# Attenuating Potential of Modified Diets on Testicular Inflammatory Biomarkers in Streptozotocin-Induced Diabetic Wistar Rats

**OSSAI Nduka Richard, NWANGWA Eze Kingsley, OJIEH Anthony Emeka, NWOGUEZE Bartholomew Chukwuebuka**
Department of Human Physiology, Delta State University, Abraka, Nigeria

**Abstract**
In vascularized tissues, inflammation is a complex and dynamic defensive response to cell injury, infection by bacteria, trauma, or toxins. Inflammatory biomarkers are molecules that regulate the inflammatory response in all immune system cells. Numerous mediators are released from the attack site, and different host immune system cells infiltrate the area during inflammation. Without the combination of regulated leukocyte population migration, different inflammatory mediators, inflammatory biomarkers (acute or systemic inflammatory marker), and subsequent physiologic changes that carry inflammatory responses, it would be impossible to assemble and regulate inflammatory responses. This study focuses on the attenuating potential of modified diets on testicular inflammatory biomarkers in streptozotocin-induced diabetic Wistar rats. Ninety-six adults male Wistar rats were divided into four units, of four groups/unit and each group consisting of six rats. Unit 1 is non-diabetic unit, whereas Units 2, 3, and 4 were induced with type 2 diabetes. While the rats in group one in all the units were fed with standard rat chaw, group 2 received HFD, group three received HPD, while groups four received HCD. Alpha lipoic acid (200 mg/kg body weight) and metformin (50 mg/kg body weight) were also given to the rats in units three and four, respectively. After 12 weeks of treatment and feeding, each rat was euthanized and testes were excised for biochemical analysis. Data were examined using GraphPad Prism. Two-way analysis of variance (ANOVA) was used to examine the mean variations between groups. The Tukey post hoc test was then performed, with p-values of 0.05 being deemed statistically significant. According to the current study's findings, high-protein meals, both by themselves and in conjunction with ALA, reduce blood glucose levels and the inflammatory damage brought on by streptozotocin toxicity. For this reason, they may be utilized to treat diabetes mellitus and autoimmune-induced inflammation.

**Keywords**: Alpha lipoic acid, Blood glucose, Carbohydrate, C-Reactive Protein, Diabetes mellitus, Diets, Fats, Inflammation, Metformin, Nutrition, Protein, Streptozotocin, Tumor Necrosis Factor-alpha, Wistar rat

## 1.0 INTRODUCTION

Diabetes Mellitus is a chronic disease associated with severe derangement of carbohydrates, protein and lipids metabolism[1, 2, 3, 4]. It continues to attract public concern, not just in developing countries but also in developed countries, and it presents usually with hyperglycemia and other metabolic abnormalities, which are due to deficiency of insulin effect[4]. According to statistics, 2.8% of the world's population suffer from diabetes and it is expected to increase to more than 5.4% by 2025[5], and globally, 4.3 million deaths have been record[6, 7, 8, 9]. Type 2 diabetes (T2D) has contributed to about 90–95% of all diabetes cases[8, 10] and it is a potential risk factor for cardiovascular disease[10], hence, it is critical to find a low-cost strategy that can help in its early prevention.

Diabetes mellitus (DM) has been reported to affect the male gonad negatively, with a decrease in fertility rate[11, 12] Mounting evidence from both clinical and experimental studies have demonstrated decreased fertility rates which has been linked with testicular inflammation[13]. The role of the immune system in development of T2D has gained interest in recent years such that a growing number of studies have highlighted the involvement of inflammatory biomarkers in the pathogenesis of T2D[14, 15]. Studies have shown that adiposity, insulin resistance, and hyperglycemia can induce systemic inflammation through stimulating the production of pro-inflammatory proteins such as C-reactive protein and cytokines including interleukin-1$\beta$ (IL-1$\beta$), interleukin-6 (IL-6), and tumor necrosis factor-$\alpha$ (TNF-$\alpha$)[16]. Additionally, environmental and behavioral factors can augment systemic inflammation in the time of stress[9, 17]. Human understanding of insulin resistance and secretion throughout the start and progression of disease has been reinforced by a number of pathophysiological investigations[18]. People at risk for type 2 diabetes first have insulin resistance, which is counteracted by excessive insulin production by beta cells. By the time diabetes is diagnosed, beta cells have lost their ability to produce enough insulin due to the pancreatic functional reserve's eventual inability to handle the necessary insulin secretion during the

clinical course of the disease[19]. The relative contributions of beta cell dysfunction and insulin resistance can vary in people with type 2 diabetes; nonetheless, low insulin sensitivity is typically identified as occurring up to 15 years before the diagnosis of diabetes[20]. Consequently, more recent research has concentrated on the routes leading to beta cell failure in addition to mechanistic investigations examining the processes behind insulin resistance[21]. In recent years, a number of therapeutic strategies that specifically target inflammatory pathways have been investigated, lending credence to the idea of using anti-inflammatory medication to treat cardio-metabolic disorders such diabetes and atherosclerotic cardiovascular disease[18, 22, 23, 24]. Salicylates, particularly aspirin, have long been used to treat rheumatic illnesses and thrombosis in the prevention of primary and secondary CVD [18, 25]. A small cohort study[26] showed that methotrexate, a disease-modifying medication, was effective in improving glycaemic control. However, the drug's effects on IL-1b, IL-6, and CRP levels were inconclusive, and further research is needed to determine its effects on type 2 diabetes[27]. To halt the onset and progression of type 2 diabetes, it has been suggested to target cytokine synthesis and secretion to inhibit additional inflammatory activation. With the exception of a randomised 6-month trial, more studies on patients with an unfavourable cardio-metabolic profile did not show sufficient results. TNF-alpha antagonists have been used to treat inflammatory conditions and have been linked to improved glycaemic control and decreased incident of diabetes[18, 28]. In people with diabetes and pre-diabetes, IL-1beta antagonists such anakinra and gevokizumab have been demonstrated to enhance beta cell secretory function, lower inflammatory biomarkers, and improve glycaemia [18, 29, 30].

One of the main variables that may be changed to affect systemic inflammation is diet[31] by influencing metabolic heath through dietary factors that exhibit anti- or pro-inflammatory properties[32]. For instance, dietary flavonoid intake has been inversely associated with inflammation[33], whereas saturated fatty acids have shown a positive association[9, 34]. However, as whole dietary patterns and indices can capture the interaction of several nutrients or foods to reflect the complexity of a diet, they may be more useful in assessing the inflammatory effect of a diet [9, 35].

The relationship between dietary intake of fat, carbohydrates, and inflammation is debatable; however, in diabetic states, a diet high in fat was not associated with a higher risk of inflammation, whereas a diet high in carbohydrates was. As a result, a diet high in carbohydrates was associated with a higher risk of inflammation than a diet high in fat, however, an inverse relationship between protein intake and changes in inflammation has been reported[36]. This suggests that dietary protein may be linked to beneficial changes in the inflammatory burden. Overall, inflammation increased less in those with the highest intake than in those with the lowest [36]. Current dietary guidelines mostly have focused on lowering dietary total fat to < 30% of total energy intake[37], while higher carbohydrate intake play an important role to incidence chronic non-communicable diseases, such as CVDs[37]. Recommendations on restricting total fat intake are largely based on observational studies performed in European and North American societies where there is relatively high intakes of energy, total fats and low intake of total carbohydrate[38, 39]. It is still unclear if these recommendations can be implemented in low- and middle-income nations where a high-carb diet and under-nutrition are even more prevalent. Additionally, the majority of these dietary recommendations are predicated on the notion that total fat consumption and low-density lipoprotein cholesterol (LDL) are positively correlated, as well as that LDL and CVD events are linked[39, 40]. It is indisputable that recommendations for fat, carbohydrate, and protein diets must take into account inflammatory indicators from human studies[39, 41]. This study therefore investigates the attenuating potential of High modified diet on testicular inflammatory biomarkers amelioration induced by streptozotocin in male rats.

## 2.0 MATERIALS AND METHODS

**Syringes And Needles (**Lot: Ww-Ag-13024**),** Production Date-Oct 2013Expiring Date-Oct 2018**, (**NAFDAC No-03-0777), Manufactured For Agary Pharmaceutical Limited By Wuxi Yushou Medical Appliances Co.Ltd, **Hand Gloves** (Lot No 2116), Nafdac No-03-3206, Production Date-04 2015, Expiring Date-04 2018, Manufactured For Longer Life Health Care Ltd, No 5 Udi Street Onitsha Anambra State Nigeria, **Incubactor** (Model TT 9052), Company Name- Techmel And Techmel Usa., **Glucometer** Accu Checkwww.acu-check.com, **Aucku Check active Strip** (Lot No-24640133), Expiring Date-10 2025, **Micropipette (**Microlux)Vol.Range 0-1000ul, **Stop Watch**

(Taksun: Ts-1809), Stop Timerq.C 2014-0907, **Oven** (Dhg-9023a), ELISA microplate (HIPO MPP-96, BIOSAN), **Centrifuge Model 800** Made In China Zhengji, UV-VIS spectrophotometer (model 752N), Digital electronic weighing balance (Model JA 2003), Plastic cages, Animal cage and plates.

**Chemicals**

**Sodium citrate** ($C_3H_4$ (OH) $(COONa)_3 2H_2O$) (BDH chemicals LTD Pools England, Batch No. 5425/18/58)**, Streptozotocin (STZ)**: Sigma. Aldrich Co.3050 Spruce Street St. Louis Mo U.S.A, Nicotinamide (NAD) Santa Cruz biotechnology St. Dallas USA (Batch number 208096).

**Drugs**

Alpha lipoic acid puritan's pride (Batch number 539608101). Metformin glucophage

**Experimental Diets**

**Purchase of Animal Feeds**

Soya beans were purchased from Dawanau market in Dawakin Tofa Local Government of Kano State, Nigeria, Groundnut seeds and Maize seed were purchased from the local market in Obiaruku, Ukwani Local Government Area, Delta State, Nigeria. Standard commercial pelleted feed was purchased from Rainbow Top Feed Company, Amukpe roundabout, Sapele, Delta State, Nigeria

**Identification**

Soya bean seed, Groundnut seed and Maize seed were identified and authentication in the Department of Agronomy, Wildlife and Forestry, Faculty of Agriculture, Delta State University, Abraka, Nigeria.

**Composition of Modified Special Diet (MSD)**

The composition of the various Nutrition Modified Diets (NMDs) formulated in the present study were based on AIN-93G rodent diet composition as recommended by the American Institute of Nutrition and adopted by Aguiar *et al.* [42] with little modifications. When carbohydrate, protein and fats components were changed, care were taken to ensure that experimental diets have a similar nutrient to calorie ratio, since it has been reported that animals will mostly eat for calories and not weight of food Aguiar *et al.* [42]. The composition of different Nutrition Modify Diets (NMDs) used is illustrated in tables below. The modified diets were fed to the rats for 12 weeks *ad libitum*.

**Preparation of Modified Animal Feeds**

**Preparation of Modified High Fat Diet (HFD)**

Groundnut Seed diet were prepared according to the methods described by Aletor[43]. The Groundnut seeds were processed into modified fat diet (MFD).

**Processing of Groundnut Seeds**

The groundnut seeds were cleaned by removing stones and dirts and air-dried. The dried cleaned groundnut seeds were milled and sieved to pass through a 0.5mm mesh[43]. The milled groundnut feeds were properly stored to avoid contamination from pests and moulds. Throughout the course of the trial, the diet was made weekly to prevent the degradation of its constituents.

**Nutritional Composition**

Eighty grams (80 g) of the milled Groundnut were added to 20 g of the normal commercial animal pellet to make a modified fat diet of nutrient composition below

| Nutrient | Composition of Groundnut in 80 (g) | Composition of Normal Animal Meal 20 (g) | Composition of Modified High Fat Diet (HFD) 100 (g) |
|---|---|---|---|
| Fat | 40.10 | 17.0 | 57.1 |
| Carbohydrate | 19.0 | 11.3 | 30.3 |
| Protein | 25.30 | 4.5 | 29.8 |

**Preparation of Modified High Protein Diet (HPD)**

Soya bean diet was prepared according to the methods described by Aletor [43]. The soya bean was processed into modified protein diet (MPD

**Processing of Soya bean**

The Soya bean seeds were cleaned by removing stones and dirts and air-dried. To pass through a 0.5mm mesh, the dried, cleaned soy bean seeds were ground and sieved[43]. The milled soya bean feeds were properly stored to avoid contamination from pests and moulds. Throughout the course of the trial, the diet was made once a week to prevent the degradation of its constituents.

**Nutritional Composition of Modified Protein Diet**

Eighty grams (80 g) of the milled soya bean were added to 20 g of the normal commercial animal pellet to make a modified protein diet of nutrient composition below

| Nutrient | Composition of Soya Bean in 80 (g) | Composition of Normal Animal Meal 20 (g) | Composition of Modified High Protein Diet (HPD) 100 (g) |
|---|---|---|---|
| Protein | 36.5 | 17.0 | 53.5 |
| Carbohydrate | 30.2 | 11.3 | 41.5 |
| Fat | 19.9 | 4.5 | 24.4 |

**Purchased and Preparation of Modified High Carbohydrate Diet (HCD)**

Maize diet was prepared according to the methods of Aletor, [43]. The raw maize seeds were processed into modified carbohydrates diet (MCD).

**Processing of Maize Seed**

The maize seeds were cleaned by removing stones and dirts and air-dried. The dried cleaned maize seeds were milled and sieved to pass through a 0.5mm mesh [43]. The milled maize feeds were appropriately stored to prevent pest and mold infection. Throughout the course of the trial, the diet was made once a week to prevent the breakdown of its components.

**Composition**

Eighty grams (80 g) of maize was added to 20 g of the normal commercial animal meal to make a modified Carbohydrate diet of nutrient composition below

| Nutrient | Composition of Maize Seeds in 80 (g) | Composition of Normal Animal Meal 20 (g) | Composition of Modified High Carbohydrate Diet (HCD) 100 (g) |
|---|---|---|---|
| Carbohydrate | 74.5 | 17.0 | 91.5 |
| Protein | 9.0 | 11.3 | 20.3 |
| Fat | 3.4 | 4.5 | 7.9 |

**Ethical Consideration**

The protocol of the experiments in this study were obtained from the Research and Ethics Committee of the Faculty of Basic Medical Sciences, Delta State University, Abraka, Nigeria **(REC/FBMS/DELSU/21/134).** This research was performed in accordance with the ethical standards on the care and use of animals as laid down by Helsinki in 1964 [44].

**Chemicals and Drugs Preparation**

**Chemicals**

**Sodium Citrate Buffer**

Two grams (2g) of Sodium Citrate were dissolved in 100ml of water to yield 2% of citrate buffer

**Streptozotocin (STZ)**

Streptozotocin (STZ) of 0.6g were dissolved in 10ml of citrate buffer to yield 60mg of stock solution (Diabetogenic agent).

**Nicotinamide**

Nicotinamide (1000 g) were dissolved in 10 ml of water and were administered to the animals fifteen (15) minutes before induction of Diabetes Mellitus (DM).

**Drugs**

**Metformin Tablet**

Five hundred milligrams (500 mg) of metformin (Glucophage) were dissolved in 10ml of distilled water, to give a solution of anti-diabetic drugs.

**Alpha Lipoic Aicd (ALA)**

Three hundred grams (300 g) of alpha lipoic acid (ALA) were dissolved in 50 ml of distil water to yield 6ml of the stock solution

**Experimental Animal**

**Purchase of Animals**

Ninety-six (96) Adult male Wistar rats for this study were purchased from the animal house of the Faculty of Basic Medical Sciences, Delta State University, Abraka, Nigeria. They were kept in a breeding chamber with a naturally controlled system (room temperature and a 12-hour light-dark cycle) after

spending seven days getting used to their surroundings in well-ventilated cages.

**Animal Grouping**

The animals were randomly divided into four (4) units of four (4) groups in each unit, with ten (10) animals in each group. Only unit(s) 2, 3 and 4 were induced with diabetes while the unit 1 rats serve as the non-diabetic control unit. Before the animals were induced diabetes, they were allowed to fast for eight to twelve hours while having unlimited access to water.

**Induction of Diabetes**

A single intraperitoneal injection of 60 mg/kg of streptozotocin (Santa Cruz Biotechnology) was given to the rats in Unit(s) 2, 3, and 4 after fifteen minutes interval of oral administration of Nicotinamide[45, 46, 47], while the rats in Unit 1 serves as the non-diabetic control rats [4, 44, 47, 48, 49, 50].

**Confirmation of Diabetes Mellitus**

The development of diabetes was checked with the aid of a glucometer (Accuk-check active, Germany) by measuring blood glucose level after 72hours of STZ injection. Diabetes Mellitus were confirmed by elevated fasting glucose over 200 mg/dL[4, 44, 50, 51, 52].

**Experimental Design**

**Unit 1**

The control unit were divided into four (4) groups as follows:

**Group 1** (n = 6): Non-diabetic Rats feed with the normal chew.
**Group 2** (n = 6): Non-diabetic Rats feed with the high fat diet (HFD)
**Group 3** (n = 6): Non-diabetic Rats feed with the high protein diet (HPD)
**Group 4** (n = 6): Non-diabetic Rats feed with the high carbohydrate diet (HFD)

**Unit 2**

The experimental unit were divided into four (4) groups as follows:

**Group 1** (n = 6): Diabetic Rats feed with the normal chew.
**Group 2** (n = 6): Diabetic Rats feed with the high fat diet (HFD)
**Group 3** (n = 6): Diabetic Rats feed with the high protein diet (HPD)
**Group 4** (n = 6): Diabetic Rats feed with the high carbohydrate diet (HFD)

**Unit 3**

The unit three diabetic rats received 200 mg/kg alpha lipoic acid daily for a period of 12 weeks and were also feed as follows:

**Group 1** (n = 6): Diabetic Rats feed with the normal chew.
**Group 2** (n = 6): Diabetic Rats feed with the high fat diet (HFD)
**Group 3** (n = 6): Diabetic Rats feed with the high protein diet (HPD)
**Group 4** (n = 6): Diabetic Rats feed with the high carbohydrate diet (HCD)

**Unit 4**

The unit four diabetic rats received 200 mg/kg alpha lipoic acid and 50 mg/kg of metformin, daily for a period of 12 weeks and were also feed as follows:

**Group 1** (n = 6): Diabetic Rats feed with the normal chew.
**Group 2** (n = 6): Diabetic Rats feed with the high fat diet (HFD)
**Group 3** (n = 6): Diabetic Rats feed with the high protein diet (HPD)
**Group 4** (n = 6): Diabetic Rats feed with the high carbohydrate diet (HCD)

**Determination of Blood Glucose Level**

Weekly assessment of blood glucose level was carried out using an accku check glucometer (Accu Check active German) and accu Check active strip. Blood was collected from the tip of the animal's tail. Values obtained were rerecorded and expressed in mg/dl.

**Euthanizing of animals and Sample Collection**

The animals were euthanized by cervical dislocation after an overnight fast but prior to this, the final body weight and fasting blood glucose check were carried out. Blood was collected via cardiac puncture, using 2ml syringes and 23G needle into plain blood sample containers for biochemical analysis.

**Biochemical Analysis**

**Assay for Testicular inflammatory Biomarkers (**TNF-alpha and CRP)

**Test principle**

This ELISA kit uses the Sandwich-ELISA principle. The micro ELISA plate provided in this kit were pre-coated with an antibody specific to Rat TNF-α and CRP. Samples (or Standards) were added to the micro ELISA plate wells and combined with the specific antibody. Then a biotinylated detection antibody

specific for Rat TNF-α and CRP and Avidin-Horseradish Peroxidase (HRP) conjugate were added successively to each micro plate well and incubated respectively. Free components were washed away. The substrate solution was added to each well. Only those wells that contain Rat TNF-α, as well as that of the CRP biotinylated detection antibody and Avidin-HRP conjugate appeared blue in color. The enzyme-substrate reaction was terminated by the addition of stop solution and the color turns yellow. The optical density (OD) was measured spectrophotometrically at a wavelength of 450 nm ± 2 nm. The OD value is proportional to the concentration of Rat TNF-α as well as CRP. The concentration of Rat TNF-α and that of the CRP were calculated in the samples by comparing the OD of the samples to the standard curve.

**Sample collection**

**Tissue homogenates**

The testes were finely chopped into tiny pieces and thoroughly rinsed in ice-cold PBS (0.01M, pH=7.4) to get rid of any extra hemolyzed blood that might have affected the outcomes. After weighing the tissue pieces, a glass homogenizer was placed on ice and used to homogenize them in PBS (tissue weight (g): PBS (mL) volume=1:9). To further degrade the cells in the suspension, an ultrasonic cell disrupter was used. To get the supernatant, the homogenates were centrifuged for 5–10 min at 5000×g at 2–8°C.

**Assay procedure**

Determine wells for diluted standard, blank and sample.

1. The standard, blank, and sample dilutions of 100 µL each were applied to the proper wells. Every sample and standard underwent duplicate analysis. After covering the plate with the kit's sealer, the samples were incubated for 90 minutes at 37°C.The micro ELISA plate well was filled with the solutions. It was avoided to touch or cause foaming on the inside wall.
2. After decanting the liquid out of each well, 100 µL of the Biotinylated Detection Ab working solution was added right away. The plate was then sealed with a fresh sealer and incubated at 37°C for an hour.
3. After each well's solution was decanted, 350 µL of wash buffer was added, steeped for a minute, and the decanted material was pat dried against fresh absorbent paper. Three times, this washing procedure was carried out.
4. Each well received 100 µL of HRP Conjugate working solution. After applying a fresh sealer, the plates were incubated at 37°C for 30 minutes. Each well's solution was decanted, and the washing procedure was carried out five times.
5. After adding ninety (90) µL of the substrate reagent to each well and covering the plate with a fresh sealer, the plate was incubated at 37°C for approximately 15 minutes.
6. Each cuvette was added with fifty (50 µL) of Stop Solution.
7. The optical density (OD) value was determined of each well at once with a micro-plate reader set to 450 nm.

**Calculation of results**

Average the duplicate readings for each standard and samples were taken and then subtracted the average zero standard optical density and plotting a four parameter logistic curve on log-log axis, with standard concentration on the x-axis and OD values on the y-axis.

**Statistical Analysis**

Data were represented as mean ± Standard Error of Mean (SEM) and analyzed using GraphPad prism version 8.0 (GraphPad Software, San Diego, CA, USA). Comparison of mean differences between groups were performed using two-way analysis of variance (ANOVA), followed by Tukey post hoc test. p - values ≤ 0.01 were considered statistically significant.

## 3.0 RESULT

### 3.1 Effect of high fat (HFD), high protein (HPD), and high carbohydrate diet (HCD) on Percentage Changes in Blood Sugar Level in non-diabetic and streptozotocin-induced diabetes Male Wistar rats co-treated with alpha lipoic acid (ALA) and Metformin (MFM)

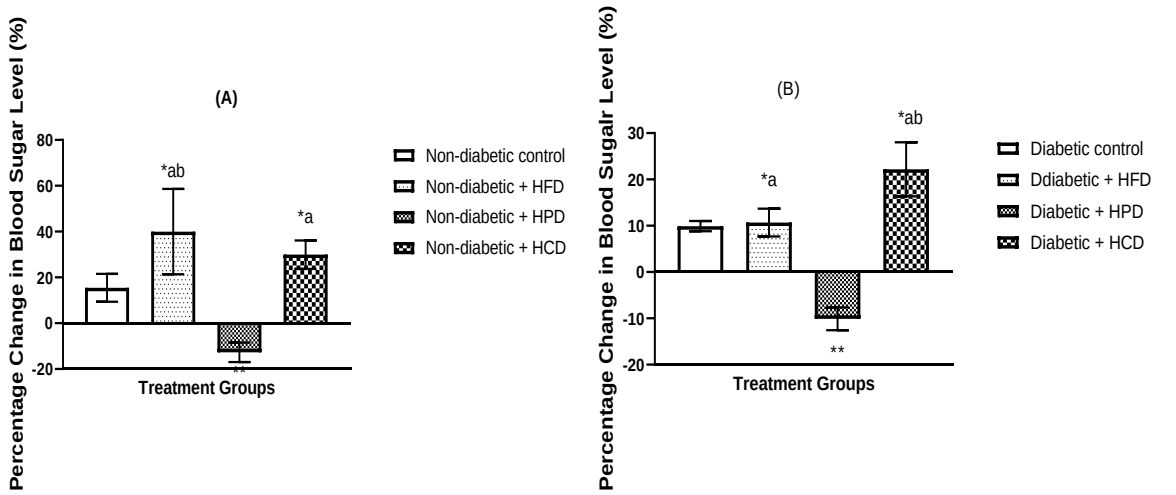


**Figure 1: Percentage Change in blood sugar level in (a) non-diabetic and (b) Streptozotocin-Induced male Wistar rats fed with high fat (HFD), high protein (HPD), and high carbohydrate diet (HCD).**

*+Bars represent Mean ± S.E.M. (n = 6) (One-way ANOVA followed by Bonferroni post hoc test). *p<0.05, relative to controls; [a] p < 0.05 relative to HFD group [b]p < 0.05, relative to HCD group.*

As shown in **figure 1a** non-diabetic rats fed HCD and HFD showed a statistically significant increased percentage change in blood glucose levels when compared to non-diabetic rats fed rat chaw. On the other hand, non-diabetic rats fed HPD showed a statistically significant decreased percentage changed in blood glucose levels when compared to non-diabetic rats fed HFD and HCD. Additionally, **figure 1b** shows that after being exposed to both the high fat and high carbohydrate diets, the percentage change in blood glucose levels of the diabetic rats fed both diets were significantly higher than those of the diabetic control rats fed rat chaw. In contrast, the percentage changed in blood glucose levels of the diabetic rats fed HPD were statistically significantly lower than those of the diabetic control rats fed rat chaw.

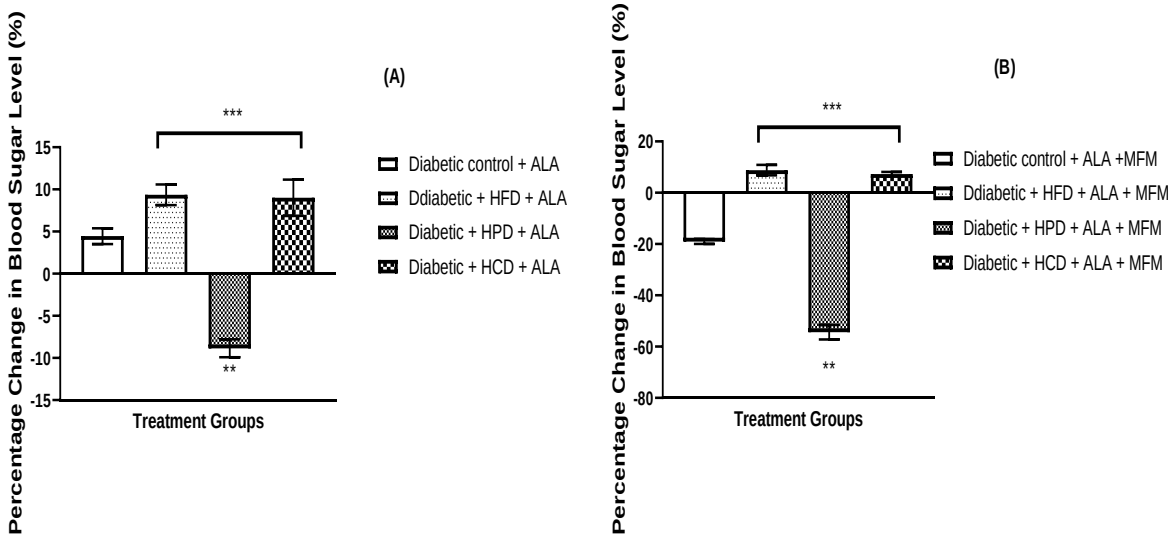


**Figure 2: Percentage Change in blood Sugar Level in diabetic male Wistar rats fed with high fat (HFD), high protein (HPD), and high carbohydrate diet (HCD) and treated with (a) (alpha lipoic acid (200 mg/kg) and (b) Alpha lipoic acid (200 mg/kg + Metformin (50 mg/kg).**

*Bars represent Mean ± S.E.M. (n = 6) (One-way ANOVA followed by Bonferroni post hoc test).* $^{*}p < 0.05$*, relative to controls;* $^{a}p < 0.05$ *relative to HFD group* $^{b}p < 0.05$*, relative to HCD group.*

**Figure 2a** shows that, in comparison to the diabetic rats given rat chaw and treated with ALA, the diabetic rats fed HFD and treated with ALA, as well as the diabetic rats fed HCD and treated with ALA, had significantly higher percentage change in blood glucose levels when compared to the diabetic rats fed rat chaw and treated with ALA, however, a statistically significant decreased percentage change in blood glucose levels was seen in the diabetic rats fed HPD + ALA when compared to diabetic rats fed with HFD + ALA, HCD + ALA as well as rats fed with rat chaw + ALA. **Figure 2b** shows that there were notable variations in the percentage change in blood glucose levels of diabetic rats fed HFD, HPD, and HCD treated with ALA and metformin, respectively. When compared to the diabetic control rats fed with rat chaw and treated with ALA and metformin, the blood glucose levels of the diabetic rats fed with HFD+ALA+MFM as well as diabetic rats fed HCD+ALA+MFM were significantly higher, whereas, diabetic rats fed with HPD+ALA+MFM demonstrated a statistically significant decreased percentage changes in blood glucose levels.

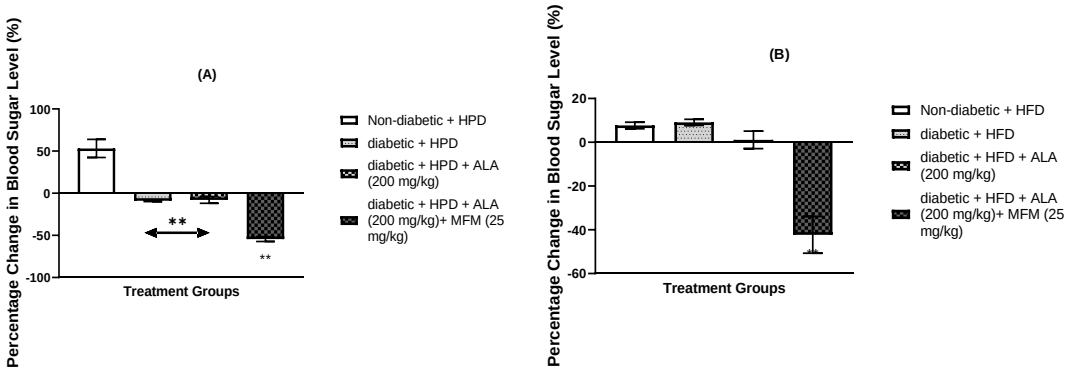


**Figure 3: Percentage Change in Blood Sugar Level in non-diabetic and streptozotocin-induced diabetic male Wistar rats fed with high protein (HPD) and HFD respectively treated with alpha lipoic acid (200 mg/kg) and metformin (50 mg/kg) respectively.**

*Bars represent Mean ± S.E.M. (n = 6) (Two-way ANOVA followed by Bonferroni post hoc test).* $^{*}p < 0.05$*, relative to Non* ***diabetic;*** $^{a}p < 0.001$ *relative to Diabetic + HFD group* $^{b}p < 0.05$*, relative to Diabetic + HFD + ALA group.*

When compared to non-diabetic rats fed HPD alone, diabetic rats fed HPD showed a significantly higher percentage change in blood glucose level **(figure 3a)**. However, when alpha lipoic acid was administered to diabetic rats fed HPD, the percentage change in blood glucose level was significantly lowered than in the diabetic rats fed HPD alone. When compared to diabetic rats fed HPD + ALA, diabetic rats receiving HPD + ALA + MFM showed even greater drop in percentage change blood glucose levels. **Figure 3b** illustrates that diabetic rats fed a high-fat diet had significantly higher percentage change in blood glucose levels than non-diabetic rats given HFD alone. When compared to diabetic rats fed HFD alone, the diabetic rats that received HFD + ALA showed a considerable drop in percentage change blood glucose levels. When compared to diabetic rats fed HFD + ALA, diabetic rats fed with HFD + ALA + MFM showed an even greater drop in percentage change in blood glucose levels.

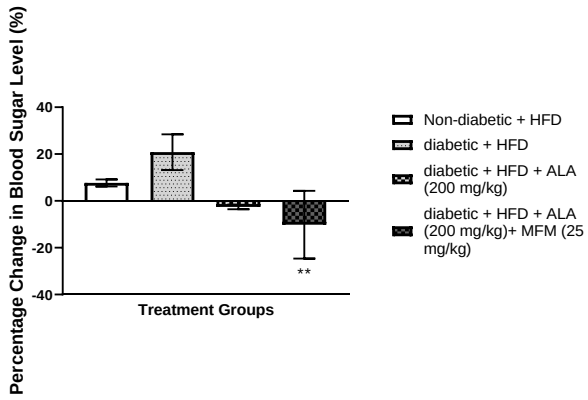


**Figure 4: Percentage Change in Blood Sugar Level in non-diabetic and streptozotocin-induced diabetic male Wistar rats fed with High Carbohydrate Diet (HCD) and treated with alpha lipoic acid (200 mg/kg) and metformin (50 mg/kg) respectively.**

*Bars represent Mean ± S.E.M. (n = 6) (Two-way ANOVA followed by Bonferroni post hoc test).* $^{*}p < 0.05$*, relative to Non* ***diabetic;*** $^{a}p < 0.001$ *relative to Diabetic + HFD group* $^{b}p < 0.05$*, relative to Diabetic + HFD + ALA group.*

A Statistically significant increased percentage change in blood glucose level in diabetic rats fed with high carbohydrate diet were observed **(figure 4)** when compared to the non-diabetic rats fed with high carbohydrate diet alone. However, diabetic rats that received HCD + ALA demonstrated a significant decreased percentage change in blood glucose level when compared to the diabetic rats fed with HCD alone. Further decreased percentage change in blood glucose level were observed in diabetic rats fed with HCD + ALA + MFM when compared to diabetic rat fed with HCD + ALA.

**3.2. Effect of high fat (HFD), high protein (HPD), and high carbohydrate diet (HCD) testicular Inflammatory Biomarkers (Tumor Necrotic Factor (TNF-alpha), and C - reactive protein (CRP) in non-diabetic and streptozotocin-induced diabetes in male Wistar rats treated with alpha lipoic acid (ALA) and Metformin (MFM).**

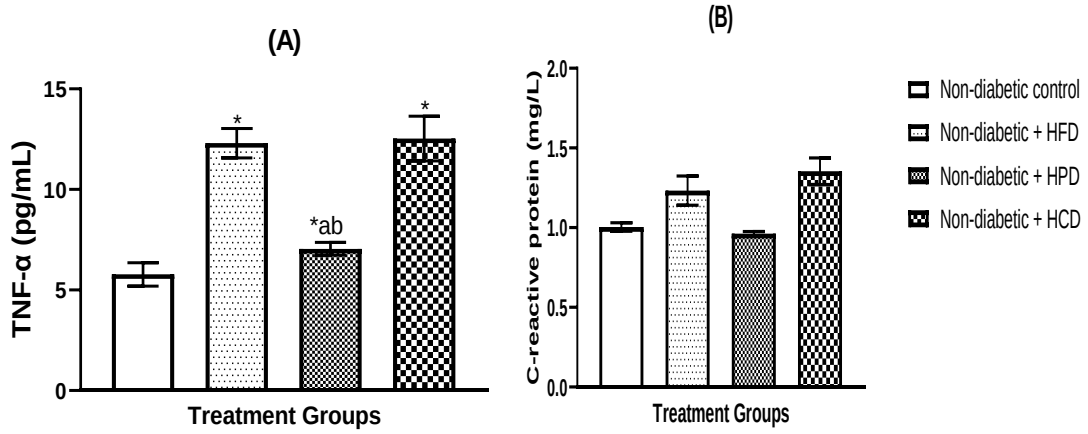


**Figure 5: Effect of high fat (HFD), high protein (HPD), and high carbohydrate diet (HCD) on (a) testicular inflammatory biomarkers (a) TNF-alpha and (b) C. Reactive Protein in non-diabetic male Wistar rats**

*Bars represent Mean ± S.E.M. (n = 6) (One-way ANOVA followed by Bonferroni post hoc test).* $^{*}p < 0.05$*, relative to controls;* $^{a}p < 0.05$ *relative to HFD group* $^{b}p < 0.05$*, relative to HCD group.*

A statistically significant increased TNF-alpha and C. Reactive Protein levels were observed in non-diabetic rats fed with HFD as well as HCD respectively when compared to the non-diabetic control rats fed with rat chaw, however, a significant decreased TNF-alpha as well as C-Reactive Protein were observed in non-diabetic rats fed with HPD when compared to non-diabetic rats fed with HFD as well as HCD respectively

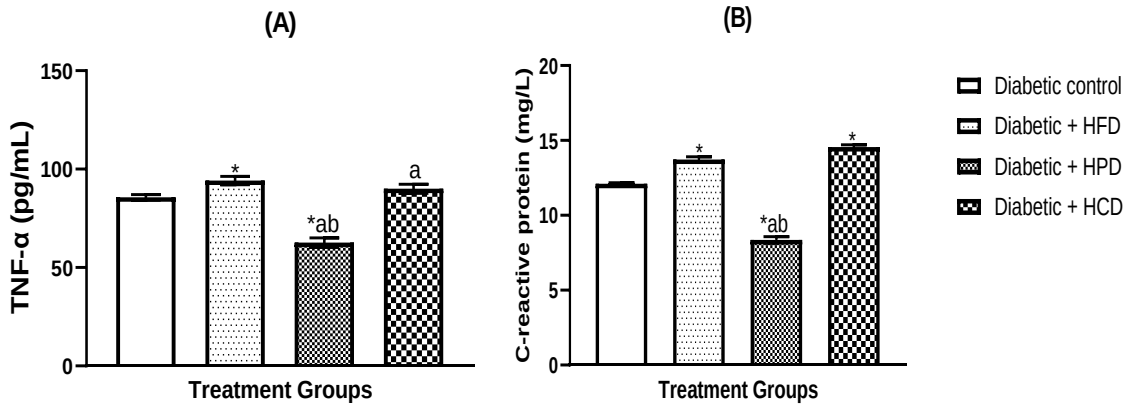


**Figure 6: Effect of high fat (HFD), high protein (HPD), and high carbohydrate diet (HCD) on testicular inflammatory biomarkers (a) TNF-alpha and (b) C. Reactive Protein in streptozotocin-induced diabetes male *Wistar* rats**

*Bars represent Mean ± S.E.M. (n = 6) (One-way ANOVA followed by Bonferroni post hoc test).* $^{*}p < 0.05$*, relative to controls;* $^{a}p < 0.05$ *relative to HFD group* $^{b}p < 0.05$*, relative to HCD group.*

A statistically significant increased TNF-alpha and C. Reactive Protein levels were observed in diabetic rats fed with HFD as well as HCD respectively when compared to the diabetic control rats fed with rat chaw, whereas, TNF-alpha and C Reactive Protein levels of diabetic rats fed with HPD where significantly decreased when compared to diabetic rats fed with HFD as well as HCD respectively.

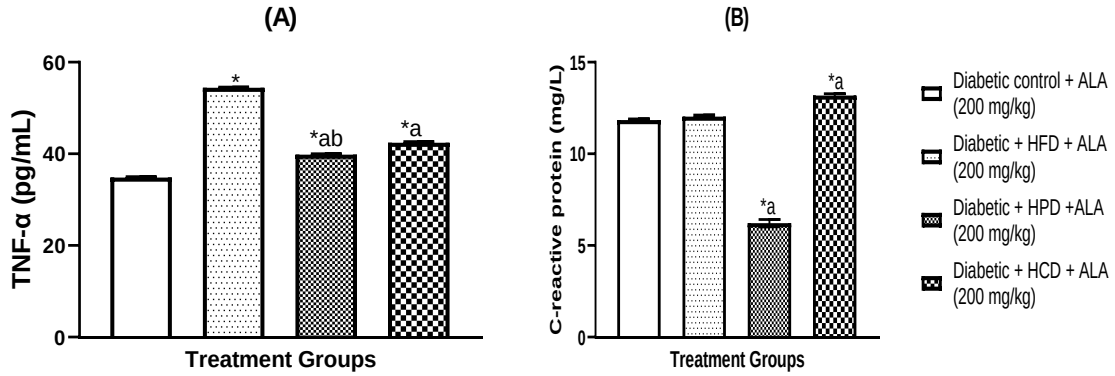


**Figure 7: Effect of high fat (HFD), high protein (HPD), and high carbohydrate diet (HCD) on testicular inflammatory bioarkers (a) TNF-alpha and (b) C. Reactive Protein in streptozotocin-induced diabetes male Wistar rats treated with alpha lipoic acid (200 mg/kg) body weight.**

*Bars represent Mean ± S.E.M. (n = 6) (One-way ANOVA followed by Bonferroni post hoc test).* $^{*}p < 0.05$*, relative to controls;* $^{a}p < 0.05$ *relative to HFD group* $^{b}p < 0.05$*, relative to HCD group.*

A statistically significant increased TNF-alpha and C. Reactive Protein levels were observed in diabetic rats fed with HFD + ALA as well as HCD + ALA respectively when compared to the diabetic control

rats fed with rat chaw + ALA, However, TNF-alpha and C Reactive Protein levels of diabetic rats fed with HPD + ALA where statistically significantly decreased when compared to diabetic rats fed with HFD + ALA as well as diabetic rats fed with HCD + ALA respectively.

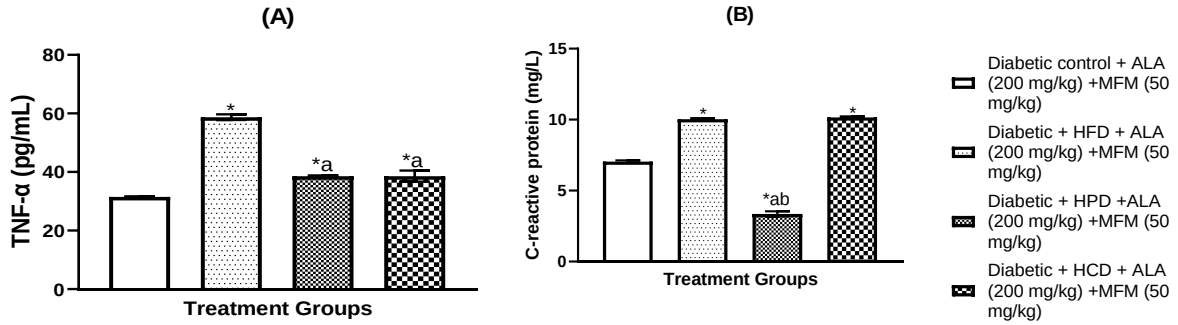


**Figure 8: Effect of high fat (HFD), high protein (HPD), and high carbohydrate diet (HCD) on testicular inflammatory biomarkers (a) TNF-alpha) and (b) C. Reactive *Protein in* streptozotocin-induced diabetic male Wistar rats treated with alpha lipoic acid (200 mg/kg) and Metformin (50 mg/kg) respectively**

*Bars represent Mean ± S.E.M. (n = 6) (One-way ANOVA followed by Bonferroni post hoc test).* $^{*}p < 0.05$*, relative to controls;* $^{a}p < 0.05$ *relative to HFD group* $^{b}p < 0.05$*, relative to HCD group.*

When compared to diabetic control rats fed rat chaw + ALA + MFM and diabetic rats fed HCD + ALA + MFM, figure 8a shows a statistically significant increase in TNF-alpha levels in the diabetic rats fed HFD + ALA + MFM. However, when comparing diabetic rats fed HPD + ALA + MFM to diabetic rats fed HCD + ALA + MFM, no statistically significant difference was seen; however, TNF-alpha levels were significantly lower in diabetic rats fed HPD + ALA + MFM than in diabetic rats fed HFD + ALA + MFM. Figure 8b shows that when diabetic rats fed HFD + ALA + MFM and HCD + ALA + MFM were compared to diabetic control rats fed rat chaw + ALA + MFM, their levels of C-reactive protein increased significantly. Conversely, when diabetic rats fed HPD + ALA + MFM were compared to diabetic rats fed HFD + ALA + MFM and diabetic rats fed HCD + ALA + MFM, respectively, their levels of C-Reactive Protein significantly decreased.

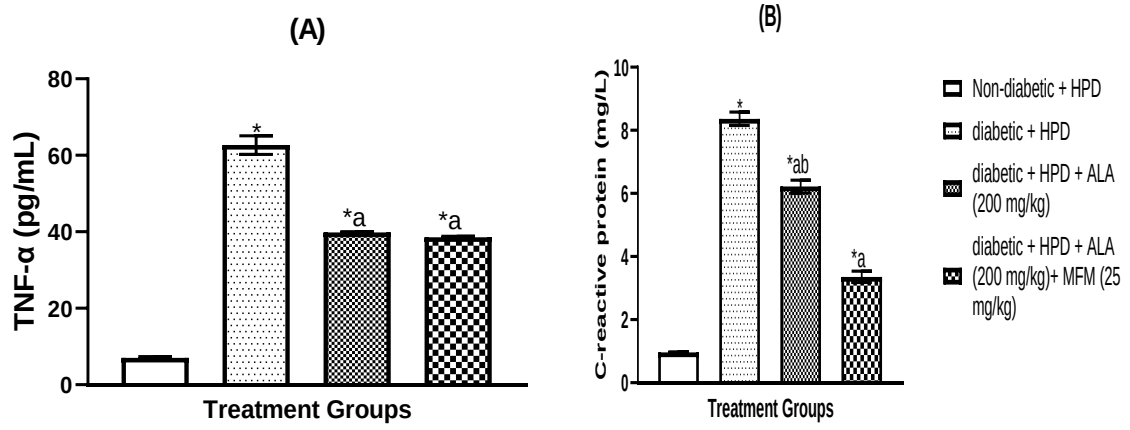


**Figure 9: Effect of alpha lipoic acid (ALA) and Metformin (MFM) on testicular inflammatory biomarkers (a) TNF-alpha and (b) C. Reactive Protein in non-diabetic and streptozotocin-induced diabetic Wistar rat fed with High Protein Diet (HPD).**

*Bars represent Mean ± S.E.M. (n = 6) (One-way ANOVA followed by Bonferroni post hoc test).* $^{*}p < 0.05$*, relative to Non* ***diabetic;*** $^{a}p < 0.001$ *relative to Diabetic + HPD group* $^{b}p < 0.05$*, relative to Diabetic*

*+ HPD + ALA group.*

A statistically significant increased TNF-alpha and C. Reactive Protein level were observed in diabetic rats fed with HPD when compared to non-diabetic rats fed with HPD, however, administration of alpha lipoic acid and metformin respectively to the diabetic rats fed with HPD, demonstrated a significant decreased TNF-alpha and C. Reactive Protein respectively when compared to diabetic rats fed with HPD only.

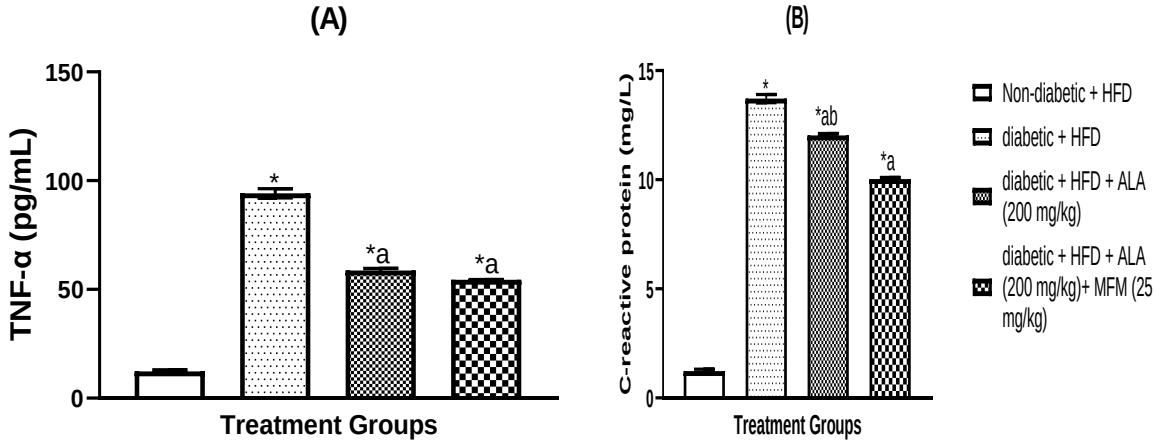


**Figure 10: Effect of alpha lipoic acid (ALA) and Metformin (MFM) on testicular inflammatory biomarkers (a) TNF-alpha) and (b) C. Reactive Protein in non-diabetic and streptozotocin-induced diabetic rat fed with high fat diet (HFD).**

*Bars represent Mean ± S.E.M. (n = 6) (One-way ANOVA followed by Bonferroni post hoc test).* $^{*}p < 0.05$*, relative to Non* ***diabetic;*** $^{a}p < 0.001$ *relative to Diabetic + HPD group* $^{b}p < 0.05$*, relative to Diabetic + HPD + ALA group.*

When compared to non-diabetic rats fed the same diet, diabetic rats fed the same diet showed a statistically significant increase in TNF-alpha and C. Reactive Protein levels; however, when alpha lipoic acid and metformin were administered to the diabetic rats fed the same diet, the levels of TNF-alpha and C. Reactive Protein significantly decreased.

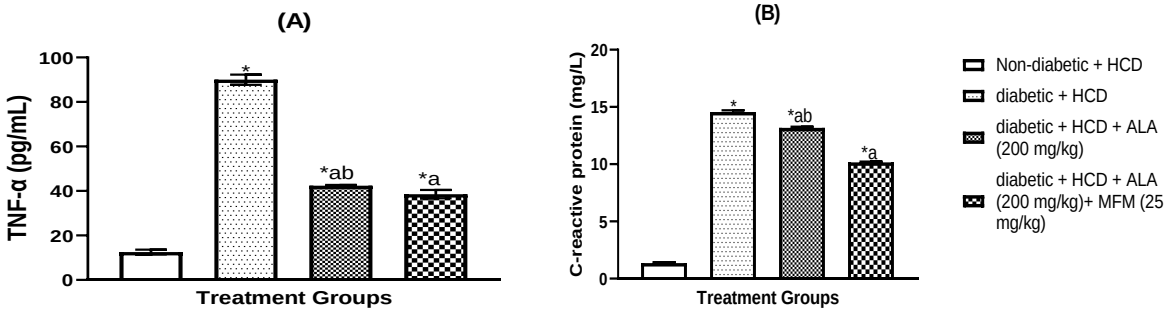


**Figure 11: Effect of alpha lipoic acid (ALA) and Metformin (MFM) on testicular inflammatory biomarkers (a) TNF-alpha) and (b) C. Reactive Protein in naïve and streptozotocin-induced diabetic rat fed with high carbohydrate diet (HCD).**

Bars represent Mean ± S.E.M. (n = 6) (One-way ANOVA followed by Bonferroni *post hoc* test). $^{*}p < 0.05$, relative to Non diabetic; $^{a}p < 0.001$ relative to Diabetic + HPD group $^{b}p < 0.05$, relative to Diabetic + HPD + ALA group.

A statistically significant increase ***was*** observed in TNF-alpha and C. Reactive Protein level in the diabetic rats fed with HCD when compared to non-diabetic rats fed with HCD, however, administration of alpha lipoic acid and metformin respectively to the diabetic rats fed with HCD, demonstrated a significant decrease in TNF-alpha and C. Reactive

Protein when compared to diabetic rats fed with HCD.

## 4.0 DISCUSSION

An important strategy in the management of type 2 diabetes mellitus (T2DM) is dietary control of blood glucose levels [4, 44]. To implement such an approach, it is imperative to understand how nutrition influences glycemic control and the underlying metabolic diseases. In **figure 1a-b**, the percentage Change in blood sugar level in non-diabetic and Streptozotocin-Induced male Wistar rats fed with high fat (HFD), high protein (HPD), and high carbohydrate diet (HCD) was assessed. When compared to non-diabetic rats fed rat chaw, non-diabetic rats fed HCD and HFD had a statistically significant higher percentage change in blood glucose levels, as illustrated in figure 1a. Conversely, when compared to non-diabetic rats given HFD and HCD, non-diabetic rats fed HPD demonstrated a statistically significant decreased percentage change in blood glucose levels. Furthermore, **figure 1b** demonstrates that the percentage change in blood glucose levels of the diabetic rats fed both diets was substantially larger than that of the diabetic control rats fed rat chaw after they were exposed to both the high fat and high carbohydrate diets. Conversely, the diabetic control rats given rat chaw had a statistically significantly higher percentage change in blood glucose levels than the diabetic rats fed HPD. The present findings are consistent with the review conducted by Russell *et al.* [53], which posits that a variety of dietary factors influences the effect of carbohydrates and fats on blood glucose levels. Nevertheless, it is important to remember that individuals with diabetes or other conditions involving impaired blood glucose regulation should not be advised to consume higher amounts due to possible negative consequences [53]. Diets high in protein had been reported to be beneficial in weight loss, improving body composition, and lowering blood sugar levels [53, 54], and better weight loss with high-protein diets has been linked to the satiating effects of dietary protein, a smaller selection of foods, and an aversion against dietary fat in the absence or smaller quantity of carbohydrates [53, 55], this further support the idea that consuming more dietary protein may aid in lowering calorie intake and thus blood sugar level.

Natural antioxidant alpha-lipoic acid (ALA) has a variety of biological activities[53]. It has been shown to operate as a metal chelator, regenerate endogenous antioxidants like vitamins C and E, and modulate signal transduction of many pathways[53, 56]. The impact of ALA on sensitivity to insulin[53, 57], insulin secretion [53, 58], the decrease in circulating lipid levels [53, 59], and the increase in nitric oxygen [53, 60] have all been highlighted in numerous studies that suggest its possible involvement in the control of glucose metabolism. Additionally, ALA appears to be helpful in reducing peripheral diabetic polyneuropathy [53, 61]. For this reason, it is commonly used for obesity, metabolic syndrome (MS), polycystic ovarian syndrome (PCOS), diabetic neuropathy, and type 1 and type 2 diabetes (T1D; T2D) [53, 62, 63, 64].

In **figure 2a**, the diabetic rats fed with HFD + ALA, as well as the diabetic rats fed with HCD + ALA, had a statistically significant increased percentage change in blood glucose levels than the diabetic rats fed rat chaw and treated with ALA, however, the diabetic rats fed with HPD + ALA showed a statistically significant decrease in percentage change in blood glucose levels when compared to the diabetic rats fed HFD + ALA, HCD + ALA, as well as rats fed rat chaw + ALA. **Figure 2b** shows that there were notable variations in the percentage change in blood glucose levels of diabetic rats fed HFD, HPD, and HCD treated with ALA and metformin, respectively. When compared to the diabetic control rats fed with rat chaw and treated with ALA and metformin, the blood glucose levels of the diabetic rats fed with HFD+ALA+MFM as well as diabetic rats fed HCD+ALA+MFM were significantly higher, whereas, diabetic rats fed with HPD+ALA+MFM demonstrated a statistically significant decreased percentage changes in blood glucose levels. When compared to non-diabetic rats fed HPD alone, diabetic rats fed HPD showed a significantly higher percentage change in blood glucose level **(figure 3a)**. However, when alpha lipoic acid was administered to diabetic rats fed HPD, the percentage change in blood glucose level was significantly lowered than in the diabetic rats fed HPD alone. When compared to diabetic rats fed HPD + ALA, diabetic rats receiving HPD + ALA + MFM showed even greater drop in percentage change blood glucose levels. **Figure 3b** illustrates that diabetic rats fed a high-fat diet had significantly higher percentage change in blood glucose levels than non-diabetic rats given HFD alone. When compared to diabetic rats fed HFD alone, the diabetic rats that received HFD + ALA showed a considerable drop in percentage change

blood glucose levels. When compared to diabetic rats fed HFD + ALA, diabetic rats fed with HFD + ALA + MFM showed an even greater drop in percentage change in blood glucose levels. A Statistically significant increased percentage change in blood glucose level in diabetic rats fed with high carbohydrate diet were observed **(figure 4)** when compared to the non-diabetic rats fed with high carbohydrate diet alone. However, diabetic rats that received HCD + ALA demonstrated a significant decreased percentage change in blood glucose level when compared to the diabetic rats fed with HCD alone. Further decreased percentage change in blood glucose level were observed in diabetic rats fed with HCD + ALA + MFM when compared to diabetic rat fed with HCD + ALA. The findings in **Figures 1, 2, 3, and 4,** respectively, are consistent with a number of previous research showing that ALA can enhance the absorption of glucose. [65, 66, 67]. Specifically, Eason et al. showed that insulin had no effect on glucose absorption; in the muscles of ob/ob mice, a model of extreme insulin resistance, ALA alone increased glucose absorption by 300% [65] and this implies that ALA has an impact on insulin resistance.

Inflammatory biomarkers are markers that modulate the inflammatory response of all cells of the immune system[68]. According to Elgazzar and Elmonayeri [69] and Zhao *et al.* [70] inflammation is a complicated, dynamic defensive reaction to vascularized tissues' damage, poisons, or microbial infection. Ansar and Ghosh [71] state that once the causal agent is diluted, eliminated, or isolated, a series of molecular events are set in motion that eventually result in the repair, healing, and reconstitution of the injured tissue. Its most important features are the response in tissues and its microcirculation, which is clinically expressed as redness (erythema), heat (hyperemia), swelling (exudation), pain (caused by nerves and chemical mediators), and loss of function (pain). Pathologically, it manifests as hyperemia, stasis, leukocyte accumulation, fluid exudation, fibrin deposition, vasoconstriction, and vasodilation. The combined vascular and cellular inflammatory responses are triggered by an inflammatory stimulus and are subsequently mediated by chemical mediators derived from specific cells or blood plasma. According to Ansar and Ghosh [71], mediators are released even by dead or damaged tissues. Inflammation can have many different causes, including microbiological infections (infections by bacteria, viruses, fungus, etc.), physical agents (such as burns, stress, trauma from cuts, radiation), chemicals (drugs, poisons, alcohol), and immunologic reactions (such as rheumatoid arthritis). Numerous mediators are released from the attack site, and different host immune system cells infiltrate the area during inflammation. Without the combination of regulated leukocyte population migration, different inflammatory mediators, inflammatory biomarkers (acute or systemic inflammatory marker), and subsequent physiologic changes that carry inflammatory responses, it would be impossible to assemble and regulate inflammatory responses. Tumour necrosis factor (TNF) is a cytokine and an adipokine that is made up of several transmembrane proteins that have a homologous TNF domain[72]. It was identified by Rolski and Błyszczuk[73] and Koelman *et al.* [74]. According to studies (Qu *et al.* [75]; and Sethi and Hotamisligil[72], TNF functions as an adipokine that increases insulin resistance and is linked to obesity-induced type 2 diabetes. On the other hand, TNF functions as a cytokine that the immune system uses for cell signalling[76]. Thus, as part of an inflammatory response, TNF is released by macrophages, which are specific types of white blood cells, upon detecting an infection[77, 78]. Immune cell modulation is TNF's main purpose[76]. As an endogenous pyrogen, TNF can cause fever, inflammation, cachexia, and apoptosis. It can also stop the growth of tumours and the spread of viruses, and it can react to sepsis by activating cells that produce IL-1 and IL-6[79]. Numerous human diseases, such as Alzheimer's disease[80], cancer[79], major depression [81], and inflammatory bowel disease (IBD)[74, 82] have all been linked to dysregulation of TNF production. Despite controversy, some research has connected elevated TNF levels to depression and inflammatory bowel disease (IBD)[82]. As seen in **figure 5a-b,** a statistically significant increased TNF-alpha and C. Reactive Protein levels were observed in non-diabetic rats given HFD as well as HCD respectively when compared to the non-diabetic control rats given rat chaw, however, a significant decreased TNF-alpha as well as C-Reactive Protein were observed in non-diabetic rats fed with HPD when compared to non-diabetic rats fed with HFD as well as HCD respectively while in **figure 6a-b,** a statistically significant increased TNF-alpha and C. Reactive Protein levels were observed in diabetic rats fed with HFD as well as HCD respectively in contrast to the diabetic control rats that received rat chaw,

whereas, TNF-alpha and C Reactive Protein levels of diabetic rats that received HPD where significantly decreased when compared to diabetic rats fed with HFD as well as HCD respectively. The findings of this investigation align with the reports of Aryani *et al.* [83] and Koelman *et al.* [74], which asserted Dietary Protein and Changes in Biomarkers of Inflammation and Oxidative Stress in the Framingham Heart Study Offspring Cohort. These reports verified that food sources exhibit varying impacts on distinct biomarkers of inflammation, and that dairy consumption is largely inconclusive concerning biomarkers like CRP, IL-6, and TNF-α. Reduced levels of inflammation have also been linked to dietary patterns high in protein sources[83, 84], substituting whole for refined grains appears to modulate select cytokines (e.g., TNF-α)[83, 85]. According to the results of an 8-week trial comparing an energy-restricted high (30% energy) protein diet with a low (15% energy) protein diet, high protein, especially meat protein—but not plant or fish protein—increases a score that includes concentrations of CRP, IL-6, TNF-α, and PAI-1[36]. The findings also indicated that while protein source and quantity may contribute to inflammation, underlying dietary and health settings may also be significant[36]. It has been established that consuming too much fat can lead to a loss of muscle mass since it can accumulate lipids and induce inflammation in the muscles[86]. High-fat diets (HFD) produce reactive oxygen species (ROS) that trigger a chronic inflammatory response and release pro-inflammatory cytokines like interleukin (IL)-1β or IL-6, as well as tumour necrosis factor (TNF)-α[86, 87]. An inflammatory response aids in muscle autophagy and regeneration under normal physiological conditions[86, 88]; on the other hand, an overabundance of pro-inflammatory cytokines may cause muscle loss, muscle atrophy, and muscle-cell apoptosis[86, 89].

Result in figure **7a-b** of this study showed a statistically significant increased TNF-alpha and C. Reactive Protein levels were observed in diabetic rats fed with HFD + ALA as well as HCD + ALA respectively when compared to the diabetic control rats that received rat chaw + ALA, However, TNF-alpha and C Reactive Protein levels of diabetic rats given HPD + ALA where statistically significantly decreased when compared to diabetic rats fed HFD + ALA as well as diabetic rats fed HCD + ALA respectively, while in **figure 8a,** a statistically significant increased TNF-alpha level were observed in diabetic rats given HFD + ALA + MFM when compared to diabetic control rats that received rat chaw + ALA + MFM as well as diabetic rats fed HCD + ALA + MFM. However, when comparing diabetic rats fed HPD + ALA + MFM to diabetic rats fed HCD + ALA + MFM, no statistically significant difference was seen; however, TNF-alpha levels were significantly lower in diabetic rats fed HPD + ALA + MFM than in diabetic rats fed HFD + ALA + MFM. **Figure 8b** shows that when diabetic rats fed HFD + ALA + MFM and HCD + ALA + MFM were compared to diabetic control rats fed rat chaw + ALA + MFM, their levels of C-Reactive Protein increased significantly. Conversely, when diabetic rats fed HPD + ALA + MFM were compared to diabetic rats fed HFD + ALA + MFM and diabetic rats fed HCD + ALA + MFM, respectively, their levels of C-Reactive Protein significantly decreased. According to studies on the impact of dietary patterns on inflammatory biomarkers in adults with type 2 diabetes mellitus, Shivappa *et al.* [90], Denova-Gutiérrez *et al.* [91], Sureda *et al.* [92], and Sánchez-Rosales *et al.* (93) all found similar results.

Vegetables and plants are rich sources of α-lipoic acid (ALA), a naturally occurring antioxidant[86]. According to earlier research[86, 94], ALA has a calming effect on inflammation and inflammatory responses in addition to diabetes and its aftereffects. In Otsuka Long Evans Tokushima Fatty (OLETF) rats, ALA was shown to maintain skeletal muscle mass[95]. Furthermore, in healthy older subjects, ALA help to maintain muscle mass and strength[96]. Many studies show that ALA reduces inflammation and oxidative stress in skeletal muscle[92, 95]. Additionally, studies have shown that ALA lowers pro-inflammatory
TNF-α and boosts the production of the anti-inflammatory IL-10 in the insulin-resistant rats' brain[86]. According to Ko *et al.* [86], ALA has multidirectional effects in the brain because it inhibits neuronal death. Remarkably, reduces oxidative/glycative stress, protein nitrosative damage, inflammation, and apoptosis while enhancing antioxidant balance[86]. Increases in TNF-alpha and C were statistically significant, as shown in **figures 9a-b, 10a-b,** and **11a-b**. Reactive protein levels were measured in the diabetic rats fed HPD, HFD, and HCD, respectively, and compared to the non-diabetic rats fed the same diets. However, when alpha lipoic

acid was administered to the diabetic rats fed HPD, HFD, and HCD, respectively, TNF-alpha and C. Reactive Protein were significantly lower than in the diabetic rats fed HPD, HFD, and HCD alone. Further decrease in TNF-alpha and C reactive proteins were observed in diabetic rats fed with HPD + ALA + MFM, HFD + ALA +MFM as well as HCD + ALA + MFM when compared to diabetic rats fed with HPD + ALA, HFD + ALA and HCD + ALA respectively. According to research, High Fat Diet causes excessive intercellular oxidative stress and stimulates pro-inflammatory cytokine secretion, resulting in the occurrence of inflammatory responses and DM[86, 97]. In addition, excessive cytokine production may had been reported to inhibit muscle regeneration by regulating mTOR expression, which may subsequently lead to muscle loss in DM[86]. The mechanism thought to be involved in the findings of this study is that ALA ha influence the nuclear factor kappa B, JNK, and PI3K/AKT signalling pathways in muscles, as reported by Hong *et al.* [95]. The current investigation revealed that ALA reduced TNF-α and C-reactive protein levels in muscle tissues, indicating that ALA may reduce inflammation via inhibiting the TNF-α/JNK pathway in type 2 diabetes rats' muscles. Since a prior study by Ko *et al.* [94] had also confirmed that ALA enhanced PI3K/AKT protein expression in the liver and brain tissues of rats with T2DM, it is assumed that ALA enhanced the protein expression of PI3K/AKT in the soleus muscle and reduced IR in rats with T2DM. In general, low-degree inflammation stimulates appropriate secretion of cytokines and subsequently stimulates insulin-like growth factor (IGF)-1 and fibroblast growth-factor secretion for satellite cell proliferation[86]. The increase in myogenic regulatory gene expression promotes the differentiation of satellite cells into myoblasts in the formation of muscle fibers[86], whereas excessive TNF-α suppresses MyoD protein and inhibits muscle regeneration[86]. On the other hand, stimulation of the PI3K/AKT pathway increases the expression of the MyoD protein and the development of satellite cells, which make up myoblasts[86]. Similarly, activation of the PI3K/AKT pathway encourages mTOR protein production and muscle growth[86].

**Summary of Findings**

1. In rats without diabetes, high protein diets (HPD) considerably reduced blood sugar levels; in contrast, these rats' blood sugar levels increased dramatically in response to HFD and HCD. HFD and HCD considerably enhance blood sugar levels in streptozotocin-induced diabetic rats, but an HPD dramatically lowers blood sugar levels in the same rats.
2. In diabetic rats that received the various modified diets and treated with ALA and MFM, blood glucose levels were significantly decreased
3. High Fat Diets (HFD) as well as High Carbohydrates Diets (HCD) significantly increases α-tumor necrosis factor as well as C-reactive protein levels in both non-diabetic and streptozotocin-induced diabetic wistar rats, while High protein diet (HPD) significantly decreases α-tumor necrosis factor as well as C-reactive protein levels in both non-diabetic and streptozotocin-induced diabetic wistar rats.
4. Treatment with ALA as well as MFM to the rats given the different modified diets significantly decreases α-tumor necrosis factor as well as C-reactive protein levels in diabetic rats.

**Contribution to Knowledge**

1. This study showed that a high-protein diet alone and in conjunction with alpha lipoic acid, lowers blood glucose levels. As a result, it may be a helpful strategy for managing blood sugar levels and, consequently, diabetes.
2. This study also demonstrated that a high-protein diet (HPD) and alpha lipoic acid supplementation reduce levels of C-reactive protein and alpha-tumor necrosis factor, respectively. This helps control the inflammation brought on by diabetes.

**Conclusion**

The current study's findings indicate that high protein diets alone and in combination with ALA decreases blood glucose level and inflammatory damage caused by streptozotocin toxicity, hence, has the potential to manage diabetes and autoimmune-induced inflammation in diabetes mellitus.

**Recommendation**

A balanced eating pattern that includes more proteins, fewer carbohydrates and fats, as well as food that falls within a reasonable calorie range, should be implemented as it provides the basis for healthy eating.

## REFERENCES

1. Modak, M., Dixit, P., Londhe, J., Ghaskadbi, S., Devasagayam, TPA. (2007). Indian herbs and herbal drugs used for the treatment of diabetes. *J Clin Biochem Nutr.* **40**(3):163.
2. Imad, M., Elhadi, M., Abd, E. (2010). Hypoglycemic Effect of *Allium cepa* in Diabetes. *Journals of U.S National Library of Medicine.* **4**:71–77.
3. Osadebe, PO., Odoh, EU., Uzor, PF. (2014). The search for new hypoglycemic agents from plant. *Afr J Pharm Pharmacol.* **8**(11):292–303.
4. Ojieh, AE; Ugorji AE; Ovuakporaye IS; Ewhre OL; Ossai NR. (2016). "Comparative Evaluation of Hypoglycemic Properties of Raw and Boiled *Allium cepa* in Alloxan- Induced Diabetes Mellitus Rats. *UK Journal of Pharmaceutical and Biosciences,* 4(3), 38-44
5. Mukesh, R. and Namita, P. (2013). Medicinal Plants with Antidiabetic Potential-A Review. *American-Eurasian J Agric Environ Sci.* **13**(1):81–94.
6. Emami, M.R., Khorshidi, M. Zarezadeh, M., Safabakhsh, M., Rezagholizadeh, F. and Alizadeh, S. (2019). "Acute effects of caffeine ingestion on glycemic indices: a systematic review and meta-analysis of clinical trials," *Complementary Therapies in Medicine*, **44**:282–290.
7. Mortazavi-Jahromi, S.S., Alizadeh, S., Javanbakht, M.H., and Mirshafiey, A. (2019). "Anti-diabetic effect of *β*-D-mannuronic acid (M2000) as a novel NSAID with immunosuppressive property on insulin production, blood glucose, and inflammatory markers in the experimental diabetes model," *Archives of Physiology and Biochemistry*; **125**(5): 435–440.
8. Saeedi, P., Petersohn, I., Salpea, P., Malanda, B., Karuranga, S., Unwin, N., Colagiuri, S., Guariguata, L., Motala, AA., Ogurtsova, K., Shaw, JE. (2019). Global and regional diabetes prevalence estimates for 2019 and projections for 2030 and 2045: Results from the International Diabetes Federation Diabetes Atlas. *Diabetes research and clinical practice.* **1**;157:107843.
9. Motamedi, A., Askari, M., Mozaffari, H., Homayounfrar, R., Nikparast, A., Ghazi, M.L., Nejad,M.M., Alizadeh, S. (2022). Dietary Inflammatory Index in relation to Type 2 Diabetes: A Meta-Analysis. *International Journal of Clinical Practice,* **2022**:1-14.
10. Mortazavi-Jahromi, S.S., Alizadeh, S., Javanbakht, M.H., and Mirshafiey, A. (2018). "Cardioprotective effect of *β*-d-mannuronic acid (M2000) as a novel NSAID on gene expression of oxLDL scavenger receptors in the experimental diabetic model," *Immunopharmacology and Immunotoxicology*, **40**(4): 284–289.
11. Kim, ST. and Moley, KH. (2007). The expression of GLUT8, GLUT9a, and GLUT9b in the mouse testis and sperm *Reprod Sci*. **14**(5):445–455
12. Nna, VU., Bakar, AB., Ahmad, A., Mohamed, M. (2019). Down-regulation of steroidogenesis-related genes and its accompanying fertility decline in streptozotocin-induced diabetic male rats: ameliorative effect of metformin. *Andrology.* **7**(1):110-23.
13. Wang, L., Tian, W., Uwais, Z., Li, G., Li, H., Guan, R., Gao, Z. and Xin, Z. (2014). AGE-breaker ALT-711 plus insulin could restore erectile function in streptozocin-induced type 1 diabetic rats. *Journal of Sexual Medicine* **11**: 1452–1462.
14. Hu, F. B. Meigs, J.B., Li, T.Y., Rifai, N. and Manson, J.E. (2004). "Inflammatory markers and risk of developing type 2 diabetes in women," *Diabetes*, **53**(3): 693–700.
15. Shoelson, S.E., Lee, J. and Goldfine, A.B. (2006). "Inflammation and insulin resistance," *Journal of Clinical Investigation*, **116**(7): 1793–1801.
16. Duncan, BB., Schmidt, MI., Pankow, JS., Ballantyne, CM., Couper, D., Vigo, A., Hoogeveen, R., Folsom, AR., Heiss, G.. (2003). Low-grade systemic inflammation and the development of type 2 diabetes: the atherosclerosis risk in communities study. *Diabetes.* **52**(7):1799-805.
17. Kang, D-H., Rice, M., Park, N.-J., (2010). A. Turner-Henson, and C. Downs, "Stress and inflammation: a biobehavioral approach for nursing research," *Western Journal of Nursing*

*Research*, **32**(6):730–760.
18. Tsalamandris, S., Antonopoulos, AS., Oikonomou, E., Papamikroulis, GA., Vogiatzi, G., Papaioannou, S., Deftereos, S., Tousoulis, D. (2019). The Role of Inflammation in Diabetes: Current Concepts and Future Perspectives. *Eur Cardiol.* **14**(1):50-59.
19. Jallut, D., Golay, A., Munger, R., Frascarolo, P., Schutz, Y., Jequier, E., Felber, JP. (1990). Impaired glucose tolerance and diabetes in obesity: a 6-year follow-up study of glucose metabolism. *Metabolism.* **39**(10):1068-1075.
20. Tabák, AG., Jokela, M., Akbaraly, TN., Brunner, EJ., Kivimäki, M., Witte, DR. (2009). Trajectories of glycaemia, insulin sensitivity, and insulin secretion before diagnosis of type 2 diabetes: an analysis from the Whitehall II study. *The Lancet.* **373**(9682):2215-2221.
21. Kahn SE., Prigeon, RL., McCulloch, DK., Boyko, EJ., Bergman, RN., Schwartz, MW., Neifing, J.L., Ward, WK., Beard, JC., Palmer, JP. (1993). Quantification of the relationship between insulin sensitivity and β-cell function in human subjects: evidence for a hyperbolic function. *Diabetes*. **42**(11):1663-1672.
22. Dinarello, CA. (2010). Anti-inflammatory agents: present and future. *Cell.* **140**(6):935-950.
23. Odegaard, JI. and Chawla, A. (2013). Pleiotropic actions of insulin resistance and inflammation in metabolic homeostasis. *Science.* **339**(6116):172-7.
24. Donath, MY. (2014). Targeting inflammation in the treatment of type 2 diabetes: time to start. *Nature reviews Drug discovery.* 13(6):465-476.
25. Guirguis-Blake, JM., Evans, CV., Senger, CA., O'Connor, EA., Whitlock, EP. (2016). Aspirin for the primary prevention of cardiovascular events: a systematic evidence review for the US Preventive Services Task Force. *Annals of internal medicine.* **164**(12):804-13.
*26.* De, Rotte MC., De, Jong PH., Den, Boer E., Pluijm, SM., Özcan, B., Weel, AE., Lindemans, J., Hazes, JM., De, Jonge R. (2014). Effect of methotrexate use and erythrocyte methotrexate polyglutamate on glycosylated hemoglobin in rheumatoid arthritis. *Arthritis & Rheumatology.* **66**(8):2026-2036.
27. Ridker, PM., Everett, BM., Pradhan, A., MacFadyen, JG., Solomon, DH., Zaharris, E., Mam, V., Hasan, A., Rosenberg, Y., Iturriaga, E., Gupta, M. (2019). Low-dose methotrexate for the prevention of atherosclerotic events. *New England Journal of Medicine.* **380**(8):752-762.
28. Stanley, TL., Zanni, MV., Johnsen, S., Rasheed, S., Makimura, H., Lee, H., Khor, VK., Ahima, RS., Grinspoon, SK. (2011). TNF-α antagonism with etanercept decreases glucose and increases the proportion of high molecular weight adiponectin in obese subjects with features of the metabolic syndrome. *The Journal of Clinical Endocrinology & Metabolism.* **96**(1):E146-E50.
29. Böni-Schnetzler, M., Thorne, J., Parnaud, G., Marselli, L., Ehses, JA., Kerr-Conte, J., Pattou, F., Halban, PA., Weir, GC., Donath, MY. (2008). Increased interleukin (IL)-1β messenger ribonucleic acid expression in β-cells of individuals with type 2 diabetes and regulation of IL-1β in human islets by glucose and autostimulation. *The Journal of Clinical Endocrinology & Metabolism.* **93**(10):4065-4074.
30. Hensen, J., Howard, CP., Walter, V., Thuren, T. (2013). Impact of interleukin-1beta antibody (canakinumab) on glycaemic indicators in patients with type 2 diabetes mellitus: results of secondary endpoints from a randomized, placebo-controlled trial. *Diabetes Metab*. **39**:524–531.
31. Mohseni, R., Abbasi, S., Mohseni, F. Rahimi, F. and Alizadeh, S. (2019). "Association between dietary inflammatory index and the risk of prostate cancer: a meta-analysis," *Nutrition and Cancer*, **71**(3): 359–366.
32. Minihane, AM., Vinoy, S., Russell, WR., Baka, A., Roche, HM., Tuohy, KM., Teeling, J.L., Blaak, EE., Fenech, M., Vauzour, D., McArdle, HJ. (2015). Low-grade inflammation, diet composition and health: current research evidence and its translation. *British Journal of Nutrition.* **114**(7):999-1012.
33. Chun, O.K., Chung, S-J. Claycombe, K.J. and Song, W.O. (2008). "Serum C-reactive protein concentrations are inversely associated with dietary flavonoid intake in U.S. Adults," *Journal of Nutrition*, **138**(4):753–760.
34. Calder, P.C. (2013). "Long chain fatty acids and gene expression in inflammation and immunity," *Current Opinion in Clinical Nutrition and Metabolic Care*, **16**(4):425–433
35. Maddahi, N., Yarizadeh, H., Aghamir, S.M.K., Alizadeh, S., Yekaninejad, M.S. and Mirzaei, K. (2020). "The association of dietary inflammatory

index with urinary risk factors of kidney stones formation in men with nephrolithiasis," *BMC Research Notes*, **13**(1):373–377.
36. Hruby, A. and Jacques, P.F. (2019). Dietary Protein and Changes in Biomarkers of Inflammation and Oxidative Stress in the Framingham Heart Study Offspring Cohort, *Current Developments in Nutrition*, **3** (5): 1-19.
37. World Health Organization WHO: (2017). World Health Organization healthy diet fact sheet number 394. WHO: Healthy diet fact sheet. http://www.who.int/mediacentre/factsheets/fs394/en/ September 14, 2015.
38. Puska, P. (2009). Fat and heart disease: yes we can make a change—the case of North Karelia (Finland) *Ann Nutr Metab.* **54**:33–38.
39. Karimi, E., Yarizadeh, H., Setayesh, L., Sajjadi, SF., Ghodoosi, N., Khorraminezhad, L., Mirzaei, K. (2021). High carbohydrate intakes may predict more inflammatory status than high fat intakes in pre-menopause women with overweight or obesity: a cross-sectional study. *BMC Res Notes.* **14**(1):279.
40. Ference, BA., Ginsberg, HN., Graham, I., Ray, KK., Packard, CJ., Bruckert, E., Hegele, RA., Krauss, RM., Raal, FJ., Schunkert, H. (2017). Low-density lipoproteins cause atherosclerotic cardiovascular disease. 1. Evidence from genetic, epidemiologic, and clinical studies. A consensus statement from the European Atherosclerosis Society Consensus Panel. *Eur Heart J.* **38**:2459–2472.
41. Sorriento, D. and Iaccarino, G. (2019). Inflammation and cardiovascular diseases: the most recent findings. *IJMS* (Multidisciplinary Digital Publishing Institute). **20**:3879.
42. Aguiar, LM., Moura, CS., Ballard, CR., Roquetto, AR., Silva, Maia JKD., Duarte, GHB., Costa, LBED., Torsoni, AS., Amaya-Farfan, J., Maróstica, Junior MR., Cazarin, CBB. (2022). Metabolic dysfunctions promoted by AIN-93G standard diet compared with three obesity-inducing diets in C57BL/6J mice. *Curr Res Physiol.* 5:436-444.
43. Aletor, O. (2013). Nutrient composition of Soya Bean Meal and Soya Protein isolate and their effects on the Growth Performance and Haematological Parameter of Albino Rats *FUTA J. Res. Sci.* **9**(2):259-268.
44. Ossai, NR; Ojieh, EA; Nwogueze, CB, Olowe, GT and Ajayi, ER (2021). Ameliorative Potentials of Methanolic Leaf Extract of *Nephrolepis undulate in* Streptozotocin-Induced Diabetic Wistar Rats. *Plant Cell Biotechnology and Molecular Biology* 22 (15 and 16):41-53.
45. Giribabu, N., Roslan, J., Rekha, SS., Salleh, N. (2016). Methanolic seed extract of Vitis vinifera ameliorates oxidative stress, inflammation and ATPase dysfunction in infarcted and non-infarcted heart of streptozotocin–nicotinamide induced male diabetic rats. *Int J Cardiol*. **222**:850–865.
46. Brahmanaidu, P., Uddandrao, VS., Sasikumar, V., Naik, RR., Pothani, S., Begum, MS. (2017). Reversal of endothelial dysfunction in aorta of streptozotocin-nicotinamide-induced type-2 diabetic rats by S-Allylcysteine. *Mol Cell Biochem.* **432**(1–2):25–32.
47. Paula, L., Cruz, IC., Moraes-Silva, AA., Ribeiro, JF., Dantas, MM., de Melo, T., dos Santos, F., da Silva, MB., Strunz, CMC., Caldini, EG. and Irigoyen, M-C. (2021). Nicotinamide attenuates *streptozotocin-induced* diabetes complications and increases survival rate in rats: role of autonomic nervous system *BMC Endocrine Disorders* **21**(133):1-10
48. Ojieh A.E. (2020). Evaluation of Antidiabetic and Antioxidant potential of *Nephrolepis unduranta* leaf extract in Streptozotocin Induced Diabetic Wistar Rats; *Int. J of Forensic Med Invest.*, **5** (1): 34-41.
49. Eke, CN., Nwogueze, BC., Ossai, NR., Nwobodo Ed. (2019), Pulmonary Function in Females with Type 2 Diabetes in AWKA, Anambra State. *International Journal of Clinical Dermatology*, 2(1): 1-6.
50. Wilson, JI., Ojieh, AE., Nwogueze, BC., Ossai, NR., and Okolocha, C. (2022). Histomorphorlogical and biochemical evaluation of oral administration of *datura metel stramonium* on the kidneys of adult male wistar rats. *International Journal of Health Sciences*, ***6***(S7), 2948–2962.
*51*. Wilson, JI., Ojieh, A., Ossai, NR., Nwogueze, BC. and Olise-Enewe, M. (2021). Histomorphological, Hypoglycaemic and Hepatoprotective effects of acute administration of Methanolic extract of Persea Americana seed in alloxan-induced Diabetic Wistar Rats. Plant Cell Biotechnology and Molecular Biology **22**(41&42):247-260.
*52*. Kahn, SE. (2020). The importance of the beta

cell in the pathogenesis of type 2 diabetes mellitus. *American Journal of Medicine **108**:2S –8S.*

53. Russell, WR., Baka, A., Björck, I., Delzenne, N., Gao, D., Griffiths, HR., Hadjilucas, E., Juvonen, K., Lahtinen, S., Lansink, M., Loon, LV., Mykkänen, H., Östman, E., Riccardi, G., Vinoy, S., Weickert, MO. (2016). Impact of Diet Composition on Blood Glucose Regulation. *Crit Rev Food Sci Nutr.* **56**(4):541-590.
54. Hession, M., Rolland, C., Kulkarni, U., Wise, A. and Broom, J. (2009). Systematic review of randomized controlled trials of low-carbohydrate vs. low-fat/low-calorie diets in the management of obesity and its comorbidities. *Obes. Rev*. **10**:36–50.
55. Weickert, M. O., Möhlig, M., Koebnick, C., Holst, J. J., Namsolleck, P., Ristow, M., Osterhoff, M., Rochlitz, H., Rudovich, N., Spranger, J. and Pfeiffer, A. F. (2005). Impact of cereal fibre on glucose-regulating factors. *Diabetologia*. **48**:2343–2353.
56. Rochette, L., Ghibu, S., Richard, C., Zeller, M., Cottin, Y., Vergely, C. (2013). Direct and indirect antioxidant properties of alpha-lipoic acid and therapeutic potential. *Mol. Nutr. Food Res.* **57**:114–125.
57. Konrad, D., Somwar, R., Sweeney, G., Yaworsky, K., Hayashi, M., Ramlal, T., Klip, A. (2001). The antihyperglycemic drug alpha-lipoic acid stimulates glucose uptake via both GLUT4 translocation and GLUT4 activation: Potential role of p38 mitogen-activated protein kinase in GLUT4 activation. *Diabetes.* **50**:1464–1471.
58. Targonsky, E.D., Dai, F., Koshkin, V., Karaman, G.T., Gyulkhandanyan, A.V., Zhang, Y., Chan, C.B., Wheeler, M.B. (2006). α-lipoic acid regulates AMP-activated protein kinase and inhibits insulin secretion from beta cells. *Diabetologia*. **49**:1587–1598.
59. Ghelani, H., Razmovski-Naumovski, V., Nammi, S. (2017). Chronic treatment of (R)-α-lipoic acid reduces blood glucose and lipid levels in high-fat diet and low-dose streptozotocin-induced metabolic syndrome and type 2 diabetes in Sprague-Dawley rats. Pharmacol. *Res. Perspect*. **2017;**5:e00306.
60. Jones, W., Li, X., Qu, Z.C., Perriott, L., Whitesell, R.R., May, J.M. (2002). Uptake, recycling, and antioxidant actions of α-lipoic acid in endothelial cells. Free Radic. *Biol. Med.* **33**:83–93.
61. Ziegler, D., Hanefeld, M., Ruhnau, K.J., Hasche, H., Lobisch, M., Schütte, K., Kerum, G., Malessa, R. (1999). Treatment of symptomatic diabetic polyneuropathy with the antioxidant alpha-lipoic acid: A 7-month multicenter randomized controlled trial (ALADIN III Study). ALADIN III Study Group. Alpha-Lipoic Acid in Diabetic Neuropathy. *Diabetes Care.* **22**:1296–1301.
62. Moffa, S., Mezza, T., Cefalo, C.M.A., Cinti, F., Impronta, F., Sorice, G.P., Santoro, A., Di Giuseppe, G., Pontecorvi, A., Giaccari, A. (2019). The Interplay between Immune System and Microbiota in Diabetes. *Mediat. Inflamm.* **2019**:9367404.
63. Genazzani, A.D., Shefer, K., Della Casa, D., Prati, A., Napolitano, A., Manzo, A., Despini, G., Simoncini, T. (2018). Modulatory effects of alpha-lipoic acid (ALA) administration on insulin sensitivity in obese PCOS patients. *J. Endocrinol. Investig.* **41**:583–590.
64. Muscogiuri, G., Policola, C., Prioletta, A., Sorice, G., Mezza, T., Lassandro, A., Della Casa, S., Pontecorvi, A., Giaccari, A. (2012). Low levels of 25(OH)D and insulin-resistance: 2 unrelated features or a cause-effect in PCOS? *Clin. Nutr.* **31**:476–480.
65. Eason, R.C., Archer, H.E., Akhtar. S., Bailey, C.J. (2002). Lipoic acid increases glucose uptake by skeletal muscles of obese-diabetic ob/ob mice. *Diabetes Obes. Metab.* **4**:29–35.
66. Yaworsky, K., Somwar, R., Ramlal, T., Tritschler, H.J., Klip, A. (2000). Engagement of the insulin-sensitive pathway in the stimulation of glucose transport by alpha-lipoic acid in 3T3-L1 adipocytes. *Diabetologia.* **43**:294–303.
67. Konrad, D., Somwar, R., Sweeney, G., Yaworsky, K., Hayashi, M., Ramlal, T., Klip, A. (2001). The antihyperglycemic drug alpha-lipoic acid stimulates glucose uptake via both GLUT4 translocation and GLUT4 activation: Potential role of p38 mitogen-activated protein kinase in GLUT4 activation. *Diabetes.* **50**:1464–1471.
68. Pinto, E.M., Neves, J.R., Laranjeira, M. and Reis, J. (2023). The importance of inflammatory biomarkers in non-specific acute and chronic low back pain: a systematic review. *Eur Spine J*. **35**(2): 346-359
69. Elgazzar, A.H., Elmonayeri, M. (2015). Inflammation. In: Elgazzar, A. (eds) The

Pathophysiologic Basis of Nuclear Medicine. Springer, Cham.
70. Zhao, H., Wu, L., Yan, G. Chen, Y., Zhou, M., Wu, Y., Li, Y. (2021). Inflammation and tumor progression: signaling pathways and targeted intervention. *Sig Transduct Target Ther* **6**, 263.
71. Ansar, W. and Ghosh, S. (2016). Inflammation and Inflammatory Diseases, Markers, and Mediators: Role of CRP in Some Inflammatory Diseases. *Biology of C Reactive Protein in Health and Disease.* **24**:67–107.
*72.* Sethi, JK. and Hotamisligil, GS. (2021). "Metabolic Messengers: tumour necrosis factor". *Nature Metabolism*. **3** (10): 1302–1312.
73. Rolski, F. and Błyszczuk, P. (2020). "Complexity of TNF-α Signaling in Heart Disease". *Journal of Clinical Medicine. **9** (10): 3267.*
*74.* Koelman, L., Rodrigues, C.E., Aleksandrova, K. (2022). Effects of Dietary Patterns on Biomarkers of Inflammation and Immune Responses: A Systematic Review and Meta-Analysis of Randomized Controlled Trials. *Adv. Nutr. Int. Rev. J.* **13**:101–115.
*75.* Qu, Y., Zhao, G., Li, H. (2017). "Forward and Reverse Signaling Mediated by Transmembrane Tumor Necrosis Factor-Alpha and TNF Receptor 2: Potential Roles in an Immunosuppressive Tumor Microenvironment". *Frontiers in Immunology*. ***8**: 1675.*
76. Saeed-Majeed, HM., Abdul-Hassan, AA., Shakir, KM. (2022).The role of TNFα in type2 diabetes mellitus. *Revis Bionatura*; **7**(2) 32.
*77.* Probert, L. (2015). "TNF and its receptors in the CNS: The essential, the desirable and the deleterious effects". *Neuroscience.* **302**: 2–22.
*78.* Heir, R. and Stellwagen, D. (2020). "TNF-Mediated Homeostatic Synaptic Plasticity: From in vitro to in vivo Models". *Frontiers in Cellular Neuroscience.* **14**: 565841.
79. Rawhia, HE., Waleed, MF., Dalia, HA., Mahmoud, ME., Noran, T., Abo E. (2020). Role of tumor necrosis factor alpha in type 2 diabetic nephropathy. *Menoufia Medical Journal.* **33**(3): 920-925.
*80.* Bobińska, K., Gałecka, E., Szemraj, J., Gałecki, P., Talarowska, M. (2017). "Is there a link between TNF gene expression and cognitive deficits in depression?". *Acta Biochim. Pol.* **64** (1): 65–73.
81. Gupta, S., Mehndiratta, M., Kalra, S., Kalra, O., Shukla, R., Gambhir, J. (2015). Association of tumor necrosis factor (TNF) promoter polymorphisms with plasma TNF-α levels and susceptibility to diabetic nephropathy in North Indian population. *J Diabetes Complications*; **29**:338–342
82. Saulnier-Blache, J., Feigerlova, E., Halimi, J., Gourdy, P., Roussel, R., Guerci, B. (2017). Urinary lysophopholipids are increased in diabetic patients with nephropathy. *J Diabetes Complications*; **31**:1103–1108.
83. Aryani, H., Santoso, B., Purwanto, B. (2020). Low Carbohydrate High Protein Diet Reduces Levels of Tumour Necrosis Factor-? (TNF-?) in Rats (Rattus norvegicus) . *Indian Journal of Forensic Medicine and Toxicology*, **14**(4), 2923–2928.
84. Silveira, BK., Oliveira, TM., Andrade, PA., Hermsdorff, HH., Rosa, CD., Franceschini, SD. (2018). Dietary pattern and macronutrients profile on the variation of inflammatory biomarkers: scientific update. *Cardiology research and practice.* **14**:2018.
*85.* Kopf, JC., Suhr, MJ., Clarke, J., Eyun, Si., Riethoven, J.-JM., Ramer-Tait, AE., and Rose, DJ. (2018). Role of whole grains versus fruits and vegetables in reducing subclinical inflammation and promoting gastrointestinal health in individuals affected by overweight and obesity: A randomized controlled trial. *Nutrition Journal*. **17**(1):72.
*86.* Ko, C-Y., Wu, C-H., Huang, W-J., Lo, MY., Lin, S-L., Wu, JS-B., Huang, W-C., Shen, S-C. (2023). Alleviative effects of α- lipoic acid on muscle atrophy via the modulation of TNF-α/JNK and PI3K/AKT pathways in high-fat diet and streptozotocin-induced type 2 diabetic rats. *Food Science and Nutrition* **2023**:00:1-9
*87.* Maciejczyk, M., Żebrowska, E. and Chabowski, A. (2019). "Insulin resistance and oxidative stress in the brain: what's new?," *International Journal of Molecular Sciences*, **20**(4): 874.
88. Clark, I. A. and Vissel, B. (2018).Therapeutic implications of how TNF links apolipoprotein E, phosphorylated tau, *α*-synuclein, amyloid-*β* and insulin resistance in neurodegenerative diseases. *British Journal of Pharmacology*. **175**(20):3859–3875.
89. Zwickey, H., Horgan, A., Hanes, D., Schiffke, H., Moore, A., Wahbeh, H., Jordan, J., Ojeda, L., McMurry, M., Elmer, P. (2019). Effect of the

Anti-Inflammatory Diet in People with Diabetes and Pre-Diabetes: A Randomized Controlled Feeding Study. *J. Restor. Med.* **8**, e20190107.

*90.* Shivappa, N., Godos, J., Hébert, J., Wirth, M., Piuri, G., Speciani, A., Grosso, G. (2018). Dietary Inflammatory Index and Cardiovascular Risk and Mortality—A Meta-Analysis. *Nutrients* **10**: 200.

91. Denova-Gutiérrez, E., Muñoz-Aguirre, P., Shivappa, N., Hébert, J.R., Tolentino-Mayo, L., Batis, C., Barquera, S. (2018). Dietary Inflammatory Index and Type 2 Diabetes Mellitus in Adults: The Diabetes Mellitus Survey of Mexico City. *Nutrients*, **10**, 385.

92. Sureda, A., Del Mar Bibiloni, M., Julibert, A., Bouzas, C., Argelich, E., Llompart, I., Pons, A., Tur, J.A. (2018). Adherence to the Mediterranean Diet and Inflammatory Markers. *Nutrients,* **10**, 62.

93. Sánchez-Rosales, A.I., Guadarrama-López, A.L., Gaona-Valle, L.S., Martínez-Carrillo, B.E., Valdés-Ramos, R. (2022). The Effect of Dietary Patterns on Inflammatory Biomarkers in Adults with Type 2 Diabetes Mellitus: A Systematic Review and Meta-Analysis of Randomized Controlled Trials. *Nutrients,* **14**:1-25

94. Ko, C. Y., Xu, J. H., Lo, Y. M., Tu, R. S., Wu, J. S., Huang, W. C., and Shen, S. C. (2021). Alleviative effect of alpha-lipoic acid on cognitive impairment in high-fat diet and streptozotocin-induced type 2 diabetic rats. *Frontiers in Aging Neuroscience*, **13**, 774477.

95. Hong, O. K., Son, J. W., Kwon, H. S., Lee, S. S., Kim, S. R., and Yoo, S. J. (2018). Alpha-lipoic acid preserves skeletal muscle mass in type 2 diabetic OLETF rats. *Nutrition & Metabolism*, **15**, 66.

96. Negro, M., Perna, S., Spadaccini, D., Castelli, L., Calanni, L., Barbero, M., Cescon, C., Rondanelli, M., and D'Antona, G. (2019). Effects of 12 weeks of essential amino acids (EAA)-based multi-ingredient nutritional supplementation on muscle mass, muscle strength, muscle power and fatigue in healthy elderly subjects: A randomized controlled double-blind study. *The Journal of Nutrition, Health & Aging*, **23**(5), 414– 424.

97. Doody, N. E., Dowejko, M. M., Akam, E. C., Cox, N. J., Bhatti, J. S., Singh, P. and Mastana, S. S. (2017). The role of TLR4, TNF-α and IL-1β in type 2 diabetes mellitus development within a North Indian population. *Annals of Human Genetics*, **81**(4), 141– 146.