



CONSEQUENCES OF DELAYED FIRST ACTIVE MEAL, A BREAKFAST SKIPPING MODEL ON INSULIN RESISTANCE AND GLUCOSE TRANSPORTER-4 IN FEMALE VERSUS MALE WISTAR RATS

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ABSTRACT

Breakfast skipping has been linked to low energy and inadequate nutrition. The metabolic responses in the female versus male are yet to be understood. This study examined how the female versus male metabolomes respond to breakfast skipping. The animals were divided into four groups (n=10, Wistar rats): female rats who ate all three meals (ControlF), female rats who skipped the first active-phase meal (NFAMF), male rats who ate all three meals (ControlM), and male rats who skipped the first active-phase meal (NFAMM). All animals were fed the same amount of food daily for 4 weeks. Fasting blood glucose, serum levels of insulin, ghrelin, lipid variables, lipid peroxidation, antioxidant markers, liver and muscle GLUT4, and glycogen were assayed. There was a significant increase in the serum insulin, HbA1c, ghrelin, LDL, MDA levels, and liver GLUT4, of female rats when compared to male rats. There was a significant increase in the liver and muscle glycogen, as well as muscle GLUT4 in the NFAMF rats when compared to the NFAMM rats. These results indicate that breakfast skip had a greater effect on female metabolism than males.

Keywords: Breakfast skipping, female rats, insulin, lipid peroxidation, male rats,

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INTRODUCTION

Feeding schedule modifications are a growing side effect of global industrialization. Thus breakfast, the first and most crucial meal of the day, is frequently skipped. Food is necessary for survival and depending on the availability of food, animals decide what to consume. The timing of meals however can synchronize several organs and tissues, including those involved in food digestion, absorption, or metabolism, such as the liver, adipose tissue, intestines, pancreas, and stomach [1].

The practice of having breakfast has been contentious since the early times. This is because breakfast ingredients, that is, bread, honey, cheese, oil, and wine do not require preparation or additional cooking, so the meal was characterized by convenience. It was meant for those who had to travel long distances after rising, such as farmers who had to tend to their livestock and crops, since convenience was crucial. As a result, breakfast was considered a meal for young children and the elderly. It was also acceptable to the working classes. Europeans started to recognize breakfast as a crucial meal for general health in the 16th century [2]. Breakfast is currently defined as the first meal of the day that breaks the fast after the longest period of sleep and is consumed within 2 to 3 hours of waking; it is comprised of food or beverage from at least one food group and may be consumed at any location. This concept was suggested by a United States review of a collaborative effort [3].

The fact that breakfast is consumed after an overnight fast, makes it essential. Breakfast eating may improve energy balance, insulin sensitivity, and glucose levels as well as increase satiety and help people lose weight [4]. Studies have reported an association between breakfast skipping and metabolic disturbances like obesity, hypercholesterolemia, and impaired glucose tolerance [5]. Others include unhealthy behaviors like inactivity and nutrient-deficient eating, which can raise the risk of developing metabolic syndrome [6]. Metabolic problems and excess body weight have been linked to skipping the first active meal of the day. According to reports, skipping breakfast more frequently affects women during their adolescent years, even harming their reproductive system [7-9]. We just conducted a study and discovered that female rats were more negatively impacted by skipping breakfast than male rats in terms of their reproductive hormones [10]. Consequently, this study aims to compare the effects of skipping the first active meal in male and female rats and the effects of delaying the first active meal on metabolic factors such as fasting blood sugar, insulin, ghrelin, liver, and muscle glycogen.

MATERIALS AND METHODS

Ethics statement

The ethical approval for this study was obtained from the College of Medicine, University of Lagos Animal Care and Use Research Ethics Committee

(CMUL/ACUREC/11/22/1111). Experimental procedures relating to the use of animals were conducted in strict accordance with internationally accepted standards and regulations. Male and female Wistar rats were used for the experiment because the study sought to compare the metabolism of both sexes after a breakfast skip. Wistar rats that were six weeks old, both male and female, were purchased from the Central Animal Home at the University of Lagos. The animals were housed at an appropriate temperature, 14 h:10 h dark-light (DL) cycle, with free access to food and water only throughout the night. The animals were habituated to the lighting and feeding circumstances for two weeks before the start of the experiment. The onset of darkness was labeled as Dark-Light Time 0 (DLT 0), and the onset of light as Dark-Light Time 14 (DLT 14), following the dark/light pattern.

Experimental design

The following divisions were made in the meal model since rats are active at night. The four groups were:

- Female rats who had all three meals (ControlF),
- Female rats who had a no-first-active meal (NFAMF),
- Male rats who had all three meals (ControlM), and
- Male rats who had a no-first-active meal (NFAMM).

The rats in each group received the same daily total amount of food, which included a standard caloric diet of 20.0% protein, 62.9% carbohydrate, and 7.0% fat (3.95 kcal/g). At DLT0-2 (33% of the total), DLT6-8 (33% of the total), and DLT12-14 (33% of the total), the rats in the Control group were fed three times daily. In the NFAM (no first active meal) group, the rats were fed twice daily between DLT 6 and 8 (50% of the total) and DLT 12 and 14 (50% of the total) [11]. The pattern of light and dark persisted. Throughout the four-week research, each rat's daily food intake and weekly body weight were measured using a scale balance. The total food intake was constrained to allow each rat to consume the full amount of food during each 2-hour feeding session. The total amount of food given to the rats in each group was the same every day and increased week after week. This total food intake was around 70-80% of the amount that rats of the same age fed ad libitum would have consumed. The body weights of the animals were measured weekly and before sacrifice.

Blood collection and serum preparation

At the end of the 4th week of the experiment, animals were fasted overnight and blood from the tail was used to determine the fasting blood glucose level (Accu-Chek Glucometer, Roche, Germany) at the time of sacrifice. The rats were then bled into bottles of ordinary serum using a heart puncture. The blood was

centrifuged at 3000 rpm for 15 minutes after being allowed to clot for at least 45 minutes.

Insulin and ghrelin estimation

Insulin and ghrelin were determined using enzyme-linked immunoabsorbent assay (ELISA) kits following the manufacturer's instruction (insulin: Rat Insulin ELISA Cat# IN374S, ghrelin: Rat Ghrelin ELISA kit, Cat# E-EL-R0842 Elabscience, China).

Serum lipid profile estimation

The total cholesterol (TC), triacylglycerol (TAG), and high-density lipoprotein cholesterol (HDL-C) were measured using commercial kits (Randox Laboratory Ltd, Crumlin, Co. Antrim, UK). Low-density lipoprotein cholesterol (LDL-C) concentration was estimated according to the formula of Friedewald et al. [12].

$$\text{LDL-C} = \text{TC} - (\text{HDL-C}) - (\text{TAG}/5)$$

Serum lactate dehydrogenase and liver enzymes estimation

Serum lactate dehydrogenase (LDH), alkaline phosphatase (ALP), aspartate transaminase (AST), and alanine transferase (ALT) were measured by standardized enzymatic colorimetric methods using reagents obtained from Randox Laboratory Ltd. (Antrim, UK).

Lipid peroxidation and antioxidant levels

Serum lipid peroxidation was determined by measuring Thiobarbituric Acid Reactive Substances (TBARS/MDA) produced during lipid peroxidation according to the method of [13]. Tissue catalase activity was assessed according to the method of [14]. Assessment of tissue superoxide dismutase (SOD) activity was determined according to the method of [15], and the reduced glutathione (GSH) level was measured by spectrophotometric assay kit (Oxford Biomedical Research, USA).

Hemoglobin A1c assay

The glycated hemoglobin A1c (HbA1c) concentration was determined using a rat HbA1c kit (Crystal Chem), following the manufacturer's instructions.

Measurement of skeletal muscle and liver glycogen contents

The estimation of glycogen concentration in the muscle and liver was measured by the amyloglucosidase method Hattori et al., [16]. Briefly, these tissues were homogenized in perchloric acid below freezing using dry ice to inhibit the breakdown of metabolites (Power Homogenizer Portable S-203, AS ONE Corporation, Osaka, Japan). After homogenization, ultrapure water was added and the tissues were re-homogenized. The lysates were separated by centrifugation (250 rpm, 15 min, 4 °C) and the supernatants were stored at -80 °C. These samples were added to 50 mM NaAC buffer and

rotary incubated at 120 rpm for 60 min at 37 °C to disassemble glycogen. Glucose reagent was added to these samples and incubated for 20 min at room temperature. Glycogen concentrations were analyzed using a fluoro-spectrophotometer.

Glucose transporter 4 (GLUT4) muscle and liver estimation

The muscle and liver were homogenized in 9-volumes of ice-cold 0.1 mM phosphate buffer saline (pH 7.4) to prepare 10% homogenate. The homogenate was then centrifuged for 5 min at 5000×g to get the supernatant used for the measurement. These parameters were estimated using Rat GLUT4 (Glucose Transporter 4) CAT # E-EL-R0430 ELISA Kit, Elabscience, China. The technique outlined in the kits' manufacturer's handbook was adhered to.

Statistical analysis

Data were presented as means \pm SEM. Statistical analysis was carried out by two-way analysis of variance (ANOVA) supported by the Newman-Keuls test when pair-wise comparison was done between the groups. The analysis was done using version 5.0 (GraphPad Software, San Diego California, USA).

The level of statistical significance was placed at $P < 0.05$.

RESULTS

Food intake and body weight

During the four weeks of skipping breakfast, the NFAM female rats' feed consumption significantly decreased in comparison to the NFAM male rats. During the first week, the female rats fed the active meal (Control) consumed more feed than the male Control rats. However, by the fourth week, the female Control rats' feed consumption had significantly decreased in comparison to the male Control rats (Table 1).

At week one there was a significant increase in the body weight of the NFAM female rats when compared to the NFAM male rats as well as in the Control female rats when compared to the Control male rats. By week four, the body weight of the NFAM female rats had significantly decreased compared to the NFAM male rats. In addition, by weeks three and four, the body weight of the female rats fed at the active mealtime (Control) was significantly lower than that of the Control male rats (Figure 1).

Table 1: Effect of No-first active meal on feed intake in male and female Wistar rats

	Control _M	NFAM _M	Control _F	NFAM _F
Week 1	101.20 \pm 0.83	165.00 \pm 2.96	132.20 \pm 2.21*	141.80 \pm 1.50 [#]
Week 2	105.86 \pm 0.34	78.00 \pm 3.74	100.14 \pm 2.77	59.00 \pm 1.68 [#]
Week 3	95.14 \pm 1.24	81.57 \pm 1.09	92.4 \pm 1.02	62.71 \pm 1.04 [#]
Week 4	126.00 \pm 0.53	79.29 \pm 1.66	99.14 \pm 3.12*	58.43 \pm 0.84 [#]

Values are expressed as mean \pm SEM of 10 rats per group, * $p < 0.05$ vs control male, [#] $P < 0.05$ vs NFAM male. NFAM (No-first active meal).

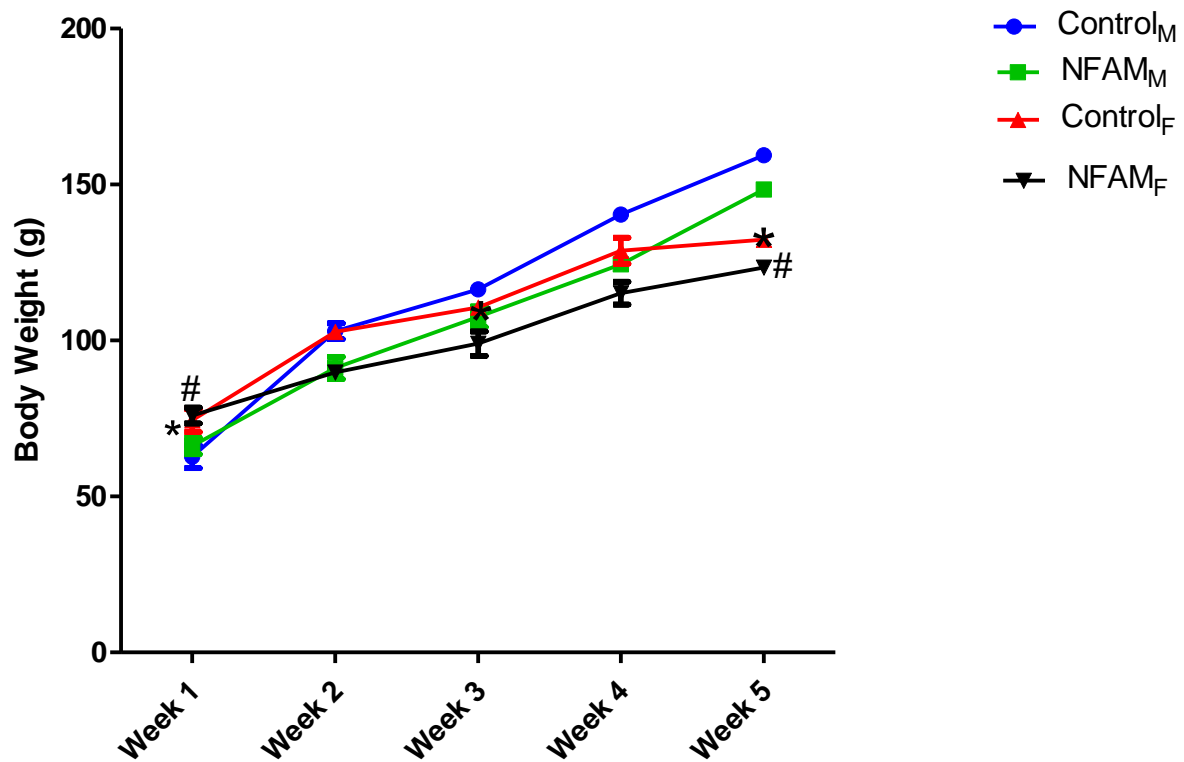


Figure 1: Effect of No-first active meal on body weight in male and female Wistar rats. Values are expressed as mean \pm SEM of 10 rats per group, * $P < 0.05$ vs control male, # $P < 0.05$ vs NFAM male. NFAM (No-first active meal).

Fasting blood glucose and Insulin

There was a significant decrease in the fasting blood glucose level of the control female rats when compared to the control male rats, as well as a significant decrease in the serum fasting blood glucose level of the NFAMF rats when compared to NFAMM rats.

There was a significant increase in the serum insulin level of the control female when compared to the control male as well as a significant increase in the serum insulin level of the NFAMF group when compared to the NFAMM group (Figure 2).

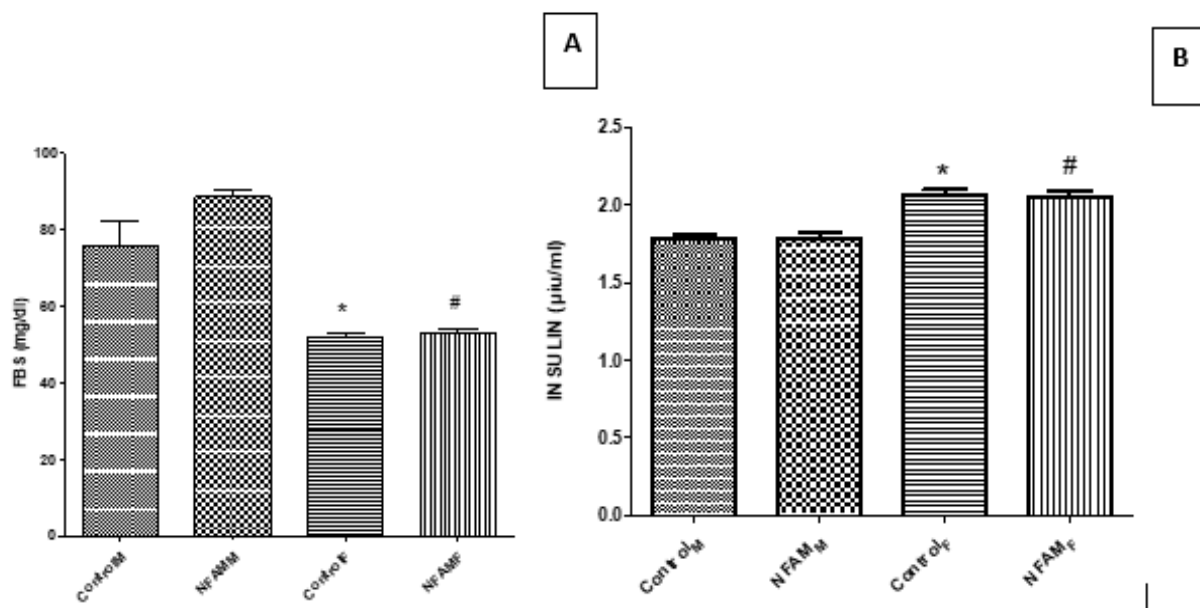
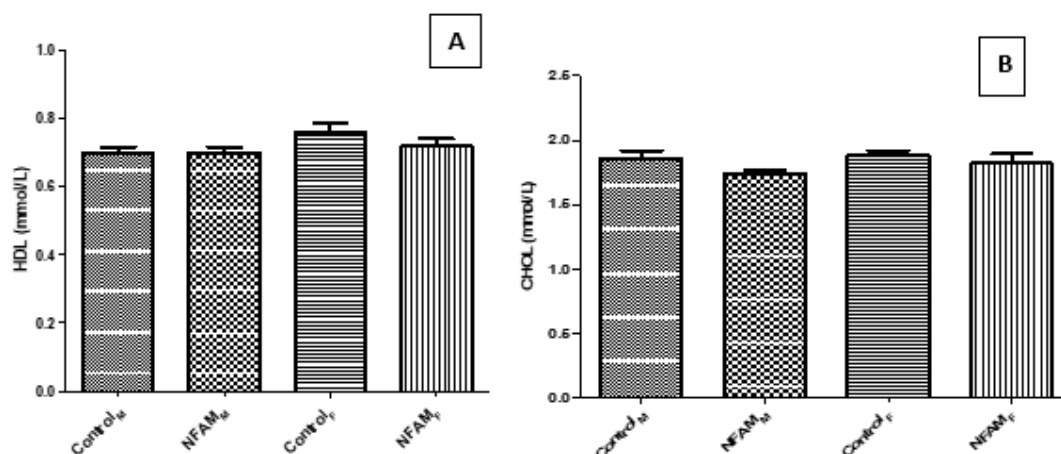


Figure 2: **A** Effect of No-first active meal on fasting blood glucose level in male and female Wistar rats. **B** Effect of No-first active meal on insulin level in male and female Wistar rats. Values are expressed as mean \pm SEM of 10 rats per group, *P < 0.05 vs control male, # P < 0.05 vs NFAM male. NFAM (No-first active meal).

Lipid profiles

There was no significant difference in the serum HDL level of the control female rats when compared to the control male rats as well as in the serum HDL level in the NFAMF rats when compared to NFAMM rats. There was also no significant difference in the serum cholesterol level of the control female rats when compared to the control male rats. There was no significant difference in the serum cholesterol level of the NFAMF rats when compared to the NFAMM rats.

There was however a significant increase in the serum LDL level of the control female rats when compared to the control male rats as well as in the serum LDL level of the NFAMF rats when compared to NFAMM rats. There was a significant decrease in the serum triglyceride level of the control female rats when compared with the control male rats, but a significant increase in the serum triglyceride level of the NFAMF rats when compared with NFAMM rats.



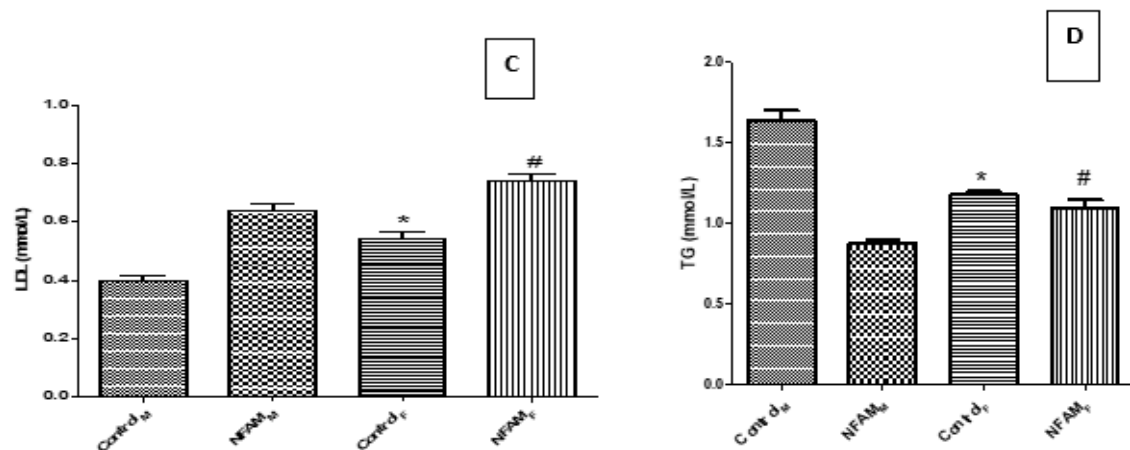


Figure 3: **A** Effect of No-first active meal on HDL in male and female Wistar rats. **B** Effect of No-first active meal on cholesterol level in male and female Wistar rats. **C** Effect of No-first active meal on LDL in male and female Wistar rats. **D** Effect of No-first active meal on TG level in male and female Wistar rats. Values are expressed as mean \pm SEM of 10 rats per group, * $P < 0.05$ vs control male, # $P < 0.05$ vs NFAM male. NFAM (No-first active meal).

Liver enzymes

There was a significant increase in the serum AST level of the control female rats when compared to the control male rats as well as in the NFAMF rats when compared to NFAMM rats. There was a significant increase in the serum ALT level of the control female rats when compared to the control male rats however

there was no significant difference in the serum ALT level of the NFAMF rats when compared to NFAMM rats. There was a significant decrease in the serum ALP level of the control female rats when compared to the control male rats as well as in the NFAMF rats when compared to NFAMM rats.

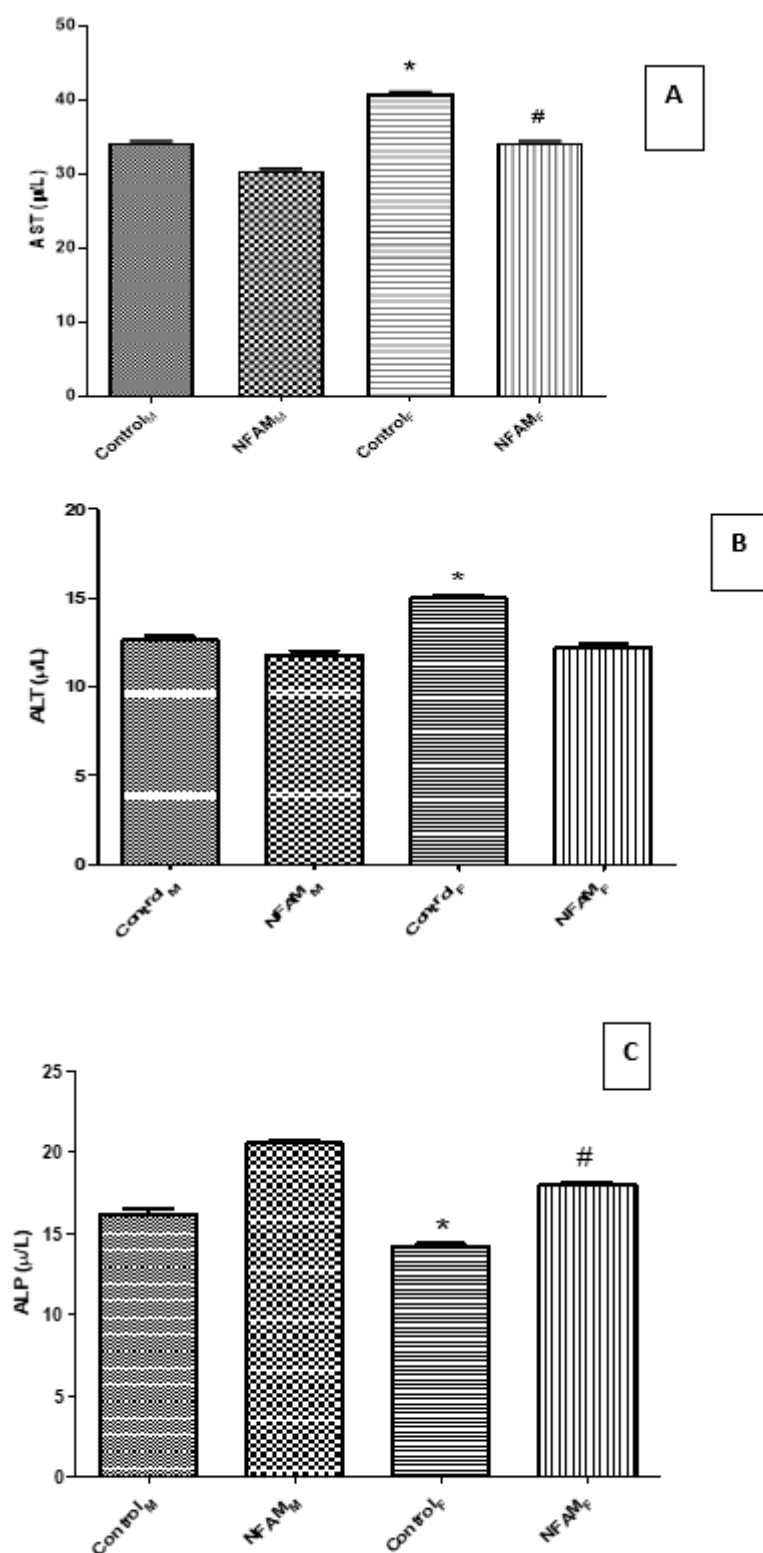
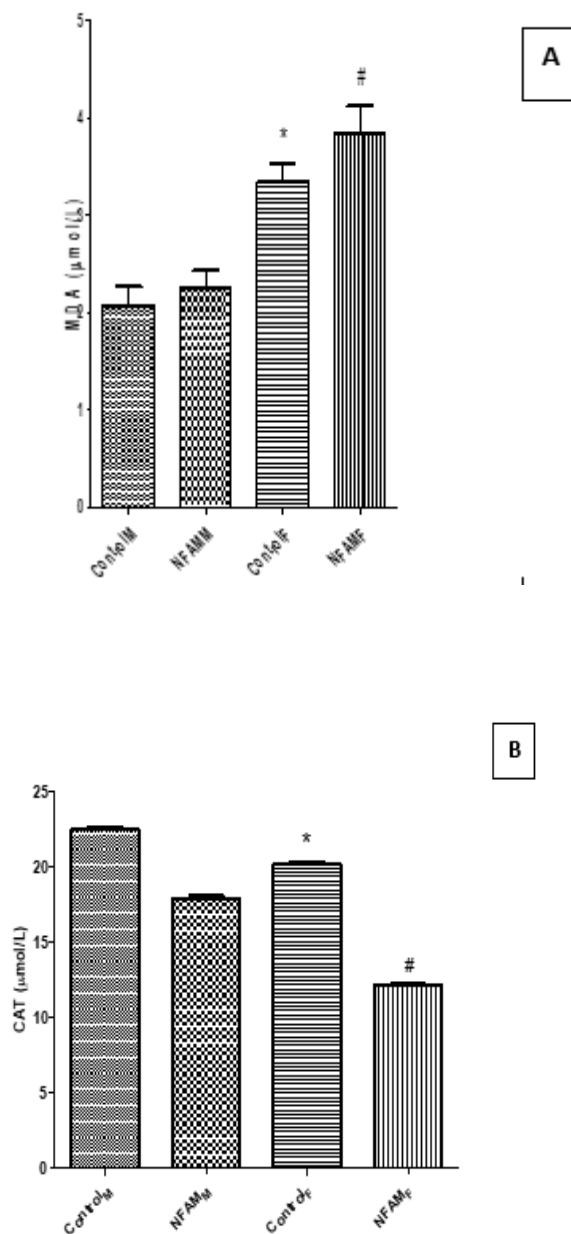


Figure 4: **A** Effect of No-first active meal on aspartate transaminase (AST) in male and female Wistar rats. **B** Effect of No-first active meal on alanine transferase (ALT) in male and female Wistar rats. **C** Effect of No-first active meal on alkaline phosphatase (ALP) in male and female Wistar rats. Values are expressed as mean \pm SEM of 10 rats per group, *P < 0.05 vs control male, # P < 0.05 vs NFAM male. NFAM (No-first active meal).

MDA level and antioxidants

There was a significant increase in the MDA level of the control female rats when compared to the control male rats. There was also a significant increase in the MDA level of the NFAMF rats when compared to NFAMM rats. There was a significant decrease in the CAT level of the control female rats when compared to the control male rats. There was also a significant decrease in the CAT level of the NFAMF rats when compared to the NFAMM rats. There was a significant

decrease in the GSH level of the control female rats when compared with the control male rats, but there was a significant increase in the serum GSH level of the NFAMF rats when compared to NFAMM rats. There was a significant decrease in the SOD level of the control female rats when compared to the control male rats. There was however no significant difference in the SOD level of the NFAMF rats when compared to the NFAMM rats.



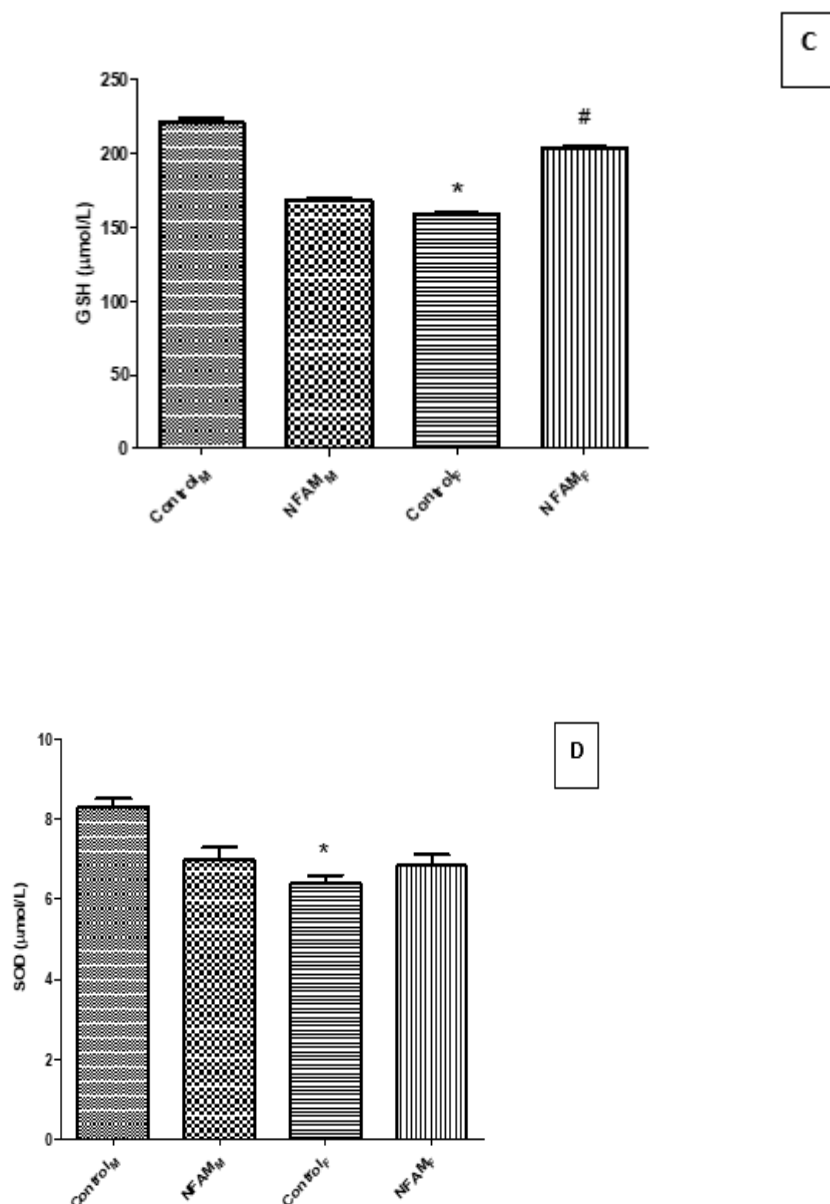


Figure 5: **A** Effect of No-first active meal on MDA level in male and female Wistar rats. **B** Effect of No-first active meal on CAT level in male and female Wistar rats. **C** Effect of No-first active meal on GSH level in male and female Wistar rats. **D** Effect of No-first active meal on SOD level in male and female Wistar rats. Values are expressed as mean \pm SEM of 10 rats per group, * $P < 0.05$ vs control male, # $P < 0.05$ vs NFAM male. NFAM (No-first active meal).

Muscle and liver glycogen levels

There was a significant decrease in the muscle glycogen level of the control female rats when compared to the control male rats. There was however a significant increase in the muscle glycogen level of the NFAMF rats when compared to NFAMM rats.

There was no significant difference in the liver glycogen level of the control female rats when compared to the control male rats. There was however a significant increase in the liver glycogen level of the NFAMF rats when compared to NFAMM rats.

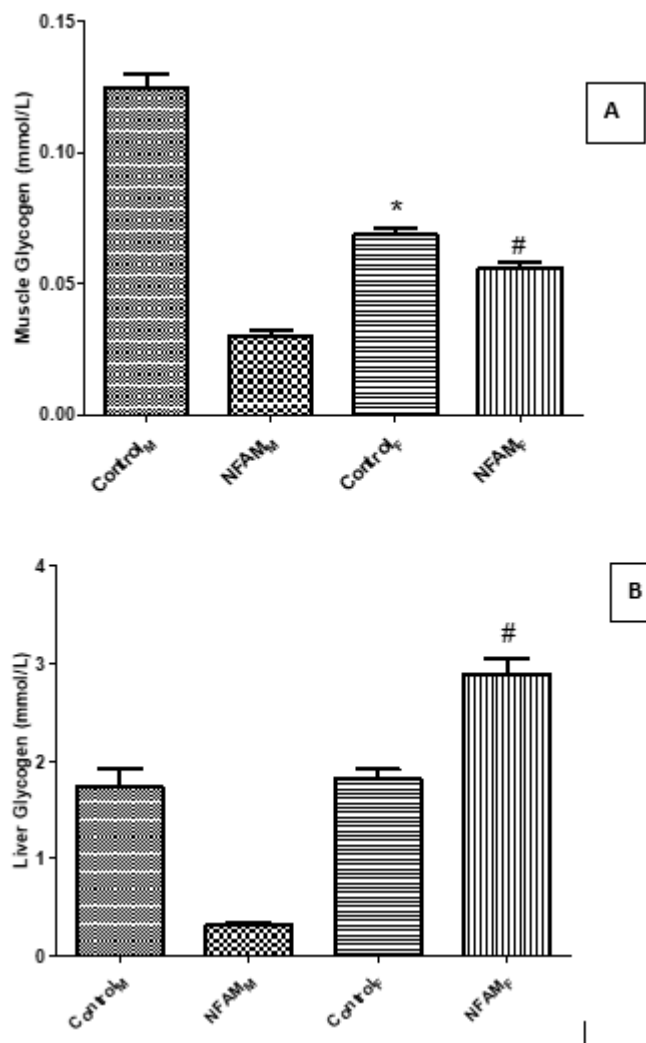


Figure 6: **A** Effect of No-first active meal on muscle glycogen level in male and female Wistar rats. **B** Effect of No-first active meal on liver glycogen level in male and female Wistar rats. Values are expressed as mean \pm SEM of 10 rats per group, * $P < 0.05$ vs control male, # $P < 0.05$ vs NFAM male. NFAM (No-first active meal).

HbA1c level

There was a significant increase in the serum HbA1c level of the control female rats when compared to the control male rats. There was also a significant increase in the serum HbA1c level of the NFAMF rats when compared to NFAMM rats.

Ghrelin level

There was a significant increase ($P < 0.05$) in the serum ghrelin level of the control female rats when compared to the control male rats. There was a significant increase ($P < 0.05$) in the serum ghrelin level of the NFAMF rats when compared to the NFAMM rats.

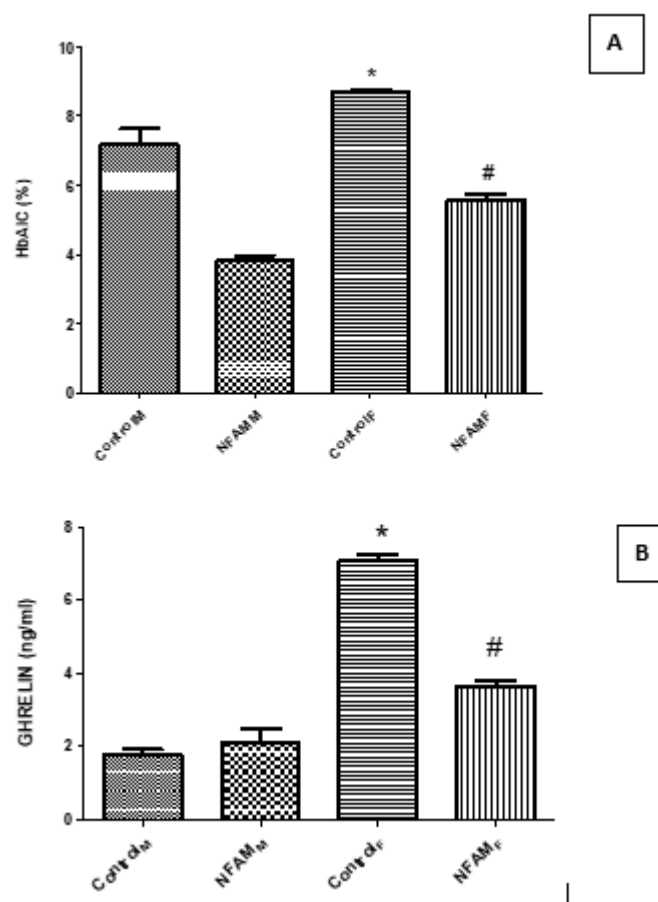


Figure 7: A Effect of No-first active meal on serum HbA1c level in male and female Wistar rats. **B** Effect of No-first active meal on serum ghrelin level in male and female Wistar rats. Values are expressed as mean \pm SEM of 10 rats per group, * $P < 0.05$ vs control male, # $P < 0.05$ vs NFAM male. NFAM (No-first active meal).

Muscle and liver GLUT 4 levels

There was a significant decrease in the muscle GLUT 4 level of the control female rats when compared to the control male rats. There was however a significant increase in the muscle GLUT 4 level of the NFAMF rats when compared to NFAMM rats.

There was a significant increase in the serum liver GLUT 4 level of the control female rats when compared to the control male rats. There was also a significant increase in the serum liver GLUT 4 level of the NFAMF rats when compared to NFAMM rats.

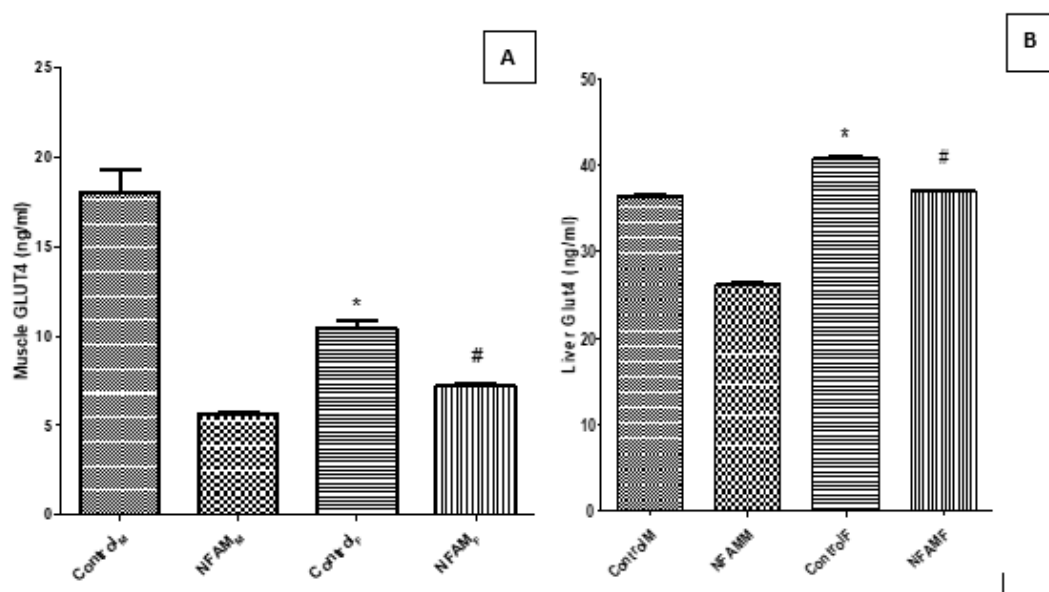


Figure 8: A Effect of No-first active meal on muscle GLUT 4 level in male and female Wistar rats. **B** Effect of No-first active meal on liver GLUT 4 level in male and female Wistar rats. Values are expressed as mean \pm SEM of 10 rats per group, * $P < 0.05$ vs control male, # $P < 0.05$ vs NFAM male. NFAM (No-first active meal).

DISCUSSION

According to research, skipping breakfast is linked to nutrition and energy deficits [17]. This study determined how missing breakfast could impact metabolism in females versus males using Wistar rats. In actuality, the timing of meals can influence food consumption and body weight. In week one of this study, the female rats fed breakfast (Control) and those deprived of breakfast (NFAMF) had an increase in body weight when compared to male rats who were fed breakfast (Control) and those deprived of breakfast (NFAMM) respectively. The body weight increase observed at week one was however not sustained throughout the four weeks of the experiment. Another study on female rats only reported that an absence of body weight difference was observed in the female rats who had delayed first active meal and westernized diet intake [18].

According to Costa et al. [19], animals that go 8 hours without eating during the dark part of the cycle consume less food overall but do not make up for the greater intake during the light phase. This is comparable to the findings of this study. Throughout the study, the animals not given the first active meal (NFAM), corresponding to the rats' dark phase, consumed less food. The NFAMF rats' food intake was lower than that of the NFAMM rats' food, and as a result, the female rats' body weights were lower than those of the male rats subsequently. The duration of meals may be shorter in females than in males, which may be the reason for the observation [20]. Female and male rats have different feeding habits [21], and sex differences and feeding habits are also present in

humans [22]. Although some studies have reported that time-restricted feeding, which is akin to breakfast skipping [23] enhances metabolic processes but does not change body weight in humans and mice, these studies were however carried out in prediabetic men [24], and in mice that were restricted feeding for 9 hours in the active period. In this study, the rats were restricted for 6 hours in the active period. Other studies have reported reduced body weight in time-restricted feeding like this study [25-26].

According to a study, animals fed a high-fat diet were protected against aberrant lipid metabolism by a model of skipping breakfast [25]. The NFAMF rats showed aberrant lipid metabolism when compared to the NFAMM rats, in this current research. The TG and LDL levels were significantly higher in the NFAMF rats than in the NFAMM rats. The LDL levels in control female rats were however significantly higher than in control male rats, TG levels were significantly lower in control female rats when compared to control male rats. The non-significant difference in the cholesterol level had been reported in male Wistar rats deprived of the first active meal [27], in this study also, the non-significant difference in the cholesterol level was observed in all groups.

Male Wistar rats who were denied the first active meal experienced hypoglycemia and hypocholesterolemic symptoms [11]. Compared to the male Wistar rats, the female Wistar rats had more hypoglycemia after the first active meal in this study. Some variables were also found to be higher in the NFAMF rats in this study when compared to the NFAMM rats, they are insulin resistance, reduced

glucose tolerance, hyperinsulinemia, and hypertriglyceridemia. These variables have also been shown to form a relationship in diet-related metabolic conditions [28].

The fasting blood glucose (FBG) was significantly lower in the NFAMF rats compared to the NFAMM rats. Aquino de Oliveira et al. [18], reported hyperglycemia in female rats who were deprived of the first active meal. This discrepancy in the results could be because this study had a four-week duration while theirs had an eight-week duration. FBG was also lower in the control female rats compared to the control male rats, however, in both groups, the insulin levels of the female rats were significantly higher than those of the male rats. Hypoglycemia results from low blood sugar induced by high blood insulin levels, which may be a result of hunger brought on by skipping meals. Because diets containing carbohydrates are broken down into simple sugars, the pancreas produces insulin, which stimulates cells to absorb blood sugar for energy and storage. This results in an increase in insulin levels in animals served the first active meal. Since estrogen has a positive impact on insulin and glucose homeostasis [29-30], it is possible that it also contributes to gender differences. Human pancreas biopsies have shown that women have more β cells than men [31].

Hepatic steatosis and poor lipid metabolism result from elevated insulin levels [32]. This is in line with the fact that serum levels of AST and ALP were significantly higher in NFAMF rats compared to NFAMM rats, while AST and ALT levels were significantly higher in control female rats compared to control male rats, which could have been because of elevated insulin levels recorded in this study in the female rats versus male rats.

There was no significant difference in the HDL levels in the female rats when compared to the male rats in both groups. The no significant difference in the HDL level with accompanying increase in the LDL and TG levels in the NFAMF rats when compared to the NFAMM rats might be due to a decrease in the activity of lipoprotein lipase as reported in obese rats [33]. Additionally, the NFAMF rats' MDA levels significantly increased when compared to the NFAMM rats and the control female rats' MDA levels significantly increased when compared to the control male rats, respectively. Two interdependent processes for atherogenesis linked to metabolic illnesses include oxidative damage and altered lipid profiles [32]. This suggests that female species are more likely than male species to develop metabolic disorders, especially by breakfast skip. MDA is the product of lipid peroxidation especially at the cell membrane which liberates superoxides. The increased MDA level and reduced antioxidant levels in the female rats when compared to the male rats in both groups indicate oxidative stress with evidence of

antioxidant depletion which may lead to inflammatory processes increasing in females than males.

The liver and muscle glycogen levels were significantly increased in the NFAMF rats when compared to the NFAMM rats. Fasting conditions cause a drop in blood sugar levels, which indicates a reduction in circulating insulin and a rise in glucagon levels. Enough blood glucose is available for the brain where it is completely oxidized to carbon dioxide and water. Glucagon stimulates the release of glucose from glycogen stores in the liver, while the decline in insulin decreases the transport of glucose into the skeletal muscle and adipose tissues [34]. Skeletal muscle also stores glycogen, but because it lacks glucose-6-phosphatase, it must first be converted to lactate before being released into the bloodstream and converted back into glucose by the liver using the Cori cycle.

Type 2 diabetes who are breakfast skippers have high glycated hemoglobin (HbA1C) levels [35-36]. However, another study reported that HbA1C was reduced because of different types of intermittent fasting among diabetes and overweight/obese adults [37]. The HbA1C level was reduced in type 2 diabetes after intermittent fasting in another study [38]. In this study, the HbA1C level was significantly higher in the female rats when compared to the male rats in both groups. In a non-diabetes state, the HbA1C level may be increased in females than in males who either skipped breakfast or otherwise.

Energy homeostasis is directly impacted by ghrelin [39]. In this study, the NFAMF rats' ghrelin levels were higher than those of the NFAMM rats. Rats on calorie-restricted diets have also shown a rise in ghrelin levels [40]. Additionally, skipping breakfast lowers daily calorie intake. There was a considerable rise in ghrelin levels in the control female rats compared to the male rats, which is consistent with studies showing that estrogen up-regulates ghrelin levels [41]. According to Gayle et al. [41], fasting similarly caused higher increases in ghrelin levels, thus the increase in ghrelin levels observed in the NFAMF rats when compared to NFAMM rats.

The completion of dietary glucose absorption occurs when fasting lasts for a specific amount of time, such as the first 24 hours, which causes the blood glucose level to drop and signals a decrease in circulating insulin and an increase in glucagon [42]. In this study, the NFAMF rats had lower blood glucose levels than the NFAMM rats, which may be explained by the association between insulin and estrogen, while the NFAMF rats had significantly higher insulin levels than the NFAMM rats. Exogenous intake, circadian, hormonal, and brain regulation are used to maintain glucose homeostasis. Insulin is the primary hormone that controls how much glucose is taken up by muscle and adipose tissue, while glucagon is the primary hormone that controls glucose levels in the blood during the post-absorptive state as well as fasting [43]. The circadian clock exerts an independent but not

exclusive influence on glucose absorption, which in turn modulates insulin secretion and tissue sensitivity to the hormone [44], by peripheral tissues reliant on insulin [45]. Insulin primarily acts on receptors in skeletal muscle, liver, and white adipose tissue to regulate glucose.

In the study, the increase in insulin level probably increased the GLUT 4 level in the muscles and liver in the same manner with a significant increase in muscle GLUT 4 and liver GLUT 4 in the NFAMF rats when compared to the NFAMM rats, as is typically the case in conditions like diabetes mellitus, defective insulin and insulin receptor activity and a decreased GLUT 4 level arising from impaired cell membrane translocation of Type 2 diabetes mellitus [46].

Skeletal muscle cells, which have the highest level of insulin-stimulated glucose absorption, consume 80% of the ingested glucose [47]. Skeletal muscles have thus emerged as the primary location of insulin resistance [48]. The activation of downstream signaling pathways, such as phosphatidylinositol 3-kinase (PI 3-kinase), facilitates GLUT4's transmembrane activity, which facilitates glucose absorption when insulin binds to its receptor in skeletal muscle [49]. In rats, insulin-mediated glucose uptake is entirely prevented by wortmannin's pharmacologic suppression of PI 3-kinase activity [50]. Therefore, the rate of glucose transfer into fat and muscle cells is controlled by the translocation of GLUT4 to the sarcolemma and the subsequent concentration of GLUT4 [51-52]. Considering the, poor insulin-insulin receptor activation and reduced GLUT4 caused by impaired GLUT4 cell membrane translocation may contribute significantly to the etiology of type 2 diabetes [46].

CONCLUSION

This study highlighted that skipping breakfast has a significant impact on both male and female metabolic responses, however, the effect was greater on the female. Female rats who skipped breakfast experienced significant changes in multiple metabolic parameters, including heightened levels of insulin, ghrelin, and liver glycogen, but reduced fasting blood glucose levels compared to the male rats. There was also an oxidative imbalance in the female rats compared to the male rats. The study also emphasizes the possible impact of hormonal variables, specifically estrogen, on ghrelin secretion and insulin levels. Overall, this study highlights the relevance of breakfast eating in controlling metabolic health as well as the need to take sex-specific responses into account in follow-up research and interventions targeted at enhancing metabolic outcomes. Given the potential implications for human health, these findings warrant further research on the impact of sex-specific responses during other mealtimes.

REFERENCES

1. LOPEZ-MINGUEZ, J., GÓMEZ-ABELLÁN, P. & GARAULET, M. (2019). Timing of Breakfast Lunch and Dinner Effects on Obesity and Metabolic Risk. *Nutrition*, **11**(11): 2624. <https://doi.org/10.3390/nu11112624>
2. ARNDT ANDERSON, H. (2013). Breakfast: a history. Lanham AltaMira Press a division of Rowman & Littlefield Publishers Inc Washington DC.
3. O'NEIL, C.E., BYRD-BREDBENNER, C., HAYES, D., JANA, L., KLINGER, S.E. & STEPHENSON-MARTIN, S. (2014). The role of breakfast in health: definition and criteria for a quality breakfast. *Journal of the Academy of Nutrition and Dietetics*, **114**(12): S8-S26. <https://doi.org/10.1016/j.jand.2014.08.022>
4. MAKI, K.C., PHILLIPS-EAKLEY, A.K. & SMITH, K.N. (2016). The Effects of Breakfast Consumption and Composition on Metabolic Wellness with a Focus on Carbohydrate Metabolism. *Advances in Nutrition*, **7**(3): 613S-621S. <https://doi.org/10.3945/an.115.010314>
5. SIEGA-RIZ, A.M., POPKIN, B.M. & CARSON, T. (2000). Differences in food patterns at breakfast by sociodemographic characteristics among a nationally representative sample of adults in the United States. *Preventive Medicine*, **30**(5): 415-424. <https://doi.org/10.1006/pmed.2000.0651>
6. SIERRA-JOHNSON, J., UNDÉN, A.L., LINESTRAND, M., ROSELL, M., SJOGREN, P., KOLAK, M., DE FAIRE, U., FISHER, R.M. & HELLÉNIUS, M.L. (2008). Eating meals irregularly: a novel environmental risk factor for the metabolic syndrome. *Obesity (Silver Spring)*, **16**(6): 1302-1307. <https://doi.org/10.1038/oby.2008.203>
7. FUJIWARA, T. (2003). Skipping breakfast is associated with dysmenorrhea in young women in Japan. *International Journal of Food Sciences and Nutrition*, **54**(6): 505-509. <https://doi.org/10.1080/09637480310001622369>
8. FUJIWARA, T. & NAKATA, R. (2010). Skipping breakfast is associated with reproductive dysfunction in post-adolescent female college students. *Appetite*, **55**(3): 714-717. [doi: 10.1016/j.appet.2010.08.005](https://doi.org/10.1016/j.appet.2010.08.005)
9. FUJIWARA, T., NAKATA, R., ONO, M., MIEDA, M., ANDO, H., DAIKOKU, T. & FUJIWARA, H. (2018). Time Restriction

- of Food Intake During the Circadian Cycle Is a Possible Regulator of Reproductive Function in Postadolescent Female Rats. *Current Developments in Nutrition*, **3**(4): nzy093. <https://doi.org/10.1093/cdn/nzy093>
10. OYELOWO, O.T., TAIRE, E.O. & AJAO, O.I. (2022). Skipping the first active meal appears to adversely alter reproductive function in female than male rats. *Current Research in Physiology*, **5**: 414-420. <https://doi.org/10.1016/j.crphys.2022.10.001>
11. WU, T., SUN, L., ZHUGE, F., GUO, X., ZHAO, Z., TANG, R., CHEN, Q., CHEN, L., KATO, H. & FU, Z. (2011). Differential roles of breakfast and supper in rats of a daily three-meal schedule upon circadian regulation and physiology. *Chronobiology International*, **28**(10): 890-903. <https://doi.org/10.3109/07420528.2011.622599>
12. FRIEDEWALD, W.T., LEVY, R.I. & FREDRICKSON, D.S. (1972). Estimation of the concentration of low-density lipoprotein cholesterol in plasma without use of the preparative ultracentrifuge. *Clinical Chemistry*, **18**(6): 499-502. <https://doi.org/10.1093/clinchem/18.6.499>
13. UCHIYAMA, M. & MIHARA, M. (1978). Determination of malonaldehyde precursor in tissues by thiobarbituric acid test. *Analytical Biochemistry*, **86**: 271–278. [https://doi.org/10.1016/0003-2697\(78\)90342-1](https://doi.org/10.1016/0003-2697(78)90342-1)
14. AEBI, H. (1984). Catalase in vitro. *Methods in Enzymology*, **105**: 121–126. [https://doi.org/10.1016/s0076-6879\(84\)05016-3](https://doi.org/10.1016/s0076-6879(84)05016-3)
15. SUN, M. & ZIGMAN, S. (1978). An improved spectrophotometric assay for superoxide dismutase based on epinephrine autooxidation. *Analytical Biochemistry*, **90**: 81–89. [https://doi.org/10.1016/0003-2697\(78\)90010-6](https://doi.org/10.1016/0003-2697(78)90010-6)
16. HATTORI, S., OMI, N., YANG, Z., NAKAMURA, M. & IKEMOTO, M. (2021). Effect of ginger extract ingestion on skeletal muscle glycogen contents and endurance exercise in male rats. *Physical Activity and Nutrition*, **25**(2): 15-19. doi: 10.20463/pan.2021.0010
17. NICKLAS, T.A., REGER, C., MYERS, L. & O'NEIL, C. (2000). Breakfast consumption with and without vitamin-mineral supplement use favorably impacts daily nutrient intake of ninth-grade students. *Journal of Adolescent Health*, **27**(5): 314–321. doi:10.1016/s1054-139x(00)00113-0
18. AQUINO, D.E., OLIVEIRA, D., ARAÚJO, N.C.M., FREIRE, A.R., ALBUQUERQUE, G.S., MUNIZ, G.S. & NASCIMENTO, E. (2021). Delay first active-phase meal breakfast-skipping model increases the risk of metabolic disorders in female rats. *Biological Rhythm Research*, **53**(10): 1587-1602. <https://doi.org/10.1371/journal.pone.0206669>
19. COSTA, A.C.O., ARAÚJO, N.C.D.M., MUNIZ, G.S., SOUZA, S.L. & NASCIMENTO, E. (2021). Timed restricted feeding in adult rats using standard chow or Western diet causes different response to food behavior. *Biological Rhythm Research*, **53**(11): 1–25. doi:10.1080/09291016.2021.1882143
20. FUNABASHI, T., HAGIWARA, H., MOGI, K., MITSUSHIMA, D., SHINOHARA, K. & KIMURA, F. (2009). Sex differences in the responses of orexin neurons in the lateral hypothalamic area and feeding behavior to fasting. *Neuroscience Letters*, **463**: 31–34. <https://doi.org/10.1016/j.neulet.2009.07.035>
21. LAVIANO, A., MEGUID, M.M., GLEASON, J.R., YANG, Z.J. & RENVYLE, T. (1996). Comparison of long-term feeding pattern between male and female Fischer 344 rats: influence of estrous cycle. *American Journal of Physiology*, **270**: R413–R419. <https://doi.org/10.1152/ajpregu.1996.270.2.R413>
22. FUKUSHIMA, A., HAGIWARA, H., FUJIOKA, H., KIMURA, F., AKEMA, T. & FUNABASHI, T. (2015). Sex differences in feeding behavior in rats: the relationship with neuronal activation in the hypothalamus. *Frontiers in Neuroscience*, **9**: 88. <https://doi.org/10.3389/fnins.2015.00088>
23. REGMI, P., CHAUDHARY, R., PAGE, A.J., HUTCHISON, A.T., VINCENT, A.D., LIU, B. & HEILBRONN, L. (2021). Early or delayed time-restricted feeding prevents metabolic impact of obesity in mice. *Journal of Endocrinology*, **248**(1): 75-86. <https://doi.org/10.1530/JOE-20-0404>
24. SUTTON, E.F., BEYL, R., EARLY, K.S., CEFALU, W.T., RAVUSSIN, E. & PETERSON, C.M. (2018). Early time-restricted feeding improves insulin sensitivity blood pressure and oxidative stress even without weight loss in men with prediabetes. *Cell Metabolism*, **27**: 1212e3–1221e3. <https://doi.org/10.1016/j.cmet.2018.04.010>
25. HATORI, M., VOLLMERS, C., ZARRINPAR, A., DITACCHIO, L., BUSHONG, E.A., GILL, S., LEBLANC, M., CHAIX, A., JOENS, M., FITZPATRICK, J.A., ELLISMAN, M.H. & PANDA, S. (2012). Time-restricted feeding without reducing caloric intake prevents metabolic diseases in mice fed a high-fat diet. *Cell*

- Metabolism*, **15**: 848–860. <https://doi:10.1016/j.cmet.2012.04.019>
26. CHAIX, A., ZARRINPAR, A., MIU, P. & PANDA, S. (2014). Time-restricted feeding is a preventative and therapeutic intervention against diverse nutritional challenges. *Cell Metabolism*, **20**: 991–1005. <https://doi:10.1016/j.cmet.2014.11.001>
27. SHIMIZU, H., HANZAWA, F., KIM, D., SUN, S., LAURENT, T., UMEKI, M., IKEDA, S., MOCHIZUKI, S. & ODA, H. (2018). Delayed first active-phase meal a breakfast-skipping model led to increased body weight and shifted the circadian oscillation of the hepatic clock and lipid metabolism-related genes in rats fed a high-fat diet. *PLoS One*, **13**(10): e0206669. <https://doi:10.1371/journal.pone.0206669>
28. MUNSHI, E.P., JOSHI, S.G. & RANE, B.N. (2014). Development of an experimental diet model in rats to study hyperlipidemia and insulin resistance markers for coronary heart disease. *Indian Journal of Pharmacology*, **46**(3): 270–276. <https://doi:10.4103/0253-7613132156>
29. MAUVAIS-JARVIS, F., CLEGG, D.J. & HEVENER, A.L. (2013). The role of estrogens in control of energy balance and glucose homeostasis. *Endocrine Reviews*, **34**(3): 309–338. <https://doi:10.1210/er.2012-1055>
30. YAN, H., YANG, W., ZHOU, F., LI, X., PAN, Q., SHEN, Z., HAN, G., NEWELL-FUGATE, A., TIAN, Y., MAJETI, R., LIU, W., XU, Y., WU, C., ALLRED, K., ALLRED, C., SUN, Y. & GUO, S. (2019). Estrogen Improves Insulin Sensitivity and Suppresses Gluconeogenesis via the Transcription Factor Foxo1. *Diabetes*, **68**(2): 291–304. <https://doi:10.2337/db18-0638>
31. MARCHESE, E., RODEGHIER, C., MONSON, R.S., MCCracken, B., SHI, T & SCHROCK, W. (2015). Enumerating beta-cells in whole human islets: sex differences and associations with clinical outcomes after islet transplantation. *Diabetes Care*, **38**(11): e176– e177. <https://doi:10.2337/dc15-0723>
32. NIMROUZI, M., ABOLGHASEMI, J., SHARIFI, M.H., NASIRI, K. & AKBARI, A. (2020). Thyme oxymel by improving of inflammation oxidative stress dyslipidemia and homeostasis of some trace elements ameliorates obesity induced by high-fructose/fat diet in male rat. *Biomedicine and Pharmacotherapy*, **126**: 110079. <https://doi:10.1016/j.biopha.2020.110079>
33. JARUKAMJORN, K., JEARAPONG, N., PIMSON, C. & CHATUPHONPRASERT, W. (2016). A High-Fat High-Fructose Diet Induces Antioxidant Imbalance and Increases the Risk and Progression of Nonalcoholic Fatty Liver Disease in Mice. *Scientifica (Cairo)*, **2016**: 5029414. <https://doi:10.1155/2016/5029414>
34. RAMNANAN, C.J., EDGERTON, D.S., KRAFT, G. & CHERRINGTON, A.D. (2011). Physiologic action of glucagon on liver glucose metabolism. *Diabetes, Obesity and Metabolism*, **13**(1): 118–125. <https://doi:10.1111/j.1463-1326.2011.01454.x>
35. REUTRAKUL, S., HOOD, M.M., CROWLEY, S.J., MORGAN, M.K., TEODORI, M. & KNUTSON, K.L. (2014). The relationship between breakfast skipping chronotype and glycemic control in type 2 diabetes. *Chronobiology International*, **31**(1): 64–71. <https://doi:10.3109/07420528.2013.821614>
36. HASHIMOTO, Y., KAJI, A., SAKAI, R., OSAKA, T., USHIGOME, E., HAMAGUCHI, M., YAMAZAKI, M. & FUKUI, M. (2020). Skipping breakfast is associated with glycemic variability in patients with type 2 diabetes. *Nutrition*, **71**: 110639. <https://doi:10.1016/j.nut.2019.110639>
37. NOWOSAD, K. & SUJKA, M. (2021). Effect of Various Types of Intermittent Fasting (IF) on Weight Loss and Improvement of Diabetic Parameters in Human. *Current Nutrition Reports*, **10**(2): 146–154. <https://doi:10.1007/s13668-021-00353-5>
38. SAEED, M., ALI, M., ZEHRA, T., HAIDER ZAIDI, S.A. & TARIQ, R. (2021). Intermittent Fasting: A User-Friendly Method for Type 2 Diabetes Mellitus. *Cureus*, **13**(11): e19348. <https://doi:10.7759/cureus.19348>
39. ALVES, M.G., JESUS, T.T., SOUSA, M., GOLDBERG, E., SILVA, B.M. & OLIVEIRA, P.F. (2016). Male fertility and obesity: are ghrelin leptin and glucagon-like peptide-1 pharmacologically relevant? *Current Pharmaceutical Design*, **22**: 783–791. <https://doi:10.2174/1381612822666151209151550>
40. MARTINS, A.D., JARAK, I., MORAIS, T., CARVALHO, R.A., OLIVEIRA, P.F., MONTEIRO, M.P. & ALVES, M.G. (2020). Caloric restriction alters the hormonal profile and testicular

- metabolome resulting in alterations of sperm head morphology. *American Journal of Physiology Endocrinology and Metabolism*, **318**(1): E33-E43. <https://doi.org/10.1152/ajpendo.00355.2019>
41. GAYLE, D.A., DESAI, M., CASILLAS, E. BELOOSESKY, R. & ROSS, M.G. (2006). Gender-specific orexigenic and anorexigenic mechanisms in rats. *Life Sciences*, **79**: L1531–L1536. <https://doi.org/10.1016/j.lfs.2006.04.015>
42. PALMER, B.F. & CLEGG, D.J. (2021). Starvation Ketosis and the Kidney. *American Journal of Nephrology*, **52**(6): 467–478. <https://doi.org/10.1159/000517305>
43. BEARDSALL, K., YUEN, K., WILLIAMS, R. & DUNGER, D. (2006). Applied physiology of glucose control. *Current Paediatrics*, **16**(6): 434–438. <https://doi.org/10.1016/j.cupe.2006.08.002>
44. LA FLEUR, S.E., KALSBECK, A., WORTEL, J., FEKKES, M.L. & BUIJS, R.M. (2001). A daily rhythm in glucose tolerance: a role for the suprachiasmatic nucleus. *Diabetes*, **50**(6): 1237–1243. <https://doi.org/10.2337/diabetes.50.6.1237>
45. SADACCA, L.A., LAMIA, K.A., DE LEMOS, A.S., BLUM, B. & WEITZ, C.J. (2011). An intrinsic circadian clock of the pancreas is required for normal insulin release and glucose homeostasis in mice. *Diabetologia*, **54**(1): 120–124. <https://doi.org/10.1007/s00125-010-1920-8>
46. YAN, S.T., LI, C.L., TIAN, H., LI, J., PEI, Y., LIU, Y., GONG, Y.P., FANG, F.S. & SUN, B.R. (2014). MiR-199a is overexpressed in plasma of type 2 diabetes patients which contributes to type 2 diabetes by targeting GLUT4. *Molecular and Cellular Biochemistry*, **397**: 45–51. <https://doi.org/10.1007/s11010-014-2170-8>
47. HONKA, M.J., LATVA-RASKU, A., BUCCI, M., VIRTANEN, K.A., HANNUKAINEN, J.C., KALLIOKOSKI, K.K. & NUUTILA, P. (2018). Insulin-stimulated glucose uptake in skeletal muscle adipose tissue and liver: a positron emission tomography study. *European Journal of Endocrinology*, **178**(5): 523–531. <https://doi.org/10.1530/EJE-17-0882>
48. WU, Y., LU, H., YANG, H., LI, C., SANG, Q., LIU, X., LIU, Y., WANG, Y. & SUN, Z. (2016). Zinc stimulates glucose consumption by modulating the insulin signaling pathway in L6 myotubes: essential roles of AktGLUT4 GSK3beta and mTOR-S6K1. *Journal of Nutritional Biochemistry*, **34**: 126–135. <https://doi.org/10.1016/j.jnutbio.2016.05.008>
49. MORAES-VIEIRA, P.M., SAGHATELIAN, A. & KAHN, B.B. (2016). GLUT4 expression in adipocytes regulates de novo lipogenesis and levels of a novel class of lipids with antidiabetic and anti-inflammatory effects. *Diabetes*, **65**: 1808–1815. <https://doi.org/10.2337/db16-0221>
50. OKADA, T., KAWANO, Y., SAKAKIBARA, T., HAZEKI, O. & UI, M. (1994). Essential role of phosphatidylinositol 3-kinase in insulin-induced glucose transport and antilipolysis in rat adipocytes: studies with a selective inhibitor wortmannin. *Journal of Biological Chemistry*, **269**: 3568–3573. [https://doi.org/10.1016/S0021-9258\(17\)41901-6](https://doi.org/10.1016/S0021-9258(17)41901-6)
51. FURTADO, L.M., SOMWAR, R., SWEENEY, G., NIU, W. & KLIP, A. (2002). Activation of the glucose transporter GLUT4 by insulin. *Biochemistry and Cell Biology*, **80**: 569–578. <https://doi.org/10.1139/o02-156>
52. WATSON, R.T., KANZAKI, M. & PESSIN, J.E. (2004). Regulated membrane trafficking of the insulin-responsive glucose transporter 4 in adipocytes. *Endocrine Reviews*, **25**: 177–204. <https://doi.org/10.1210/er.2003-0011>