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Contribution of high fat diet to the development of gestational diabetes mellitus in rats

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Gestational diabetes mellitus (GDM) is a glucose intolerance resulting in hyperglycemia with onset or first recognition during pregnancy. High fat diet (HFD) is one of the contributing factors to GDM. This study evaluated the contribution of HFD in differential adipose tissue (AT) expansion and subsequent development of GDM in Wistar rats. Pregnant and non-pregnant rats were given streptozotocin (STZ) by a single intraperitoneal injection or HFD throughout the experiment. The animals were sacrificed on day 1, 8, 15, or 21 of the experiment. Blood, adipose tissue and pancreas were collected and analyzed. In this study, STZ treated animals had a significant ($p<0.05$) increase in serum glucose and a decrease in insulin, without changes in the size of adipocytes. The levels of both serum glucose and insulin were significantly high in HFD fed animals ($p<0.05$); being higher in pregnant ($p<0.05$) than non-pregnant rats. The increase in glucose and insulin levels was associated with increase in the size (hypertrophy) than number (hyperplasia) of adipocytes. The increase in size of adipocytes was higher in viscera (VAT) than subcutaneous (SAT) adipose tissue and related to insulin resistance and GDM development. Histologically, the number of β-cells was decreased and deformed in STZ groups while maintained in HFD groups in both pregnant and non-pregnant animals. The findings of this study show that, intake of HFD during pregnancy may lead to AT expansion and thus insulin resistance, which is one of the risk factors for the hyperglycemia and development of gestational diabetes mellitus.

Key words: Pregnancy, insulin-resistance, adipose-tissue, subcutaneous, visceral, streptozotocin.

INTRODUCTION

Gestational diabetes mellitus (GDM) is any degree of impaired glucose tolerance recognized during pregnancy (Punthakee et al., 2018; Lin et al., 2022), or a complication in which spontaneous hyperglycemia advances during pregnancy (Nanobashvili et al., 2018). The condition is due to impairment of insulin secretion or

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metabolism during pregnancy (WHO, 1999; Punthakee et al., 2018). It is reported to be a serious metabolic disorder affecting women in both developed and developing world (Zhu and Zhang, 2016). Ineffective management of GDM may represent an early stage of future obesity, type 2 diabetes and other long-term health effects in the mother as well as the infant (Sugiyama, 2011; Macaulay et al., 2014; Ngala et al., 2017).

Several studies have reported that, insulin resistance and subsequent development of GDM are influenced by adipose tissue (AT) expansion among other factors (Simas and Corvera, 2014; Aziz et al., 2016; Li et al., 2016; Ngala et al., 2017). During pregnancy, AT expands in response to increased fetal growth (Simas and Corvera, 2014) and can expand further due to consumption of a high fat diet (HFD) (Musial et al., 2017). Fetal growth and HFD mediated AT expansion induce insulin resistance during pregnancy and are pivotal to the development of GDM (Aziz et al., 2016). The AT expansion may occur in subcutaneous (SAT) or visceral (VAT) adipose tissue (Kansu-Celik et al., 2018). Normally fat storage occurs within SAT, when its storage capacity is exhausted, the fats are stored in ectopic depots around the visceral organs (Smith and Kahn, 2017). The ability of SAT and VAT to accommodate fat storage with hyperplasia rather than hypertrophic expansion may determine the risk for GDM (Simas and Corvera, 2014).

While SAT or VAT expansion contributes to GDM development, it is not known as to how the differential fat deposition can contribute to GDM. Moreover, information on AT metabolic alterations and its contribution to GDM development are limited, thus the need for further investigation. Therefore, the current study investigated the contribution of differential AT deposition and expansion due to HFD in GDM development using Wistar rats. Knowledge on the mechanisms of the AT expansion and its contribution to the development of GDM is crucial in the search for management strategies (Simas and Corvera, 2014).

MATERIALS AND METHODS

Study area

The study was conducted at Small Animal Research Unit (SARU), at the College of Veterinary Medicine and Biomedical Sciences (CVMBS), Sokoine University of Agriculture (SUA), Morogoro, Tanzania.

Experimental animals

A total of ninety-six (96) female Wistar rats aged 8-10 weeks, weighing 130-160 g were used in the experiments. Animals were housed in a room with controlled temperature (22 ± 2°C), relative humidity (40% - 60%), and light cycle (12/12 h light/dark). Animals were maintained on pelleted feed before commencement of the experiment and given drinking water ad-libitum.

Ethical clearance

All experimental procedures in this study were approved by the Animal Research Ethics Committee (RPGS/R/ETHICS) of SUA. Guidelines for care and use of laboratory animals were effectively followed.

Experimental diet

The HFD and low fat diet (LFD) were prepared by using maize flour (carbohydrate), fish meal (protein) and beef tallow (fat), as per Gohir et al. (2019) with some modifications (Table 1). These ingredients were mixed and boiled while stirring constantly to prepare stiff porridge.

A standard pelletizer machine (KENWOOD, type MG51, designed in Hampshire, PO 9NH UK and made in China) was used to prepare feed pellets from the stiff porridge.

Experimental setup and animal treatment

Animals were allowed to acclimatize to the diet for two weeks before mating. A total of 32 animals were given HFD and 64 animals given LFD. In the course of acclimatization, animals were examined for estrous cycle as per Aziz et al. (2016). Those following a 4-day cycle were mated at 1:2 male to females. Mating was confirmed by the presence of vaginal plug, and noted as gestational day 0 (GD 0) of the experiment. Those confirmed mated were categorized as pregnant and the rest non-pregnant (Table 2).

Induction of experimental GDM

Experimental GDM was induced to 32 GD 0 LFD animals by single intraperitoneal (IP) injection of 50 mg/kg streptozotocin in 0.1 mol/l citrate buffer (pH 4.5). Nicotinamide (NA) at 120 mg/kg was injected intraperitoneally 15 min before STZ to protect pancreatic cells against the severe cytotoxic effects of STZ. The remaining control and HFD rats received citrate buffer only (Table 2). In the course of gestation, samples were collected on days 1, 8, 15, and 21 by sacrificing 4 animals from each group following intramuscular injection of combination of ketamine and xylazine at 50 and 5 mg/kg, respectively (Table 2).

Sample collection

From the sacrificed animals, whole blood (by cardiac puncture) for glucose and insulin analyses as well as AT and pancreas for histological analyses were collected. SAT and VAT were obtained from lateral abdomen and the visceral organs (omenta), respectively.

Blood samples were collected into plain and heparinized vacutainers, followed by centrifugation at 2500 rpm for 12 min by using centrifuge (MPW M-Diagnostic, model; M-universal, Poland). Serum and plasma were transferred into eppendorf tubes and kept at -20°C until further analysis.

Biochemical analyses

Glucose levels were determined using Trinder's method, End point kit (Erba Mannheim GmbH, India).

The absorbance of standard and samples were read at a wavelength of 505 nm. The concentration of glucose was calculated as:

Table 1. Composition of HFD and LFD.

HFD		LFD	
Composition	**Kcal/1000 g**	**Composition**	**Kcal/1000 g**
Carbohydrate 20% (200 g)	823.2	Carbohydrate 54% (537 g)	2150.40
Protein 20% (200 g)	819.88	Protein 29% (291 g)	1167.04
Fat 60% (600 g)	5743.35	Fat 17% (172 g)	1553.04
Total energy (kcal/1000 g)	7386.43	Total energy (kcal/1000 g)	4870.48

Source: Author

Table 2. Experimental setup.

Group	Treatment/Status	Number of animals Sacrificed				Total
		Day1	Day 8	Day 15	Day 21	
1	Pregnant (P+) *	4	4	4	4	16
2	Non - pregnant (P-)*	4	4	4	4	16
3	pregnant (HFDP+)	4	4	4	4	16
4	non- pregnant (HFDP-)	4	4	4	4	16
5	STZ pregnant (STZP+) *	4	4	4	4	16
6	STZ non- pregnant (STZP-)*	4	4	4	4	16
	Total	24	24	24	24	96

*LFD.
Source: Author

$$\text{Glucose conc}\left(\frac{\text{mg}}{\text{dl}}\right) = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \times \text{Conc of standard}\left(\frac{100\text{mg}}{\text{dl}}\right)$$

Insulin levels were determined using Rat Insulin ELISA kit (Colorimetric) NBP3-00515 (Bio-Techne, UK). The optical density (OD) of the samples was determined spectrophotometrically at a wavelength of 450 nm. The duplicate readings of each standard and sample were then averaged. The average standard OD were subtracted by the average zero standard OD to get the corrected OD. A standard curve was plotted then used to calculate the concentrations of insulin from the samples.

Histopathological analysis of adipose tissue and pancreas

The obtained SAT, VAT and pancreas samples were fixed in 10% neutral phosphate buffered formalin overnight and then washed for 2 h. Processing involved dehydrating the tissue in serial graded ethanol (70, 80, 90%, and absolute), then clearing in xylene, and embedding in paraffin wax. The produced tissue blocks were sectioned to obtain 4 µm tissue sections that were stained with Hematoxylin and Eosin then mounted using permanent optical grade glue to insure adhesion. The sizes of adipocytes were measured using micrometer of the microscope eyepiece as per Khan et al. (2009) and pancreatic tissue cells morphology were viewed using a binocular light microscope (Olympus Corporation, U-DO3, S/N 9M11951, Tokyo, Japan).

Data analysis

Statistical package for Social Sciences (SPSS version 25) was used for data analysis. The normality of the data for qualitative variables was demonstrated using histogram. All quantitative variables were presented as mean ± SEM. The univariate general linear model (GLM) analyzed the association of the dependent and independent variables. The factorial analysis of variance (ANOVA) was used to compare means among the six treatment groups in different days. Post hoc Turkey's test was used for multiple comparisons among the groups which showed significant difference. The differences were considered statistically significant at 95% confidence interval ($p < 0.05$).

RESULTS

Glucose and insulin levels

The levels of glucose and insulin in pregnant (P+) and non-pregnant (P-) Wistar rats treated with STZ or given HFD are as shown in Figure 1. In both P+ and P- groups, STZ induced highest levels of glucose throughout the experiment than HFD, which was higher than the controls ($p<0.05$) (Figure 1A). These levels were higher in STZP+ than STZP- animals beyond day 8 of the experiment ($p<0.05$). On day 1, the levels of glucose in STZP+ animals were $2.49\pm0.3\times10^2$ mg/dl equivalent to 2.2 folds over the controls (P+) value. The levels peaked on day 15 to $3.7\pm0.45\times10^2$ mg/dl equivalent to 3.4 folds over their controls (P+), and remained the same to the end of the gestation (day 21). In STZP-, the levels of glucose were $2.3\pm0.08\times10^2$ mg/dl, equivalent to 2.3 folds over the control (P-) value on day 1. These levels peaked on day

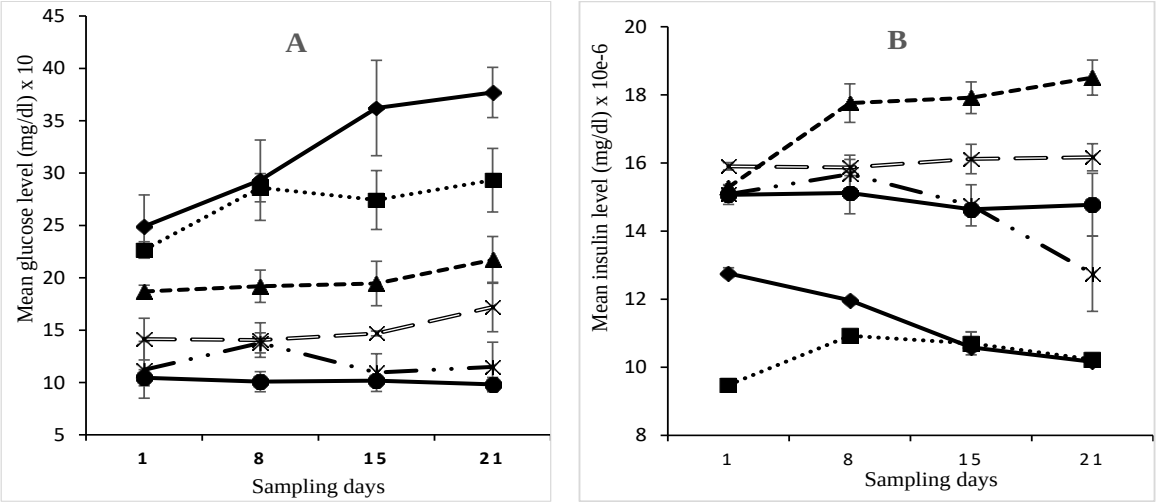

Figure 1. Mean serum levels of glucose (A) and insulin (B) from pregnant and non-pregnant Wistar rats treated with streptozotocin (STZ) or given high fat diet (HFD). Pregnant rats treated with STZ (▪◆▪), non-pregnant rats treated with STZ (▪■▪), pregnant rats treated with HFD (▬▲▬), non-pregnant rats treated with HFD (═✕ ▬), P+ (▬✕ ▪) and P- (▬●▬)
Source: Author

Figure 1A also shows that, HFD induced elevated levels of glucose throughout the experiment, with HFDP+ animals showing significantly higher levels ($p<0.05$) than HFDP- animals. The levels in HFDP+ animals were$1.9\pm0.15\times10^{2}$ mg/dl, equivalent to 1.7 folds over the controls (P+) throughout the experiment. In HFDP- animals, levels of glucose on days 1, 8 and 15, were $1.4\pm0.16\times10^{2}$ mg/dl equivalent to 1.4 folds over the controls (P-). These levels increased on day 21 up to $1.7\pm0.24\times10^{2}$ mg/dl equivalent to 1.7 folds over their controls.

Figure 1B shows that, the levels of insulin in STZP+ animals were $1.28\pm0.017\times10^{-5}$ mg/dl on day 1, lower than the controls (P+) value of $1.5\pm0.006\times10^{-5}$ mg/dl. The levels decreased with gestation, reaching the lowest ($1.0\pm0.004\times10^{-5}$ mg/dl) on day 21. In STZP- animals, insulin levels were lowest on day 1, $0.9\pm 0.017\times10^{-5}$ mg/dl, equal to 0.6 folds below the controls (P-) ($1.49\pm0.03\times10^{-5}$ mg/dl). It slightly increased on day 8 to $1.05\pm0.008\times10^{-5}$ mg/dl, equivalent to 0.7 folds below the controls (P-) and remained the same throughout the experiment. STZP+ animals showed significantly high levels of insulin ($p<0.05$) than STZP- animals from day 1 to day 8 of the experiment. HFDP+ animals had a significant increase in insulin levels on day 8 to $1.78\pm0.05\times10^{-5}$ mg/dl, which was maintained to day 15 and increased on day 21 to $1.85\pm0.05\times10^{-5}$ mg/dl, equal to 1.5 folds over their controls (P+). The levels of insulin in HFDP- animals were $1.6\pm0.03\times10^{-5}$ mg/dl, equivalent to 1.1 folds over their controls (P-) throughout the experiment. The HFDP+ animals showed significantly higher levels of insulin ($p<0.05$) than the HFDP- animals throughout the experiment except on day 1. In both pregnant and non-pregnant animals, the levels of insulin were significantly higher in HFD and lower in STZ treated animals ($p<0.05$) than their controls throughout the experiment.

Histopathology of the pancreas

The structure of pancreatic islets and number of β-cells in rats treated with STZ or given HFD are asshown in Figures 2 (P+) and 3 (P-). In STZ treated P+ and P- animals, there was distortion of the structure of islets with progressive decrease in the number of β-cells with time of experiment (from day 1 to 21) (Figures 2C and 3C, respectively). On the other hand, the structure of Islets and number of β-cells were maintained in the HFD groups (P+ and P-) throughout the experiment (Figures 2B and 3B, respectively). Generally, there was high number of normal β-cells in HFD than STZ groups.

Histopathology of adipose tissue during gestation

The size of adipocytes from animals treated with STZ or given HFD is as shown in Figures 4 and 5. The size of VAT (Figure 4) and SAT (Figure 5) adipocytes increased with time of experiment, which was significantly higher ($p<0.05$) in HFD group compared to their counterpart controls and STZ groups ($p<0.05$); VAT adipocytes being slightly higher than the SAT ($p<0.05$). Furthermore, pregnant groups given HFD, showed insignificant ($p>0.05$) increase in the size of VAT and SAT adipocytes compared to non-pregnant groups towards the end of the

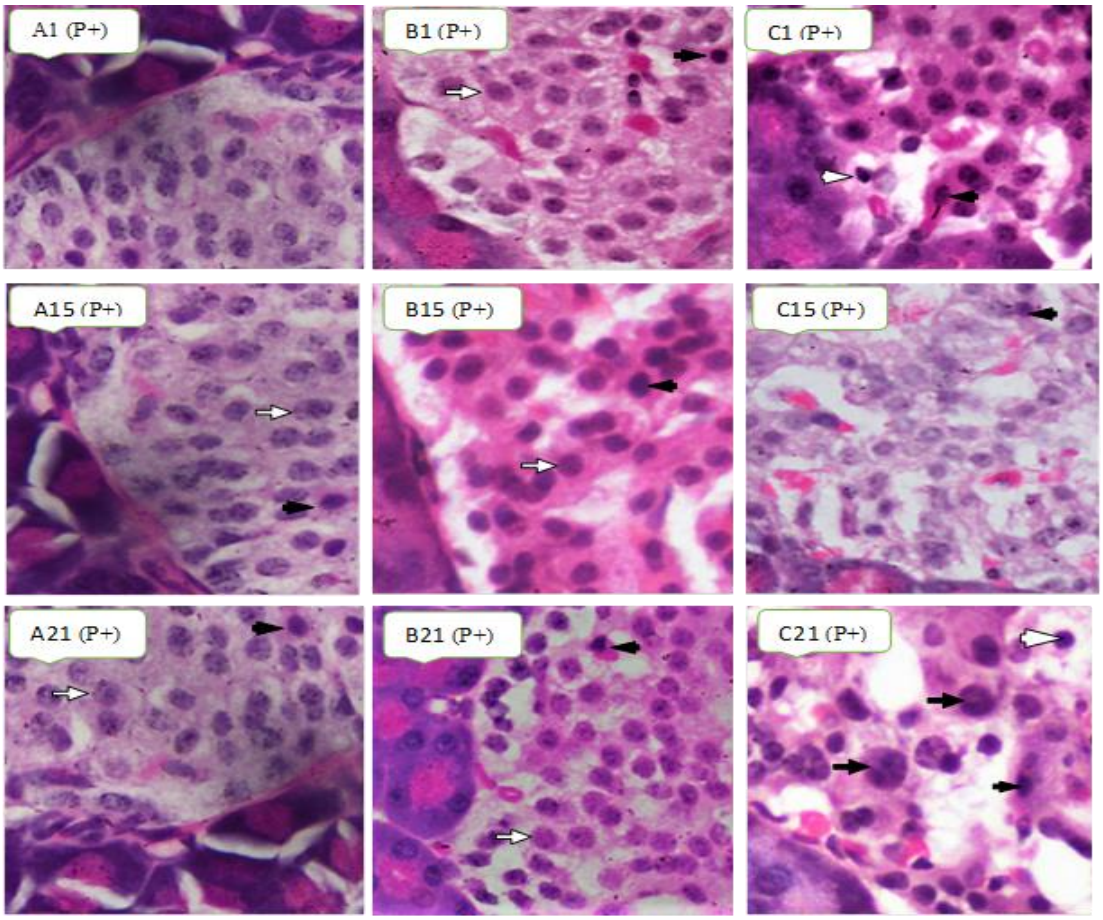

Figure 2. Histopathology of the pancreas (H &E, X 400) from pregnant Wistar rats. (A) section from control animals, showing normal islets of Langerhans and β-cells. (B) section from HFD given animals, showing normal islets of Langerhans and β-cells. (C) Section of STZ treated animals, showing degeneration of Langerhans, small number of β-cells, vacuolation and pyknosis, and giant cells formation. Sections 1: from day 1, 15: from day 15 and 21: from day 21 of the experiment. (⇨) normal cells, (➡) giant cells, (➡) cells that undergo pyknosis, (⇨) cells that undergo vacuolation.
Source: Author

experiment (day 21).

DISCUSSION

The study provides data that shows biochemical parameters (glucose and insulin), histopathology of SAT, VAT and the pancreas on both pregnant and non-pregnant animals. The levels of glucose were higher in STZ treated animals than the HFD given groups, which had also higher levels of glucose than the control groups. And the levels of insulin were higher in HFD and lower in STZ than the control groups.

The high levels of glucose observed in STZ treated animals might be attributed to the destruction of the pancreatic β-cells by the drug. This resulted into failure in insulin production and finally low levels of insulin. This is in agreement with other studies (Saad et al., 2015; Waer and Helmy, 2017; Özdek et al., 2020; Hrachik et al., 2020) which reported high levels of glucose and low levels of insulin in rats treated with STZ. However, from days 1 to 8 of the experiment the levels of insulin in STZ treated animals were high, then decreased to day 21 of the experiment. This can be explained from the fact that, at the early days of STZ injection insulin levels are high as a result of massive streptozotocin-induced necrosis of β-cells resulting into release of insulin into the blood circulation. This finding is further linked with histopathology of the pancreas from STZ treated animals, which showed distortion of the structure of islets of Langerhans, marked decrease in the number of β-cells on days 15 and 21 of the experiment. This is comparable to Waer and Helmy (2012), who observed disorganization of the structure of the endocrine and exocrine cells of pancreas in a group of albino rats treated with STZ. This destruction of the β-cells is the possible cause of the

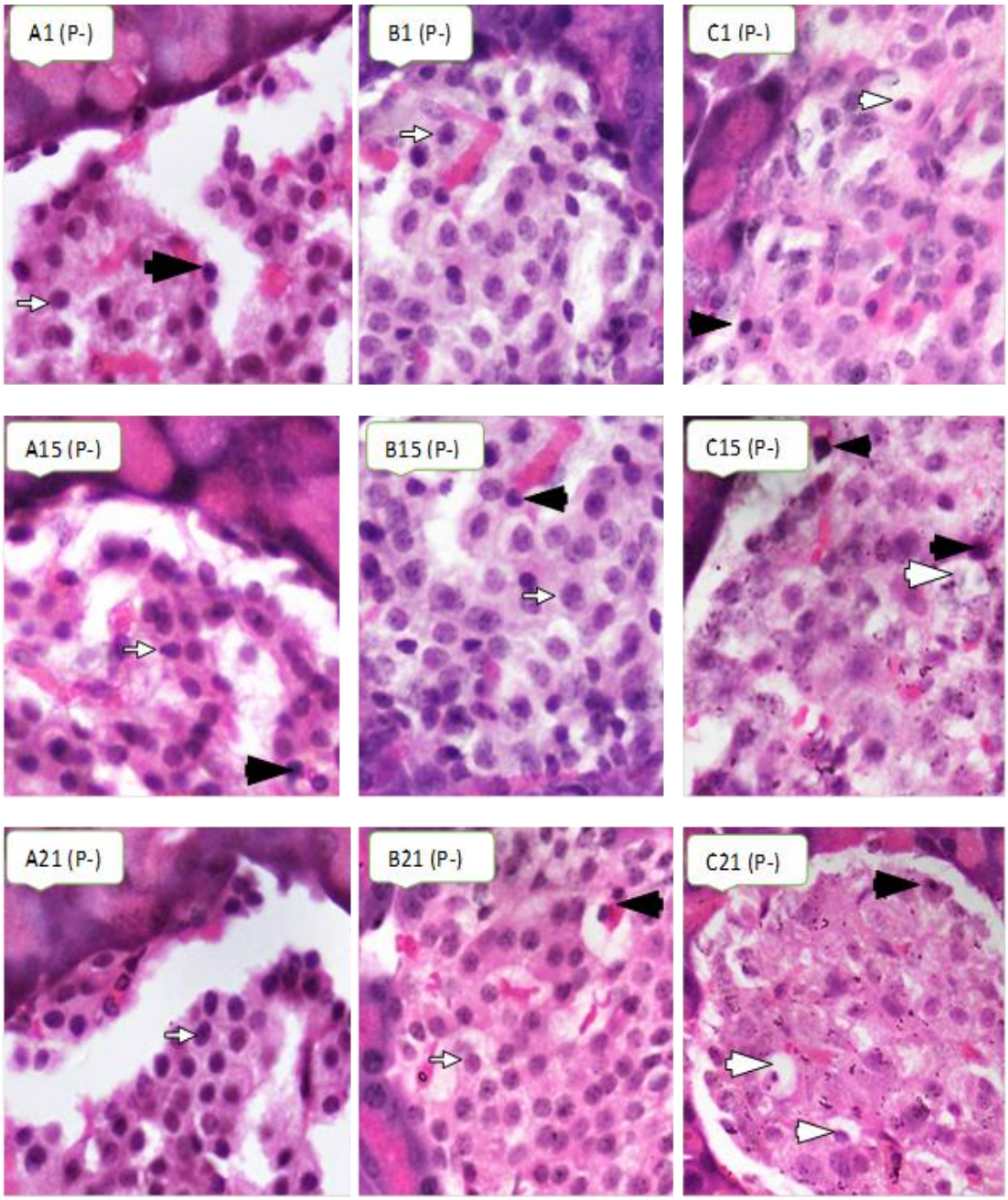

Figure 3. Histopathology of the pancreas (H &E, X 400) from non-pregnant Wistar rats. (A) sections from control animals, showing normal islets of Langerhans and β-cells. (B) sections from HFD given animals, showing normal islets of Langerhans and β-cells. (C) sections from STZ treated animals, showing degeneration of Langerhans, small number of β-cells, vacuolation and pyknosis, and giant cells formation. Sections 1: from day 1, 15: from day 15 and 21: from day 21 of the experiment. (⇨) normal cells, (➨) cells that undergo pyknosis, (⇨) cells that undergo vacuolation.
Source: Author

decrease in the levels of insulin and increase in glucose levels observed in STZ treated groups. This agrees with Holemans et al. (2004), Saad et al. (2015) and Hrachik et al. (2020) who described the destruction of pancreatic β-cells in rats caused by STZ and its effects on the levels of insulin and glucose.

In HFD groups, the levels of glucose were high despite the high levels of insulin. This could be due to insulin resistance as a result of fat accumulation in the body. Increased accumulation of fat in the body results into

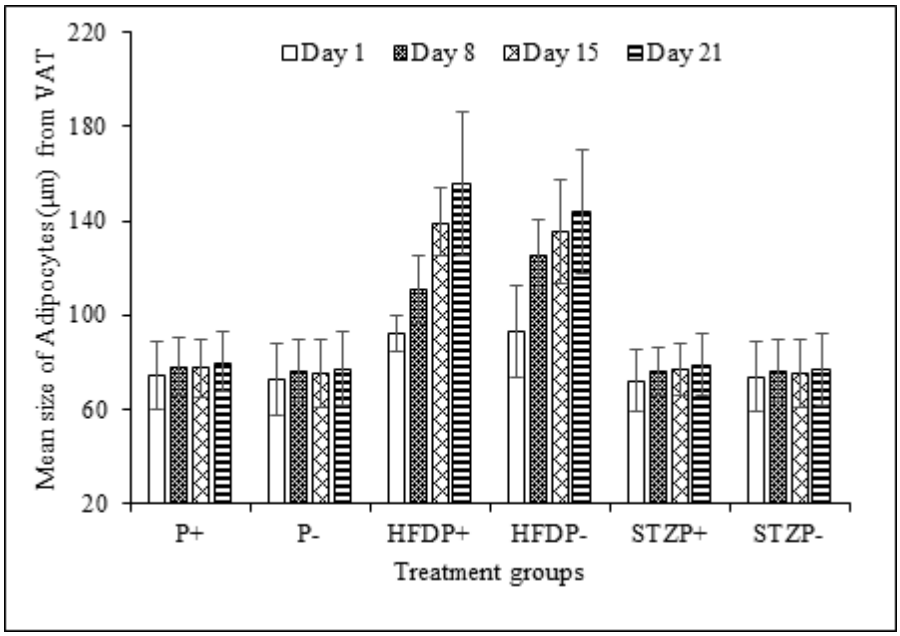

Figure 4. Size of VAT adipocytes from pregnant and non-pregnant rats treated with streptozotocin (STZ) or high fat diet (HFD)
Source: Author

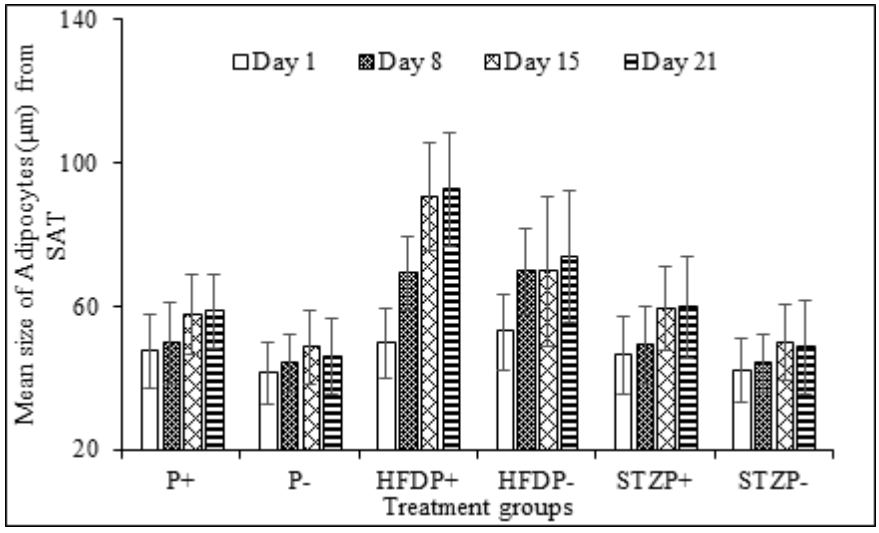

Figure 5. Size of SAT adipocytes from pregnant and non-pregnant rats treated with streptozotocin (STZ) or high fat diet (HFD).
Source: Author

obesity, one of the predictive factors for diabetes. This finding is similar to Holemans et al. (2004) and Kampmann et al. (2019) who reported that, obesity due to fat diet is one of the conditions which resulted into insulin-resistance and hyperinsulinemia. Fat accumulation in the body may induce insulin resistance; therefore the secreted insulin is not used by the cells to convert excess glucose into glycogen which can be stored into the body.Furthermore, increase in glucose and insulin levels was observed in P+ than P- groups, this might have been contributed by hormones produced during pregnancy. Similarly, Holemans et al. (2004) observed an increase in insulin levels in non-pregnant animals and further increase with pregnancy development in rats treated with a cafeteria diet (containing 33% ground commercial rat chow, 33% full fat sweetened condensed milk, 7%

sucrose and 27% water) with high components of fat. On the contrary, Qiao et al. (2021) reported that, in human beings, fat dietary intake one year before pregnancy or during pregnancy does not associate with risk for GDM, as compared to high intake of fat from 12 to 22 weeks of gestation. However, the measure of HFD intake was not exactly the same to all participants, and therefore was not under control. On the other hand, the pancreas of HFD fed animals were the same as those of control animals. The islets' structure and number of β-cells were maintained throughout the experiment with low rate of apoptosis as the control. This resulted into continuous production of insulin by the pancreas. Therefore, hyperglycemia despite of higher levels of insulin observed in the HFD fed animals was probably due to insulin resistance caused by the accumulation of fat in the body. This is in agreement with Pennington et al. (2017), who observed the same number of β-cells in animals given high fat high sucrose diet as their control, with increased insulin concentration and glucose intolerance.

The increase in the size of the VAT and SAT adipocytes might have contributed to hyperglycemia and GDM development; however, the increase in size was significantly higher in VAT than the SAT. This suggests that, visceral AT expansion associates more with obesity and insulin resistance which lead to increase in glucose and insulin levels, predictive factors for GDM. This is supported by Ambrosi and Colosi, (2017); Chait and Hartigh, (2020); Benevides et al. (2020); who explained that VAT expansion is associated with insulin resistance and GDM development than SAT. Furthermore, the result indicated that, the increase in VAT is in the form of size (hypertrophy) rather than number of adipocytes (hyperplasia). This shows that when animals are given HFD, the SAT fat storage becomes rapidly overwhelmed thus leading to accumulation of fat in the VAT. This is supported by Wang et al. (2013) who observed hypertrophy in VAT due to HFD within a month and associated with insulin resistance in mice while hyperplasia occurred after two months of HFD intake. In addition, the present study observed that, pregnant groups had a slight increase in VAT and SAT compared to non-pregnant groups. This is supported by Ambrosi and Colosi (2017) who observed high levels SAT and VAT in diabetic pregnant women compared to non-pregnant women. Adipose tissue must expand to support the growing fetus and future nutritional needs of the offspring. However, poor diet with high fat may result into AT expansion which play role in the development of GDM.

Conclusion

The findings of this study have shown clearly that, when pregnant rats are maintained on HFD, it results into hypertrophy of adipocytes from VAT which leads to insulin resistance with gestational time. Insulin resistance is one of the risk factors for the hyperglycemia and development of GDM.

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CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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