam flour had the lowest mean weight gain when compared with other groups. This weight gain was significantly lower than that obtainable from the control group. This is an unexpected result considering the fact that yam flour is purely carbohydrate diet, which is expected to yield a higher quantity of energy. However, the fact that the yam flour was uncooked may provide explanation for the unexpected result. Cooked yam flour as found in Amala and pounded yam yield more glucose than uncooked yam flour, which is pure starch with complex carbohydrate chain. This result is expected considering the fact that uncooked starch has lower glycemic index than cooked. In a previous study, amala made form dried yam flour had a lower glycemic index than boiled yam which also had lower glycemic index than pounded yam. In this study there was a negative correlation between weight gain and fasting blood sugar r = -0.032; **Figure 3**. There was also a negative correlation between body weight and fasting blood glucose at 21st day after constant administration of experimental diets (r=-0.279). This is not consistent with previous studies where fat mass was found to be positively correlated with fasting blood glucose [35]. This is probably because the weight gain was mainly from increase in muscle and bone weight rather than fat mass. It is known that decreased fat mass and increased fat free mass are associated with positive change in the control of blood glucose [36]. This goes to support the fact that even lean people with less fat mass

Table 4: Showing the relationship between fasting blood glucose concentration and weight gain for Sprague Dawley rats on controlled diet (grower's mash), ground palm kernel, yam flour and crayfish. P>0.05: Not significant.

Diet Administered	No of SD Rats	Mean FBG (mmol/l)	Mean weight gain (g)	Correlation	P vale	Remark
Grower's mash	12	5.63 ± 0.58	30.67 ± 9.95	20.79%	0.136	P>0.05
Ground Palm kernel	6	6.04 ± 1.27	11.67 ± 5.75	17.72%	0.405	P>0.05
Yam Flour	6	5.75 ± 0.95	1.83 ± 12.54	27.77%	0.283	P>0.05
Crayfish	6	5.54 ± 1.16	4.67 ± 10.56	5.34%	0.660	P>0.05

Table 5: Showing the relationship between the two parameters (weight gained against fasting blood glucose (FGB) concentration) mulled over in this experimental study.

Diet Administered	Grower's mash (Control)	Ground Palm kernel	Yam flour	Crayfish	Correlation coefficient
No of SD Rats	12	6	6	6	-
Mean weight gain (g)	30.67 ± 9.95	11.67 ± 5.75	1.83 ± 12.54	4.67 ± 10.56	-
Mean FBG (mmol/l)	5.63 ± 0.58	6.04 ± 1.27	5.75 ± 0.95	5.54 ± 1.16	- 0.178

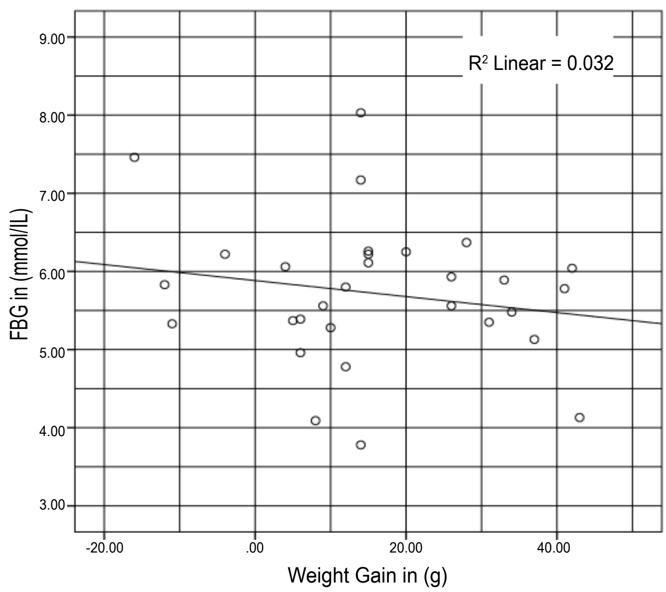

Figure 3 Showing the correlation of fasting blood glucose (FBG) concentration with weight gain for this study.

can be diabetic though obese people are more predisposed to diabetes.

Conclusion

In this study, we discovered that weight gain is related to dietary intake and that dietary fibre modulates weight gain. There was no statistical significant difference between the fasting blood glucose levels in the rats fed with the various classes of macronutrients (balanced diet, carbohydrate, protein and lipid diets) provided the quantity of food is limited. In other words, restricted calorie intake of any of the macronutrients does not lead to glucose intolerance. However, a similar study for a longer duration with higher quantity of the various types of diets is being considered.

References

1 Venn BJ, Green TJ (2007) Glycemic index and glycemic load: measurement issues and their effect on diet–disease relationships. Eur J Clin Nutr 61: S122–S131.

2 Jenkins DJ, Wolever TM, Jenkins AL, Thorne MJ, Lee R, et al. (1983) The glycaemic index of foods tested in diabetic patients: a new basis for carbohydrate exchange favouring the use of legumes. Diabetologia 24: 257-264.

3 FAO (1998) Carbohydrates in human nutrition Report of a Joint FAO/WHO Expert Consultation FAO. Food Nutr Pap 66: 1-140.

4 Foster-Powell K, Holt SH, Brand-Miller JC (2002) International table of glycemic index and glycemic load values. Am J Clin Nutr 76: 5-56.

5 Jenkins DJ, Wolever TM, Jenkins AL, Giordano C, Giudici S, et al. (1986) Low-glycemic response to traditionally processed wheat and rye products: bulgur and pumpernickel bread. Am J Clin Nutr 43: 516-520.

6 Liljeberg H, Granfeldt Y, Bjorck I (1992) Metabolic responses to starch in bread containing intact kernels versus milled flour. Eur J Clin Nutr 46: 561-575.

7 Granfeldt Y, Liljeberg H, Drews A, Newman R, Bjorck I (1994) Glucose and insulin responses to barley products: influence of food structure and amylose-amylopectin ratio. Am J Clin Nutr 59: 1075-1082.

8 Granfeldt Y, Hagander B, Bjorck I (1995) Metabolic responses to starch in oat and wheat products On the importance of food structure, incomplete gelatinization or presence of viscous dietary fibre. Eur J Clin Nutr 49: 189-199.

9 Liu S, Willett WC (2002) Dietary glycemic load and atherothrombotic risk. Curr Atheroscler Rep 4: 454-461.

10 Ludwig DS (2002) The glycemic index: physiological mechanisms relating to obesity, diabetes, and cardiovascular disease JAMA 18: 2414-2423.

11 Cordain L, Eades MR, Eades MD (2003) Hyperinsulinemic diseases of civilization: more than just syndrome X Comp Biochem Physiol A 136: 95-112.

12 Eaton SB, Konner M, Shostak M (1988) Stone agers in the fast lane: Chronic degenerative diseases in evolutionary perspective. Am J Med 84: 739-749.

13 Das SK, Gilhooly CH, Golden JK, Pittas AG, Fuss PJ, et al. (April 2007) Long-term effects of 2 energy-restricted diets differing in glycemic load on dietary adherence, body composition, and metabolism in CALERIE: a 1-y randomized controlled trial. Am J Clin Nutr 85: 1023-1030.

14 Villegas R, Liu S, Gao Y, Yang G, Li H, et al. (2007) Prospective Study of Dietary Carbohydrates, Glycemic Index, Glycemic Load, and Incidence of Type 2 Diabetes Mellitus in Middle-aged Chinese Women. Arch Intern Med 6167: 2310-2316.

15 Krishnan S, Rosenberg L, Singer M, Hu FB, Djoussé L, et al. (2007) Glycemic Index, Glycemic Load, and Cereal Fiber Intake and Risk of Type 2 Diabetes in US Black Women. Arch Intern Med 167: 2304-2309.

16 Liu S, Manson JE, Stampfer MJ, Holmes MD, Hu FB, et al. (2001) Dietary glycemic load assessed by food-frequency questionnaire in relation to plasma high-density lipoprotein cholesterol and fasting plasma triacylglycerols in postmenopausal women. Am J Clin Nutr 73: 560-566.

17 Amano Y, Kawakubo K, Lee JS, Tang AC, Sugiyama M, et al. (2004) Correlation between dietary glycemic index and cardiovascular disease risk factors among Japanese women. Eur J Clin Nutr 58: 1472-1478.

18 Slyper A, Jurva J, Pleuss J, Hoffmann R, Gutterman D (2005) Influence of glycemic load on HDL cholesterol in youth. Am J Clin Nutr 81: 376-379.

19 Murakami K, Sasaki S, Takahashi Y, Okubo H, Hosoi Y, et al. (2006) Dietary glycemic index and load in relation to metabolic risk factors in Japanese female farmers with traditional dietary habits. Am J Clin Nutr 83: 1161-1169.

20 Ma Y, Li Y, Chiriboga DE, Olendzki BC, Hebert JR, et al. (2006) Association between carbohydrate intake and serum lipids. J Am Coll Nutr 25: 155-163.

21 Ford ES, Liu S (2001) Glycemic index and serum high-density lipoprotein cholesterol concentration among US adults. Arch Intern Med 161: 572-576.

22 Kelly S, Frost G, Whittaker V, Summerbell C (2004) Low-glycaemic index diets for coronary heart disease. Cochrane Database Syst Rev 18: CD004467.

23 Opperman AM, Venter CS, Oosthuizen W, Thompson RL, Vorster HH (2004) Meta-analysis of the health effects of using the glycaemic index in meal-planning. Br J Nutr 92: 367-381.

24 Collier G, O'Dea K (1983) The effect of coingestion of fat on the glucose, insulin, and gastric inhibitory polypeptide responses to carbohydrate and protein. Am J Clin Nutr 37: 941-944.

25 Brand-Miller JC, Holt SH, Pawlak DB, McMillan J (2002) Glycemic index and obesity. Am J Clin Nutr 76: 281S-285S.

26 Sun N, Wu T, Chau C (2016) Natural Dietary and Herbal Products in Anti-Obesity Treatment. Molecules 21: 1351.

27 White BD, Porter MH, Martin RJ (2000) Effects of age on the feeding response to moderately low dietary protein in rats. Physiol Behav 68: 673-681.

28 White BD, Porter MH, Martin RJ (2000) Protein selection, food intake, and body composition in response to the amount of dietary protein. Physiol Behav 69: 383-389.

29 Solon-Biet SM, McMahon AC, Ballard JW, Ruohonen K, Wu LE, et al. (2014) The ratio of macronutrients, not caloric intake, dictates cardiometabolic health, aging, and longevity in ad libitum-fed mice. Cell Metab 19: 418-430.

30 Pezeshki A, Zapata RC, Singh A, Yee NJ, Chelikani PK (2016) Low protein diets produce divergent effects on energy balance. Sci Rep 6: 25145.

31 Sluijs I, van der Schouw YT, van der ADL, Spijkerman AM, Hu FB, et al. (2010) Carbohydrate quantity and quality and risk of type 2 diabetes in the European prospective investigation into cancer and nutrition-netherlands (EPIC-NL) study. Am J ClinNutr 92: 905-911.

32 Sluijs I (2011) Diet, Intermediate Risk Markers and Risk of Type 2 Diabetes PhD Julius Center, Utrecht University, UMCU trecht.

33 Greenwood DC, Threapleton DE, Evans CEL, Cleghorn CL, Nykjaer C, et al. (2013) Glycemic index, glycemic load,carbohydrates, and Type2 diabetes: systematic review and dose-response meta-analysis of prospective studies. Diabetes Care 36: 4166-4171.

34 Jain C, Singh A, Kumar P, Gautam K (2014) Anti-diabetic potential of flavonoids and other crude extracts of stem bark of Mango Linn: a comparative study. J Sci Innov Res 3: 21-27.

35 Schaeffer O (1971) When the Eskimo comes to town. Nutr Today 6: 8-16.

36 Trowell HC (1980) From normotension to hypertension in Kenyans and Ugandans 1928-1978. East Afr Med J 57: 167-173.