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## Weight Gain and Adipose Tissue Accumulation in Diabetic and Prediabetic Rats Fed with Palm Olein enriched High Fat Diet

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### ABSTRACT

Diabetic is known to be the main non-commutable public health concern. In this study, we evaluated the relationship between diet, body weight and accumulation of adipose tissue in healthy, diabetic and prediabetic rats. Physiologically, adipose tissue plays a central role in lipid and glucose metabolisms. Therefore, accumulation of adipose tissue in diabetic and prediabetic may lead to others non-commutable diseases. The rats in control, diabetic and prediabetic groups were fed with normal and high fat diets for 6 consecutive weeks, while the rats in 4 sub-groups of diabetic and prediabetic groups also received metformin, a common drug to control diabetes. The results indicated that in diabetic rats, a small increase of food calories by high fat diet caused a significant gained in body weight and a significant increase in adipose tissue accumulation with or without metformin treatment, however in prediabetic rats, rats with metformin treatment shown to be beneficial in maintaining body weight and adipose accumulation. The data of the study imply that balance diet at high calories should be practiced by patients as high fat diet with similar calories can caused a significant health deterioration to the patients in term of body weight gained and adipose tissue accumulation.

### INTRODUCTION

Diabetes Mellitus, an epidemic chronic disease affecting a major population of the world, is the most common non-communicable diseases of present era. It is a metabolic disease characterized by hyperglycemia with the symptoms include weight loss, polyuria, polydipsia and polyphagia. Individuals may experience sudden weight loss when ones develop diabetes mellitus. Diabetic patients are usually restricted by diet intake in order to regulate their blood sugar. Palm oil has a balanced ratio of unsaturated and saturated fatty acids. It contains 40% oleic acid (monounsaturated fatty acid), 10% linoleic acid (polyunsaturated fatty acid), 45% palmitic acid and 5% stearic acid (saturated fatty acid), it is the most consumed fat in Asia countries [1].

High intake of energy and reduction in energy consumption leads to lipid accumulation, while excess lipid accumulation causes obesity, which resulted in the body weight increase and subsequently lead to adipose tissue accumulation. Adipose is an

active endocrine organ exists in adipocytes and it is part of vascular-stromal fraction in which macrophages, fibroblasts, endothelial cells and preadipocytes are present. Pre-adipocytes produce new fat cells throughout the entire human life [2], they are derived from a multipotent stem cell of mesodermal origin. The primary function of adipose tissue is to protect and cushion the body. Adipose tissue in the form of free fatty acids is formed after food intake, and during fasting phase the free fatty acids are released to maintain sufficient energy level of body. Besides serving as a storage place for fatty acids, adipose tissue has a central role in lipid and glucose metabolism and produces various hormones and cytokines, e.g. angiotensinogen, tumour necrosis factor- $\alpha$  (TNF- $\alpha$ ), interleukin-6, adiponectin, leptin, and plasminogen activator inhibitor-1 [3-5]. Excessive accumulation of lipid species is potentially toxic which may lead to over-activation of lipid signalling pathways. The toxic lipid species may cause cellular distress and dysfunction which sometimes results in apoptotic cell death or lipoapoptosis [6]. Obesity, a highly complex multifaceted disease, is one of the major

contributors to cardiovascular diseases, and it is the strongest risk factor for development of type 2 diabetes mellitus. Progression in obesity promotes significant cellular changes in adipose tissue like triggering of hypertrophic adipocyte expansion and immune cell infiltration [7-9]. Enlarged adipocytes demonstrate decreased insulin responsiveness, decreased glucose uptake, and increased secretion of proinflammatory adipokines [10-13]. Moreover, secretion of free fatty acids and TNF- $\alpha$  are upregulated in enlarged adipocytes, which plays a prominent role in the development of insulin resistance. Free fatty acids decrease insulin sensitivity and as insulin is the main regulator to limit enzyme activity in triglyceride hydrolysis, lipolysis in adipocytes increases. Thus, when energy input is more than output, blood glucose and blood triglycerides will be elevated, and the insulin resistance will lead to fat accumulation in liver and muscle [14].

Dietary and lifestyle modifications can be effective for the treatment and prevention of diabetes as dietary components have significant and clinically relevant effects on blood glucose regulation. However, difficulties in maintaining these styles for long terms is a hurdle. Although there is no specific recommended diet for the prevention of type 2 diabetes. In this study, we examine the effect of fat from the most consumed plant oil on adipose accumulation in diabetic rats, which is hoped to be helpful in the management of diabetes diet amongst the patients.

## MATERIALS AND METHODS

### Materials

Ketamine was purchased from Troy Laboratories Australia, while Xylazine was obtained from Indian Immunological Limited India. Metformin tablets were procured from Dynapharm, Malaysia. All other chemicals used in this study were purchased from Sigma Aldrich USA.

## RESULTS AND DISCUSSION

### Animals and housing

A total of 76 male Sprague Dawley rats weighing between 300g to 350g were obtained from the Animal Research Center and Service, Universiti Sains Malaysia. The rats were randomly divided and housed individually in plastic cages under controlled environment at 22-24 °C with 12 hours light/dark cycles. Prior to starting of the experiment, all the rats were quarantined for at least 7 days for environmental adaptation and stabilization. All the experimental procedures were approved by Animal Ethics Committee of Universiti Sains Malaysia (USM/Animal Ethics Approval/2016/ (717)).

### Diets Preparation

The customized feed powder was purchased from Altormin (Lage, Germany). The self-made normal diet pellets were prepared by mixing the feed powder and distilled water at 1:1. Meanwhile, high fat diet (HFD) pellets were prepared by adding cooking oil, consisting of 100% palm olein (22.4 % v/w) to the feed powder followed by addition of distilled water (1:1 ration). Both diets were baked in oven overnight at 40 °C.

### Induction of Diabetes

Seventy-six male Sprague Dawley rats were randomly divided into control (non-diabetic) and experimental groups. Twelve rats were segregated as control group while experimental groups consisted of 48 animals. In experimental groups, diabetes was induced in animals by intraperitoneal injection (IP) of streptozotocin (STZ). Prior to induction, the rats were kept on fasting for 12 hrs. On completion of fasting period, nicotinamide

(NA) dissolved in normal saline was administered to the peritoneal cavity of rats at dose of 110 mg/kg. After 15 minutes of NA administration, STZ (dissolved in 0.1M sodium citrate dihydrate, pH 4.5) was administered in animals of experimental groups through IP injection at a dose of 65mg/kg. On the other hand, the animals in the control group (non-diabetic) were injected with vehicle (saline and citric buffer). The animals in both control and experimental group were monitored for 4 weeks. Fasting blood glucose (FBG) and body weight (BW) of animals were monitored and recorded weekly throughout the monitoring period.

### Experimental Design

All the rats in experimental and control groups were fed with normal diet during the monitoring period. After 4 weeks of the monitoring period, rats in control group (non-diabetic) were subdivided as Group 1 (G1) and Group 2 (G2), where each group consisted of six to nine animals. Furthermore, based on the rats' FBG level, the experimental groups were categorized as prediabetic and diabetic groups. The rats with FBG level of > 7 mmol/L were classified as diabetics while those with FBG level between 5.6 - 6.9 mmol/L were grouped as prediabetics category [15]. Prediabetic and diabetic groups were further subdivided into four groups as G3- G10 (Group 3 till Group 10) according to their dietary plans. Animals in all groups were fed with their respective diets either as normal diet or HFD. Table 1 describes the groups with their diet treatment.

Metformin drug was introduced in diet treatment period by suspending metformin tablets (250 mg/kg) in 1% carboxymethylcellulose and administered orally twice a day. In both monitoring and diet treatment periods, water was given ad libitum and approximately 25 - 26g of food pellets were supplied to the rats daily. FBG and body weights were monitored once a week for 6 consecutive weeks.

### Collection of adipose tissue

On completion of 6 weeks period of diet treatment, all the rats were euthanized by injecting ketamine (75mg/kg) and xylazine (8g/kg) cocktail IP. The adipose tissues were harvested and weighed.

### Statistical Analysis

The data are presented as mean  $\pm$  standard deviation. All the data were statistically analysed by using Graph Prism version 5. One-way analysis of variance (ANOVA) followed by Tukey's Test was applied for assessment of data. The Significance level was set as 95%, 99% and 99.9% confidence level.

## RESULTS

**Table 2** shows the calories of the food consumed by the rats (mean  $\pm$  SEM, n= 6 to 9). The same amount of food was fed to all the rats in this study, the unfinished food was measured by end of each feeding. The normal diet was purchased from Altormin, Germany whilst the HFD was modified from normal diet by adding 22.4% palm oil. The calories of normal diet is 3.61 kcal/g while the calories for HFD is 4.575 kcal/g. The difference in calories between normal diet and HFD was 96.5 Kcal per 100g. **Table 3** shows the weight gained of the rats after week 6<sup>th</sup> being fed with normal diet and HFD (mean  $\pm$  SEM, n= 6 to 9). The highest increased in body weight was seen in diabetic rats fed with HFD (G4) while diabetic rats fed with normal diet (G4) showed the greatest loss in body weight. **Table 4** shows the weight of adipose tissue after week 6<sup>th</sup> of the study while **Fig. 1** and **Fig. 2** show the pictures of adipose tissue accumulations for each group of rats (mean  $\pm$  SEM, n= 4 to 9)

**Table 1.** Experimental design, grouping and dietary plan of the study.

| Rats                | Pathological condition | Group | Diet        | Metformin |
|---------------------|------------------------|-------|-------------|-----------|
| Control Group       | Non-diabetic           | G1    | Normal diet | No        |
|                     |                        | G2    | HFD         | No        |
|                     |                        | G3    | Normal diet | No        |
|                     | Diabetic               | G4    | HFD         | No        |
|                     |                        | G5    | Normal diet | Yes       |
|                     |                        | G6    | HFD         | Yes       |
| Experimental Groups | Prediabetic            | G7    | Normal diet | No        |
|                     |                        | G8    | HFD         | No        |
|                     |                        | G9    | Normal diet | Yes       |
|                     |                        | G10   | HFD         | Yes       |

**Table 2.** Calories of the food consumed by the rats (mean  $\pm$  SEM, n= 6 to 9).

| Group                  | Diet                    | Food intake (g/day) | Food calories intake (kcal/day)       |
|------------------------|-------------------------|---------------------|---------------------------------------|
| G1 (Control Rats)      | Normal diet             | 19.93               | 71.95 $\pm$ 1.69 <sup>a</sup> (n=7)   |
| G2 (Control Rats)      | HFD                     | 17.52               | 80.15 $\pm$ 2.06 <sup>b,c</sup> (n=6) |
| G3 (Diabetic Rat)      | Normal diet             | 24.48               | 87.80 $\pm$ 0.95 <sup>a,d</sup> (n=9) |
| G4 (Diabetic Rat)      | HFD                     | 21.64               | 99.55 $\pm$ 2.20 <sup>b,d</sup> (n=9) |
| G5 (Diabetic Rat)      | Normal diet + Metformin | 23.23               | 83.56 $\pm$ 1.83 (n=9)                |
| G6 (Diabetic Rat)      | HFD + Metformin         | 20.97               | 95.39 $\pm$ 2.62 <sup>c</sup> (n=8)   |
| G7 (Prediabetic rats)  | Normal diet             | 20.49               | 74.07 $\pm$ 1.09 (n=8)                |
| G8 (Prediabetic rats)  | HFD                     | 18.57               | 84.95 $\pm$ 4.44 (n=6)                |
| G9 (Prediabetic rats)  | Normal diet + Metformin | 20.63               | 74.47 $\pm$ 2.79 <sup>e</sup> (n=7)   |
| G10 (Prediabetic Rats) | HFD + Metformin         | 19.76               | 90.40 $\pm$ 3.31 <sup>e</sup> (n=7)   |

Means with the same letter significantly different from each other (P<0.05 ANOVA followed by Tukey post test)

<sup>a</sup> group 1 vs group 3 showed significant level of P<0.001

<sup>b</sup> group 2 vs group 4 showed significant level of P<0.001

<sup>c</sup> group 2 vs group 6 showed significant level of P<0.01

<sup>d</sup> group 3 vs group 4 showed significant level of P<0.01

<sup>e</sup> group 9 vs group 10 showed significant level of P<0.001

**Table 3.** Weight gained of the rats after week 6<sup>th</sup> being fed with normal diet and HFD (mean  $\pm$  SEM, n= 6 to 9).

| Group                  | Diet                    | Weight Gained in gram (Final Body Weight – Initial Body Weight before feeding programme) |
|------------------------|-------------------------|--|
| G1 (Control Rats)      | Normal diet             | +16.3 $\pm$ 3.94 (n=7)   |
| G2 (Control Rats)      | HFD                     | +24.28 $\pm$ 4.49 (n=6)  |
| G3 (Diabetic Rat)      | Normal diet             | -9.72 $\pm$ 6.63 (n=9)   |
| G4 (Diabetic Rat)      | HFD                     | +31.69 $\pm$ 5.27 (n=9)  |
| G5 (Diabetic Rat)      | Normal diet + Metformin | -7.78 $\pm$ 6.02 (n=9)   |
| G6 (Diabetic Rat)      | HFD + Metformin         | +16.35 $\pm$ 6.83 (n=8)  |
| G7 (Prediabetic rats)  | Normal diet             | +15.50 $\pm$ 5.64 (n=8)  |
| G8 (Prediabetic rats)  | HFD                     | +33.02 $\pm$ 9.95 (n=6)  |
| G9 (Prediabetic rats)  | Normal diet + Metformin | +12.40 $\pm$ 8.56 (n=7)  |
| G10 (Prediabetic Rats) | HFD + Metformin         | +11.34 $\pm$ 15.62 (n=7)   |

**Table 4.** Adipose tissue weight after week 6<sup>th</sup> of the study (mean  $\pm$  SEM, n= 4 to 9).

| Group                  | Diet                    | Weight of Adipose Tissue Accumulated (g)          |
|------------------------|-------------------------|---|
| G1 (Control Rats)      | Normal diet             | 6.14 $\pm$ 1.67 <sup>a</sup> (n=5)                |
| G2 (Control Rats)      | HFD                     | 7.96 $\pm$ 3.00 <sup>b,c</sup> (n=5)              |
| G3 (Diabetic Rat)      | Normal diet             | <sup>1</sup> 0.83 $\pm$ 1.30 <sup>a,d</sup> (n=9) |
| G4 (Diabetic Rat)      | HFD                     | 4.33 $\pm$ 1.08 <sup>b,d</sup> (n=9)              |
| G5 (Diabetic Rat)      | Normal diet + Metformin | <sup>2</sup> 0.79 $\pm$ 1.19 (n=8)                |
| G6 (Diabetic Rat)      | HFD + Metformin         | 3.00 $\pm$ 0.89 <sup>c</sup> (n=8)                |
| G7 (Prediabetic rats)  | Normal diet             | 4.05 $\pm$ 0.83 (n=6)                             |
| G8 (Prediabetic rats)  | HFD                     | 7.00 $\pm$ 0.25 (n=5)                             |
| G9 (Prediabetic rats)  | Normal diet + Metformin | 3.95 $\pm$ 0.98 (n=6)                             |
| G10 (Prediabetic Rats) | HFD + Metformin         | 5.38 $\pm$ 0.68 (n=4)                             |

Means with the same letter significantly different from each other (P<0.05 ANOVA followed by Tukey post test)

<sup>a</sup> group 1 vs group 3 showed significant level of P<0.001

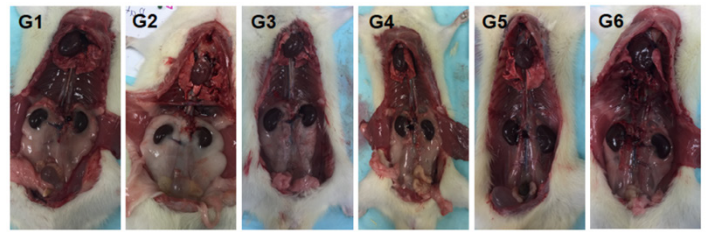
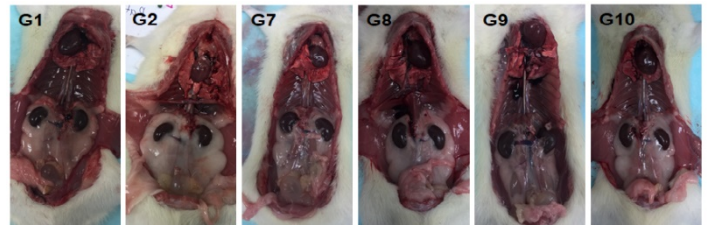
<sup>b</sup> group 2 vs group 4 showed significant level of P<0.001

<sup>c</sup> group 2 vs group 6 showed significant level of P<0.01

<sup>d</sup> group 3 vs group 4 showed significant level of P<0.05

<sup>1</sup> only 5 rats out of 9 rats formed adipose tissue

<sup>2</sup> only 3 rats out of 8 rats formed adipose tissue

**Fig. 1.** Effect of diets on adipose tissue accumulation in abdominal cavity of non-diabetic and diabetic rat. G1 and G2 represent the control rats fed with normal diet and HFD respectively. While G3, G4, G5 and G6 represent the diabetic rats fed with normal diet, HFD, normal diet + metformin and HFD+ metformin.**Fig. 2.** Effect of diets on adipose tissue accumulation in abdominal cavity of non-diabetic and prediabetic rats. G1 and G2 represent the control rats fed with normal diet and HFD, respectively. While G7, G8, G9 and G10 represent the prediabetic rats fed with normal diet, HFD, normal diet + metformin and HFD+ metformin respectively.

## DISCUSSION

In this study, the rats with FBG level of > 7 mmol/L were considered as diabetics, following the diabetes guideline from America Diabetes Association. Besides the FBG level, the diabetic rats displayed symptoms of diabetes, which include polydipsia, polyuria and polyphagia. Nevertheless, there are also reports to suggest FBG level of 7.5 mmol/L as the minimum cut off point for diabetic rats [16, 17].

### The effect of high fat diet on the accumulation of adipose tissue in normal and diabetic rat

Diabetic patients are required to follow a low carbohydrate and a low-fat diet [19]. Strict diet in the form of controlled energy consumption is usually implied on diabetic patients in order to regulate their blood glucose for better management of the disease. Nevertheless, very few patients can abide to the strict diet requirement. Food fried in oil gives a better taste to the taste buds; therefore, these foods are more favourable by patients. Palm oil is commonly used in food preparation.

In this study, we used palm oil to increase calorie in animal feed and examine its effect on the body weight and adipose tissue accumulation in diabetic rats. A difference of 26.80% in calories between normal diet and HFD was used in this study. In general, diabetic rats are consuming more calories than control groups. When comparing the quantity of food consumption by animal, G4, diabetic rats fed with high fat diet appeared to be the rats that consumed the most food given, which is equivalent of 99.55  $\pm$  2.20 kcal calories. G1, control rats with normal diet was the group consumed the least amount of calories (71.95 $\pm$ 1.69 kcal). The uptake of the energy is not directly proportional to the increase in body weight. Although G1 animal consumed the least amount of calories, they shown an increase of 4.4% in body weight, which is equivalent to 16.3 $\pm$ 3.94g. The group that loss the highest body weight is G3, diabetic rats fed with normal diet (reduce 9.72 $\pm$ 6.63g), which show a greater loss in body weight when compared to G5, diabetic rats fed with normal diet with

metformin treatment (reduce  $7.78 \pm 6.02$ g of body weight). In this study, we have observed that in term of food calories, G2 animal (control rats with HFD) consumed 8.2 Kcal more energy compared to G1 animal (control rats with normal diet) and an increment of 7.98g of body weight and 1.82g (33% increment) of adipose tissue were recorded by G2 as compared with G1, where energy intake to weight gain ration is ~ 1:1 ration, this is in agreement with Warwick and Schiffman (18). Comparatively although the intake of total calories by diabetic rats fed with HFD (G4) was relatively low ( $< 10$  Kcal) when compared to those fed with normal diet (G3), nevertheless, this small difference in calories resulted in clear difference in the body weight gained by G4, where a total of 41.4g of body weight gain (1:4 ratio of calories intake to body weight gain) and 421.69% adipose tissue gained was recorded when comparing G4 to G3 rats. The body weight and adipose tissue in the body are closely associated [19], where the higher increase in body weight also resulted in increased accumulation of adipose tissue. Different kind of fats and feeding duration will affect the body weight gained and fat accumulation of the rats. A study had showed that similar weight gain was recorded when T2DM mice were fed with palm oil, lard, rapeseed oil and safflower oil although much higher weight gained was observed in soybean oil. In the same study, The parametrial WAT wet weight also reported to show parallel result to the body weight [20].

In current study, it was observed that the diabetes mellitus caused the adipose tissue weight to decrease significantly regardless the diet fed (showed in Table 3 and Figure 1). There was a 86.48% ( $P < 0.001$ ) reduction in the adipose tissue weight in G3 as compared to G1 rats. The same observation was seen between G2 and G4, control and diabetic rats fed with HFD, G2 control rats had higher content of adipose tissue ( $7.96 \pm 1.34$ g) as compared to diabetic rats (G4), which was equivalent to 45.60% ( $P < 0.01$ ) reduction in adipose tissue. However, not all diabetic rats accumulated adipose tissue when fed with normal diet. Only 5 rats out of 9 in G3 (diabetic rat fed with normal diet). The drop in adipose tissue deposits mainly due to insulin deficiency or cell insensitivity to insulin. Insufficient insulin or the insensitivity drive the body to gain the energy from burning fat or muscle instead of glucose from the blood. In addition, It was also reported that the insulin deficiency inhibited the proliferation of white adipose tissue cells and stimulated the degeneration process of adipocytes which in turn reduced the accumulation of adipose tissue [21].

We observed that in both control and diabetic groups, the animals fed with HFD showed an increased accumulation of the adipose tissue as compared to those fed with normal diet. Statistical analysis of the results revealed that the accumulation of adipose tissue in diabetic rats fed with HFD (4.33g) was 5.22 times higher as compared to those with normal diet (0.83g) (G3). Earlier study had reported that HFD enhanced the adipocyte size and also increased the number [22]. As the matter, the accumulation of fat mass increased in the body causing obesity. Numerous studies have shown that obesity, especially abdominal fat depots cause various diseases. Adiposity, especially abdominal adiposity increased the risk of insulin resistance, diabetes, cardiovascular disease, hypertension, non-alcoholic fatty liver disease, kidney disease, infertility, osteoporosis and inflammation. Besides all these, studies have also shown that obesity and its related diabetes are also risk factors for all types of cancer [23].

One of the most common drugs to treat diabetes mellitus is metformin (dimethylbiguanide) as it shows clear benefits in diabetes treatment. Metformin is an antihyperglycemic agent

which acts by decreasing hepatic glucose production, increasing peripheral glucose uptake and halting gastrointestinal glucose intake [24, 25]. Metformin's effectiveness reduced in the absence of insulin as it improves insulin sensitivity but not the insulin production, like sulfonylurea [25]. Compared to sulfonylurea and insulin treatments, no elevation in body weight or adipose tissue accumulation is the "positive" side effect of metformin [26, 27]. Instead, metformin treatment has shown some weight loss [28]. The influencing characteristic of metformin on AMP-activated protein kinase, major player in lipid metabolism, leads to different effect on adipose tissue and muscle mechanism [29-32]. In this study the effect of diet on adipose tissue accumulation in diabetic rats which received the metformin as treatment drug was also observed. Similar to G3, it was observed that only 3 rats out of 8 not all rats in the diabetic group fed with normal diet and treated with metformin (G5) formed adipose tissue. The similar observation with diabetic groups without treatment was observed, where a slight increase in food calories intake (12 Kcal) in diabetic group fed with HFD and treated with metformin (G6) when compared to those with normal diet (G5), resulted in clear increase in rats body weight by 24.1g (1:2 ratio of calories intake to body weight gain) comparing to G5 while the accumulation of adipose tissue was 279.75% higher in G6 as compared to G5.

Comparatively, metformin reduced the accumulation of adipose tissue in both diabetic groups fed with normal diet and HFD. In those diabetic rats fed with normal diet, animals in G5 who received additional metformin treatment showed 4.82% less adipose tissue accumulation as compared to animals in G3 who did not receive metformin. Similar trend was showed in the ones fed with HFD, the animal G6 treated with metformin showed 30.72% less adipose tissue accumulation as compared to animals of G4 who without metformin treatment. The results of study are in accordance and supports outcomes of the previous studies that metformin prevents the adipose tissue accumulation [24, 33, 34].

#### Adipose tissue accumulation in Prediabetic rats

Prediabetic rats are rats with the fasting blood glucose level in the range of 5.6 to 6.9 mmol/L, it is of upmost importance to study this group of animals as the possibility of prediabetic to turn to diabetic is 70% higher than healthy people [35]. Prediabetes condition had also been associated with early nephropathy, increased kidney diseases, macrovascular disease and retinopathy [36]. Therefore, it would be interesting to look into the effect of HFD and the accumulation of adipose tissue in prediabetic rats as prediabetic patients have the same dietary recommendation as diabetic patients [37]. In general, prediabetic animal are taking relatively similar amount of food with control animal (Table 2), where the rats regardless control or prediabetic that were fed with HFD consumed slightly higher calories of food (+10 kcal) when comparing with those fed with ND. Nevertheless, the increased in body weight of prediabetic rats with HFD (G8) was 18g (1:1.8 ratio of calories intake to body weight gain) higher than those with normal diet (G7), which is directly proportional to adipose tissue accumulation (72.84%) higher in prediabetic rats with HFD.

Among the prediabetic groups fed with the same diet, the rats in group treated with metformin showed lower adipose tissue weight regardless of food (normal diet or HFD). The mean adipose tissue weight of the prediabetic group fed with normal diet and treated with metformin (G9) dropped 2.47% from  $4.05 \pm 0.83$ g to  $3.95 \pm 0.98$ g when compared to the prediabetic group fed with normal diet without metformin treatment (G7). On the other hand, the rats of prediabetic group fed with HFD (G8) accumulated 30.11% higher adipose tissue compared with

prediabetic group fed with HFD and treated with metformin (G10). In general, the accumulation of abdominal adipose tissue increased when the prediabetic rats were fed with HFD although not significant. Comparing between G9 and G10, rats treated with metformin and fed with normal diet and HFD, respectively, G10 animal consumed higher energy by 10 Kcal, nevertheless a loss of body weight by 1g and adipose accumulation increased by 36% were observed. Metformin treatment on prediabetic rats seems to be necessary as metformin treatment causes the prediabetic rats to be better than the control rats in term of body weight gained and adipose tissue accumulation regardless of type of diet fed.

The main intervention for diabetes and prediabetes treatment is lifestyle changes aiming weight reduction. Previous study showed that lifestyle intervention such as weight loss (7% of body weight) and physical activity (150 minutes per week) had positive effect, and there was 58% reduction of the risk of diabetes [38]. Another study showed that the risk of developing diabetes reduced 16% with every decrease of 1 kg body weight [39]. Therefore, prediabetes individuals are encouraged to reduce total fat intake less than 30 % of energy intake, saturated-fat intake less than 10 percent of energy intake.

## CONCLUSION

The results of this study showed that regardless of the quantity of food calories intake, the HFD enhanced the body weight and adipose tissue accumulation in diabetic, non-diabetic and prediabetic conditions. The fat used in this study is of plant origin, where the composition of saturated fatty acids is relatively low as compared to the animal fat, and in many occasions, plant fat is recommended as it was considered as healthy fat. Nevertheless, this study has shown that the use of HFD, although may be little in quantity in term of food calories, can cause a dramatic increase in body weight and adipose tissue accumulation. The use of metformin in prediabetic rats was found to be beneficial to the rats, where the body weight gained, and adipose tissue accumulation were under well controlled regardless of the type of diet fed. The findings of this study provide support for the development of proper dietary plan for the diabetes patients with therapy. Besides, this further knowledge about the mechanistic effects of diet on body weight and adipose tissue development and its pharmacological function can provide new and alternative targets in the drug development for the cure and management of diabetes.

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