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CHARACTERIZING THE ADIPOKINES, INFLAMMATORY CYTOKINES RESPONSE IN ENDOTHELIAL DYSFUNCTION IN HIGH-FAT DIET AND SINGLE DOSE OF STZ IN WISTAR ALBINO RAT MODELS

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Keywords:

Streptozotocin, Endothelial Dysfunction, Adipokines, Cytokines, Obesity, Inflammatory

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ABSTRACT: Introduction: Endothelial dysfunction and diet-induced diabetes may be significantly impacted by changes in Adipokine and inflammatory cytokine secretion linked to obesity. Our study's objective was to determine the effect of a high-fat diet also on concentrations of leptin, adiponectin, as well as other inflammatory indicators in type 2 diabetes mellitus brought on by streptozotocin (STZ)-induced endothelial dysfunction (T2DM). **Material and Method:** This study examined how endothelial dysfunction rats responded to a high-fat diet and Streptozotocin by producing more adipokines and inflammatory cytokines. The 32 rats of four groups of adult male Wistar rats utilized in this investigation were as follows: group 1 received the diet that was 60% fat (HFD) (n = 8); group 2 received a high-fat diet plus, STZ (STZ-HFD) (n = 8); as well as group 3 received a normal diet plus, STZ (STZ-ND) (n = 8). The fourth groups ate a normal diet for six weeks. Using a single intraperitoneal STZ injection, (40mg/kg), type 2 diabetes was induced. a 12-hour fast was imposed on all of the ratsprior to the drawing of serum sample to determine the serum concentrations of tumour necrosis factor (TNF), leptin, adiponectin, CRP, ICAM, and VCAM endothelial markers using an ELISA. Using the Student's t-test, four categories were compared. Using just one variable. Multiple comparisons against the control group were made using a one-way ANOVA followed by a Dunnett post hoc test. **Results:** Mean serum glucose leptin, TNF –α, CRP, ICAM, VCAM concentrations were substantially higher in HFD + STZ compared to Normal Diet + STZND) and HFD ($p<0.001$). The average serum concentration of adiponectin was significantly lower in HFD + STZ compared to Normal Diet + STZ ND and HFD ($p<0.001$). Serum GAMMA INTERFERON level was significantly higher in Normal Diet + STZ compared to HFD + STZ, ND and HFD ($p<0.001$). **Conclusion:** Inflammation is a major factor in endothelial dysfunction correlated with type 2 diabetes and obesity, and is protected by anti-inflammatory adipokines like adiponectin.

INTRODUCTION: Hyperglycemia (DM) is a severe global problem affecting low-, middle-, and high-income societies. Type 1 and type 2 metabolic diseases, including abnormalities in insulin activity, are collectively referred to as diabetes. Episodes of hyperglycemia brought on by a relative or absolute absence of sufficient insulin is a sign of insulin resistance and insensitivity, which are also characterized by pancreatic dysfunction and elevated blood sugar levels [1].

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Focusing on diabetes is important for a number of reasons, including the fact that it is a growing epidemic due to the increase in global deaths of about 70% since 2000, making diabetes the 9th leading cause of death worldwide. Premature deaths are on the rise (up 5% since 2010). 420 million people have diabetes today; 570

million will have it by 2030; and 700 million will have it by 2030 (2045) [2]. According to Mutie, *et al.* (2017), using biomarkers as precision medicine for diabetes detection and prevention is a focused therapeutic approach. Although type 2 diabetes mellitus (T2DM) treatment is currently limited pharmacotherapy, using biomarkers to tailor lifestyle recommendations is becoming a viable option [3].

Due to the several (cardiovascular) comorbidities that are extremely common, Precision and adaptive medical strategies are effective in treating type 2 diabetes mellitus patients. Should be crucial and urgent consideration Pleiotropic polypeptides called cytokines act on cells to control inflammatory and immune reactions. They contribute immensely to the pathophysiology of numerous diseases, including diabetes. Numerous pathogenic reactions, including chronic inflammation and innate immune system stimulation, are brought on by diabetes and microvascular effects.IL-6 and TNF are a few examples [4].

Obesity and the development of numerous disorders, such as diabetes mellitus (DM), are caused by a high-fat diet [5]. The potential processes by which this disease may manifest are currently unclear. It is well known that the production to fatty acids, hormones, other chemicals derived from fat tissue rises because of overweight, which alters its energy metabolism and hormonal function. Pro-inflammatory molecules, all of which lead to the challenges brought on by obesity. Diet-induced diabetes may be significantly influenced by changes in adipokine production linked to obesity. Leptin and adiponectin, two of the main adipocytokines, are believed to be crucial in controlling metabolic and cardiovascular homeostasis [6].

Adipocytes secrete the hormone leptin, a 16-kDa protein. To sustain body fat stores, plasma leptin concentration regulates caloric intake and energy usage and rises according to body fat mass. The blood is secreted with leptin, which then travels throughout the body, passing across the brain-blood barrier (BBB) and cerebrospinal fluid (CSF) barrier to reach the brain. Leptin suppresses neuropeptide Y (NPY) neurons in the hypothalamus, which results in anorexia [7]. While leptin's peripheral action controls the activity of, adipose tissues, muscle tissue, and pancreatic cells, its central action controls hemodynamics, bone density, and immunity [8]. Plasma leptin concentrations are much greater depending on the level of obesity. In obese individuals, Hyperleptinemia might be a contributing factor to the etiology of problems with obesity.

Hence, body fat may affect the connection between plasma leptin and diabetes mellitus. Higher leptin levels, obesity, and weight increase [9] likely influence the following development of diabetes. Regarding the impact of HF on leptin levels, earlier research has produced mixed findings [10]. Many investigations have discovered that leptin levels positively correlate with insulin resistance in non-diabetics (IR) [11]. In the limited studies that looked at the association between leptin concentration and IR in people with type 2 diabetes, inconsistent results were discovered [12].

Rajkovic *et al.* found no evidence of a significant people with type 2 diabetes and their leptin levels and IR [13]. According to Gulturk *et al.* leptin was related to IR, insulin, and body mass indices in people with type 2 diabetes (T2DM). Researchers have shown that leptin concentration differs between various ethnic groups when type 2 diabetic patients are contrasted with healthy volunteers [14].

Adiponectin controls lipid and glucose metabolism by focusing on the liver and skeletal muscle. Most of the time, adiponectin, an insulin-sensitive hormone essential for proper glucose and lipid metabolism, is the most abundant plasma protein synthesized in adipose tissue [15]. Adiponectin is a cytokine that only exists in adipose tissue.

It appears to protect against metabolic diseases and has anti-inflammatory and protective effects against insulin resistance. Obesity causes changes in adiponectin. It has been demonstrated that animals fed on HF have significantly lower adiponectin levels than the control group. A lower incidence of T2DM was linked to high adiponectin levels. Plasma, The degree of adiponectin, was found to be negatively connected with obesity, insulin sensitivity, diabetic complications, and metabolic syndrome in studies using regularly

collected intravenous glucose tolerance screening and clamps, but highly connected with markers of insulin sensitivity [16].

Long-term inflammatory response has been linked to obesity, which frequently coexists with metabolic issues like diabetes. Obesity and accompanying metabolic problems like diabetes are exceedingly frequent and have been linked to long-term inflammation [17].

One of the crucial adipokines adipocytes generate is leptin, and its level rises with an increase in body mass index (BMI). Due to persistent inflammation, adipocytes and inflammatory cells release the cytokine tumour necrosis factor-alpha (TNF-). There is a theory that type 2 diabetic mellitus have a connection to low-grade chronic inflammation (T2DM) [18].

Owing of diabetes's widespread prevalence as well as its potentially fatal adverse effects, new methods for identifying and treating diabetic patients had to be developed. Therefore, the focus of this study was on determining whether or not circulating biomarkers would be a reliable indicator of both the disease's development and the effectiveness of its therapy [19].

One of the main contributors to vascular complications, oxidative stress, and inflammation is endothelial dysfunction. Endothelial dysfunction is increased in diabetic and obese/insulin-resistant conditions that promote growth and dissemination of vascular conditions. Diabetes, obesity and insulin resistance are all defined as condition of underlying inflammatory process. High sensitivity C-reactive protein (hsCRP) and the inflammatory score, which is made up of the anti-inflammatory adiponectin and the pro-inflammatory plasma cytokines interleukin (IL)-6, tumour necrosis factor (TNF), can both be used to monitor inflammation [20].

We suggested that adiponectin may mediate the relationship between signs of inflammation, endothelial dysfunction, obesity, and the future likelihood of developing type 2 diabetes. As a result, we examined how adiponectin is linked to several biomarkers of endothelial dysfunction and inflammation within the same stacked study based that was previously addressed and analyzed how some markers connected to the subsequent occurrence of diabetes [21].

MATERIAL AND METHOD:

Experimental Animals: We procured 32 male wistar albino rats from the Biogen Laboratory Animal Facility Bangalore in India, weighing between 170-230 Gms. Eight rats per cage, clean polypropylene cages, were utilized to house and care for them. During the 12:12 hour day and dark cycle, the main animal housing is kept at a constant room temperature of 25° and has a relative humidity of 45–55%. Before the experiment began, they were kept for a week to get used to the lab setting and were provided with regular lab feed and water as needed. Prior to the trial, the strategy for said experiment was authorized by the I.A.E.C. of the Saveetha Institute of Medical and Technological Sciences (SIMATS), Chennai-602105. The research was done in conformity with CCSEA guidelines, registration number 1183/PO/Re/S/08/CPCSEA. We chose to use male mice because it has been demonstrated that these animals will exhibit diabetic symptoms such hyperglycemia, glucosuria, and elevated HbA1C levels.

Experimental Design: Following acclimation, each of the 32 animals was randomly divided into four groups of 8 and treated as follows:

Group I: HFD for 42 days.

Group II: HFD for 42 day and STZ 40mg/kg

Group III: Normal Diet for 42 days and STZ 40mg/kg.

Group IV: Normal Diet for 42 days.

Rats in groups I and II were given a 60% HFD (Protein 20%, Fat 60%, and Carbohydrate 20%; Research Diet) for 42 days, while rats in groups III and IV were given a conventional diet (Fat% kcal - 16. Carbohydrate % k cal)-64% Protein% kcal 2%). (Purina rations) [22]. Rats were given the proper meals for 42 days. The diet's composition is shown in the table below. The HFD was bought from VRK Nutritional Solutions in Sangli, Maharashtra. All of the remaining chemicals belonged to the analytical category. All biochemical assays were conducted using double-distilled water.

Development of Type 2 Diabetes Model Caused by HFD/STZ: The rats received a single intraperitoneal injection of streptozotocin (STZ) (Ref no JPS 22-23 1386 Code S0130 Sigma Aldrich, USA) at a low dose (40 mg/kg body weight, dissolved in 0.05 M citrate buffer, pH 4.5) after 42 days of consuming a high fat diet. The HFD (the selected HFD) was administered to the rats for 42 days in order to produce type 2 diabetes. The effects of STZ (40 mg/kg body weight) and HFD were both studied to find the optimum dose for producing T2DM.

One week following the injection of STZ, the plasma glucose levels in the control groups (ND and HFD) were determined, and rats with a glucose concentration we got greater than 250 mg/dl the vehicle (0.9% saline solution).

The rats' individual diets were permitted to be consumed until the study's conclusion. Because of their HFD, the diabetic group of rats developed insulin resistance; as a result, even a little insult from a modest dose of STZ would impair -cell activity and cause hypoinsulinemia.

All of the rats were starved for the duration of the experiment, and then they were all given an overdose of the anaesthetic ketamine hydrochloride (Ketalar, Pf) before being euthanized. Microcapillary tubes will be used to retro-orbitally plexus collect blood. To examine serum Fasting plasma glucose, cytokinesand endothelial markers in serum were separated by centrifugation (4000 rpm, 10 min), after the blood collection.

Assay of Cytokine and Endothelial Dysfunction Markers: The levels of leptinadiponectin, TNF-α, CRP, I -CAM and V-CAM were measured by ELISA and according to the method adopted by Whiteside using the kits following the guidelines from the manufacturer investigations And use a commercially available ELISA kit (using commercial Millipore® ELISA kit.) and following the manufacturer's instructions, the serum level ofmarkers - was evaluated. The findings of each sample's analysis were provided as picograms per milliliter (pg/mL).

Statistic Evaluation: In the current study, descriptive and inferential statistical analysis was completed. Results for categorical data are reported in Number (%), whereas results for continuous measurements are presented as Mean ± SD (Min-Max). The 5% level of significance is used to determine significance. To ascertain whether there are any statistically significant differences between the means of three or more independent (unrelated) groups, the one-way analysis of variance (ANOVA) is used. After an ANOVA, use a posthoc Tukey test.

Statistical Software: SPSS 22.0 and R environment ver for the data analysis. 3.2.2 were utilized, and Microsoft Word and Excel were used to produce graphs, tables, and other output.

RESULTS:

Effect of Experimental Diet and STZ (40mg /kg BW) on Cytokines and Endothelial Dysfunction Parameters: The findings displayed in Figure 1 in **Table 1** reveal the considerable variation between averages of the four groups, with the HFD + STZ group and Normal Diet + STZ group's mean values of (320.1±48.87) with a p-value of <0.001 being significantly higher than the HFD and ND group's (129.1±11.5).

These findings show that a high fat diet and streptozotocin result in significantly higher glucose levels, suggesting that a person's risk of developing diabetes may be increased by both an unhealthy diet and environmental factors.

Overall, the results suggest that there is a statistically considerable variation between the four values according to fasting plasma glucose a 48^{th} day later of the experiment; these findings demonstrate that fasting plasma glucose levels vary depending on the group and are highly statistically significant when comparing the four groups.

Mean serum leptin concentrations was substantially higher in HFD + STZ (2332.63±834.43 pg/mL) compared to Normal Diet + STZ **Table 1 Fig. 2** (663.48 ± 105.83 pg/mL), ND (6179.5 ± 130.78pg/mL) and HFD (612.48±149.24pg/mL) (p<0.001) is shown in **Table 1 Fig. 3.**

This show possibility that leptin contributes to the development of heart failure in diabetic rats caused by STZ. More research is required to learn more about the potential mechanisms underlying this connection. The average serum concentration of

adiponectin was significantly lower in HFD + STZ (1.21±0.41 ng/mL) compared to Normal Diet + STZ (3.32±0.42 ng/mL), ND (3.32±0.42 ng/mL) and HFD (3.1±0.43ng/mL) ($p<0.001$) is mention in **Table 1 Fig. 4**. In HFD + STZ group, a Leptin and glucose levels showed a strong positive association. The results suggest that STZ-induced diabetes leads to a decrease in serum adiponectin levels, which could result in the appearance of several diseases.

Diet-induced diabetes may be significantly influenced by changes in adipokine secretion linked to obesity. Leptin and adiponectin, two major adipocytokines, are thought about participating significant roles in the control of cardiovascular and metabolic homeostasis [23].

While adiponectin improves insulin sensitivity and has anti-inflammatory properties, leptin regulates appetite and energy expenditure. As a result, dysregulation of these adipokines may play a role in developing diseases linked to obesity, such as type 2 diabetes and cardiovascular disease.

Mean serum TNF -α level was significantly higher in HFD + STZ (8.3±0.8 ng/mL) contrast to Normal Diet + STZ (7.06±0.7ng/mL), ND (7.98±1.02 ng/mL) and HFD (ng/mL) (6.63 ± 0.62)($p<0.001$)the vales describe in **Table 1 Fig. 5**.

The results suggest that a high-fat diet, in addition to STZ administration, leads to an increase in serum TNF-α levels, which may bring an aspect to the development of insulin resistance and diabetes.

The median level of serum CRP was much higher in HFD + STZ (0.85±0.08 ng/mL) compared to Normal Diet + STZ (0.79±0.02ng/mL), ND (0.51±0.03 ng/mL) and HFD (0.64±0.03ng/mL) ($p<0.001$) According to **Table 1** and **Fig. 6**. This suggests that Consuming excess fat and taking STZ administration can lead to increased inflammation, as indicated by the higher CRP levels. More research is required to learn more about the various mechanisms causing this effect.

Mean serum Gamma Interferon level was significantly higher in Normal Diet + STZ (76.77±3.61 pg/mL) compared to HFD + STZ (71±2.58pg/mL), ND(72.2±3.47 pg/mL) and HFD (52.46±2.78g/mL) ($p<0.001$). The values were describe in **Table 2 Fig. 7.**

I-CAM levels in serum were noticeably higher on average in HFD + STZ (263.55±33.43pg/mL ompared to Normal Diet +STZ (221.91±13pg/mL), ND (182.88±21 pg/mL) and HFD (150.36±4.36 pg/mL) ($p<0.001$).

Table 1 Fig. 8 suggests that a high-fat diet and streptozotocin treatment may increase inflammation and endothelial dysfunction, as indicated by elevated levels of I-CAM. More research is required to examine the underlying mechanisms and potential treatment approaches.

Mean serum V-CAM levels were noticeably increased in HFD + STZ (774.36±51.26 pg/mL) compared to Normal Diet STZ (646.95 ± 61.17pg/mL), ND (530.81±58.63pg/mL) and HFD (441.15±25.99 pg/mL) ($p<0.001$). **Table 1 Fig. 9.**

This shows that a high-fat diet mixed with STZ therapy may cause a rise in inflammation and endothelial dysfunction, which may help to cause diabetes and its consequences. More research is required to understand the underlying mechanisms and potential therapeutic strategies.

TABLE 1: COMPARISON OF STUDY VARIABLES IN FOUR GROUPS

Biochemical parameter	High Fat Diet	High Fat Diet and STZ	Normal Diet and STZ	Normal Diet	P Value
Glucose mg/dl	165.03±5.27	376.83±47.38	320.16±48.87	129.11±15.99	<0.001**
Leptin pg/ml	612.48±149.24	2332.63±834.43	663.48±105.83	617.5±130.78	<0.001**
Adiponectin ng/ml	3.1±0.43	1.21±0.41	2.65±0.75	3.32±0.42	<0.001**
TNF –αng/ml	6.63±0.62	8.3±0.8	7.06±0.7	7.98±1.02	<0.001**
CRP ng/ml	0.64±0.03	0.85±0.08	0.79±0.02	0.51±0.03	<0.001**
Gamma Interferon pg/ml	52.46±2.78	71±2.58	76.77±3.61	72.2±3.47	<0.001**
I-CAMpg/ml	150.36±4.36	263.55±33.43	221.91±13.4	182.88±21.3	<0.001**
V-CAM pg/ml	441.15±25.99	774.36±51.26	646.95±61.17	530.81±58.63	<0.001**

The data is displayed as mean SEM (n = 8). Significant variations between columns are denoted by different letters ($p < 0.001$).

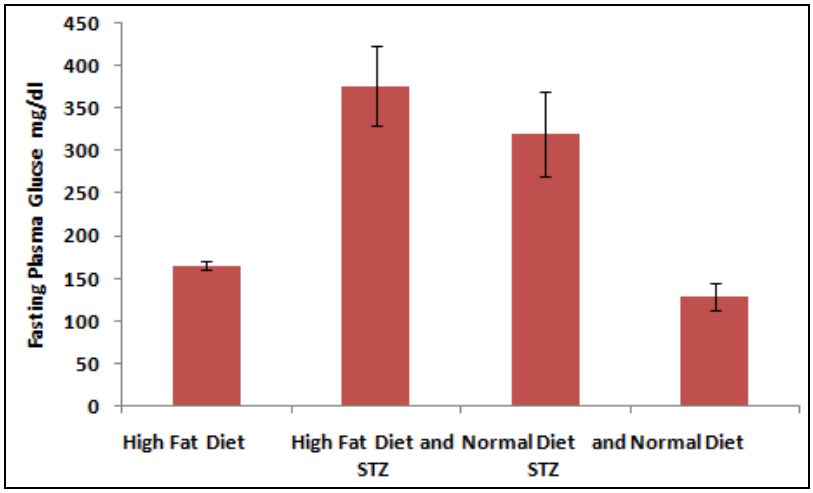

FIG. 1: IMPACT OF DIET AND STZ ON FASTING PLASMA GLUCOSE IN THE EXPERIMENTAL GROUPS. THE HIGHS AND LOWS WITH AND WITHOUT STZ IN WISTAR RATS AND A P-VALUE LOWER THAN 0.001 WAS CONSIDERED SIGNIFICANT

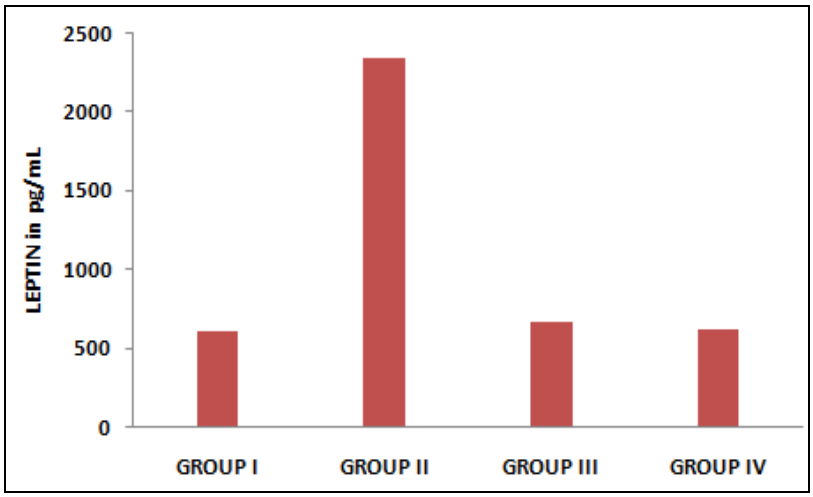

FIG. 2: IMPACT OF DIET AND STZ ON LEPTIN IN THE EXPERIMENTAL GROUPS.THE HIGHS AND LOWS WITH AND WITHOUT STZ IN WISTAR RATS AND A P-VALUE LOWER THAN 0.001 WAS CONSIDERED SIGNIFICANT

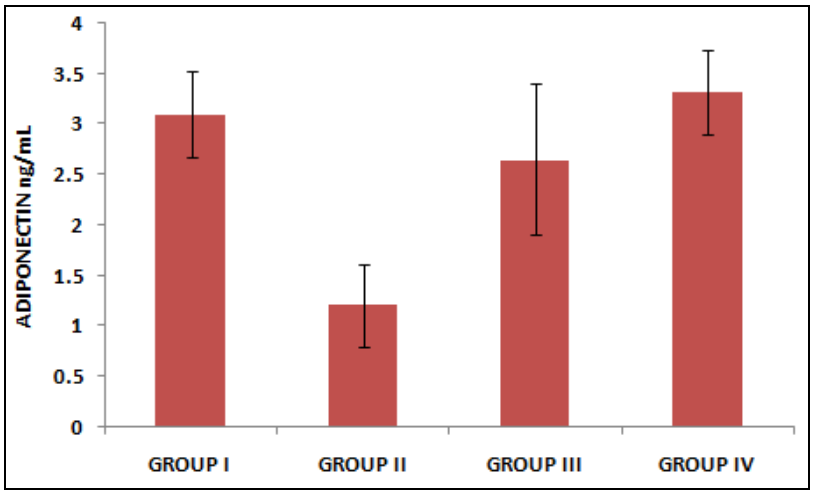

FIG. 3: IMPACT OF DIET AND STZ ON ADIPONECTIN IN THE EXPERIMENTAL GROUPS. THE HIGHS AND LOWS WITH AND WITHOUT STZ IN WISTAR RATS.AND A P-VALUE LOWER THAN 0.001 WAS CONSIDERED SIGNIFICANT

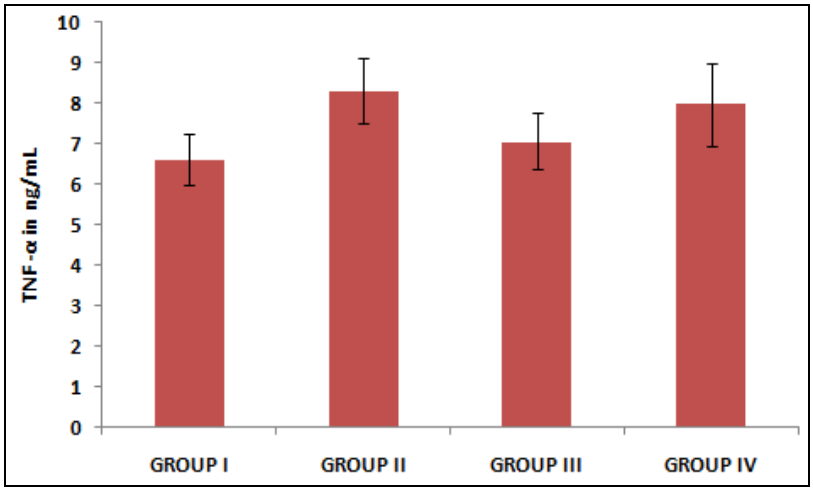

FIG. 4: IMPACT OF DIET AND STZ ON TNF –α IN THE E EXPERIMENTAL GROUPS. THE HIGHS AND LOWS WITH AND WITHOUT STZ IN WISTAR RATS AND A P-VALUE LOWER THAN 0.001 WAS CONSIDERED SIGNIFICANT

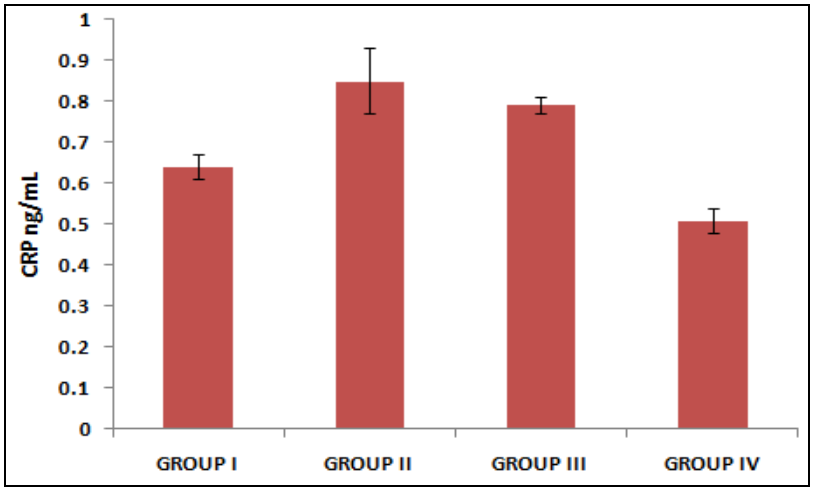

FIG. 5: IMPACT OF DIET AND STZ ON CRP IN THE EXPERIMENTAL GROUPS. THE HIGHS AND LOWS WITH AND WITHOUT STZ IN WISTAR RATS AND A P-VALUE LOWER THAN 0.001 WAS CONSIDERED SIGNIFICANT

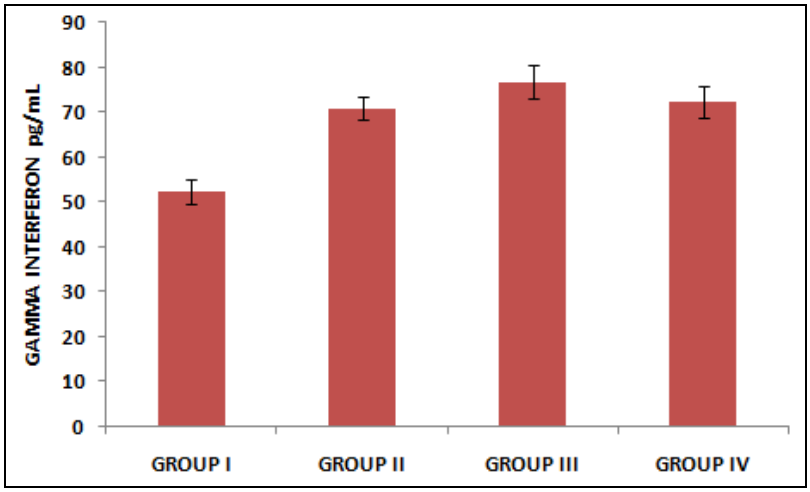

FIG. 6: IMPACT OF DIET AND STZ ON GAMMA INTERFERON IN THE EXPERIMENTAL GROUPS. THE HIGHS AND LOWS WITH AND WITHOUT STZ IN WISTAR RATS AND A P-VALUE LOWER THAN 0.001 WAS CONSIDERED SIGNIFICANT

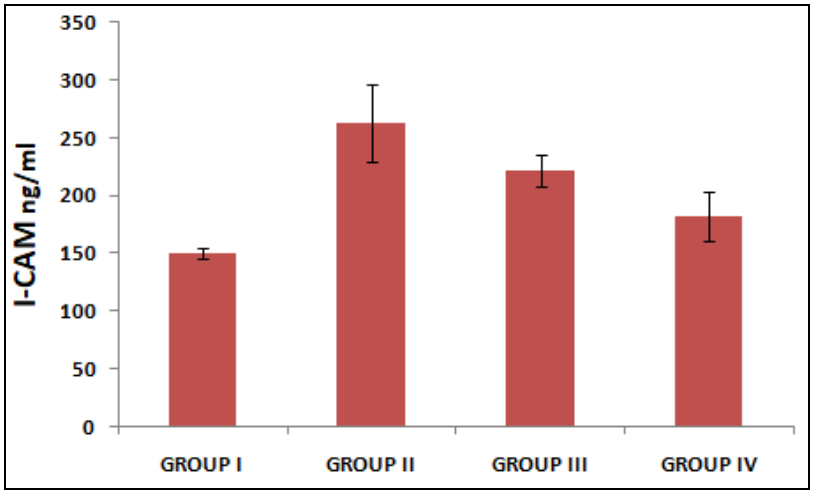

FIG. 7: IMPACT OF DIET AND STZ ON I-CAM IN THE EXPERIMENTAL GROUPS. THE HIGHS AND LOWS WITH AND WITHOUT STZ IN WISTARRATS AND A P-VALUE LOWER THAN 0.001 WAS CONSIDERED SIGNIFICANT

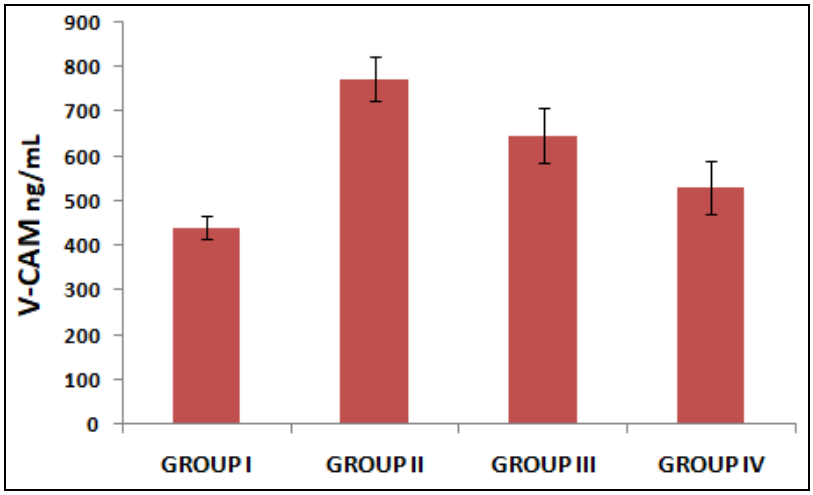

FIG. 8: IMPACT OF DIET AND STZ ON V-CAM IN THE EXPERIMENTAL GROUPS. THE HIGHS AND LOWS WITH AND WITHOUT STZ IN WISTAR RATS AND A P-VALUE LOWER THAN 0.001 WAS CONSIDERED SIGNIFICANT

DISCUSSION: Obesity is a widely recognised contributor to chronic diseases like coronary artery disease and diabetes, particularly type 2 diabetes (T2DM), as well as an important global cause of morbidity in humans. 24A high-fat diet is administered, which directly affects pancreatic beta-cell degeneration, which starts IR and T2DM.The metabolic status of the precursor throughout HF diet administration and the duration of damage determine the extent of beta-cell failure. 25Adipose tissue grows too much when someone is obese. It is understood that white adipose tissue is a functioning endocrine organ that can produce and releasing a variety of bioactive polypeptides, including adipokines [26]. Leptin is one of numerous adipokines in humans whose tissue expression and circulating levels rise with increasing obesity Leptin capacity to limit eating and reduce body fat is compromised obesity, which is characterized hyperleptinemia in both humans and animals fed an HF diet [27]. According to our findings, the STZ-HF group's blood leptin level was significantly greater than that of the STZ-SF and HFC groups. When contrasted with the control group, rats fed a high-fat diet revealed a substantial increase in total body weight. According to research by Handjieva-Darlenska T *et al* [28]. Hyperleptinemia was brought on by a high-fat diet consumed over time. According to the authors, the amount of fat in the epididymis, the liver, and the heart all significantly correlated with plasma leptin levels. A high-fat diet resulted in greater body weight and glucose intolerance when compared to a low-fat diet. Leptin is a powerful insulin sensitizer that enhances

glucose tolerance in leptin-deficient, insulin-insensitive Lep (ob/ob) mice. Suggesting a connection between leptin resistance and insulin sensitivity and obesity. Hyperleptinaemia and inlammation have been connected to leptin resistance brought on by a high-fat diet as potential causative factors 29.However, Ainslie *et al.* discovered a connection between fast, high-fat diets and decreased leptin secretion. They found evidence that the high-fat diet may have caused the rats to gain weight *via* reducing leptin secretion. It has been shown that isolated adipocytes' expression and secretion of leptin fall as insulin-stimulated glucose metabolism rises. Leptin levels decrease during fasting, which may be attributed to adipocytes' increased ability to absorb glucose when it is triggered by insulin [30].

Hyperleptinemia, however, may influence the aetiology of obesity-related problems due to obesity. The processes that lead to diabetes and Leptin resistance and the ensuing dysregulation altered hepatic metabolism, reduced insulin production, decreased whole-body glucose utilisation, lipotoxicity, ectopic fat deposition, and altered leptin function are all connected to a number of related illnesses in humans [31]. Leptin can also affect mechanisms that promote platelet aggregation, which may lead to thrombosis, reduce endothelial function, promote inlammation and angiogenesis, and enhance immune system activity. These activities may contribute to or exacerbate diabetes problems or other disorders [32].

In contrast, leptin was found by Kusakabe *et al.* to improve glycaemic and lipid management in a mouse model of T2DM with increased adiposity caused by STZ and a high-fat diet. In a study using a model animal that mimics human T2DM (STZ/ HFD), researchers demonstrated that continuous leptin infusion improved lipid and glucose metabolism, increased insulin sensitivity, and decreased food consumption. Triacylglycerol levels in the liver and skeletal muscle were decreased by leptin, while alpha2 AMPK activation was elevated in the muscle. In pair-feeding experiments, authors showed that leptin improved lipid and glucose metabolism regardless of decreased meal consumption. Our results demonstrated the potential clinical relevance of leptin as a novel glucose-lowering medication in humans by highlighting the positive effects. Leptin's effects on lipid and glycaemic regulation in a mouse model of type 2 diabetes and increasing obesity. In the STZ-HF group, leptin and glucose levels were shown to be significantly positively associated ($r=0.71$; $p=0.048$) [34]. Adiponectin controls numerous metabolic activities, including those that lead to T2DM, coronary artery disease, metabolic syndrome, and obesity. Unexpectedly, obesity results in a drop in adiponectin [35].

Our results showed that the STZ-HFD group's serum adiponectin level was considerably lower than those of the STZ-ND, ND, and HFD groups. These findings support past studies that found adiponectin reduces body weight by raising energy expenditure Adiponectin therapy reduces body weight and fat percentage. Many indicators point to the possibility that adiponectin increases energy expenditure by working through the hypothalamus [36].

As shown by its catabolic effect on adipose tissue and enhanced peripheral glucose uptake after TNF was neutralised in obese rats, TNF plays a significant role in the onset of insulin resistance and diabetes as a result of obesity [37]. According to earlier studies, TNF- causes insulin resistance and T2DM, both of which are connected to fat. 38 Nevertheless, Miyazaki et al. refuted this assertion 39. Our investigation discovered that the HFD + STZ group had a considerably higher TNF- level than the usual diet group. We also noted a greater TNF-level in the HFD +STZ group compared to the HFD group.

The body uses cytokines, which are immune system molecules, for a number of purposes, including cellular injury, wound healing, and inflammation. The results showed that the DH group had significantly greater serum levels of IFN- than the other groups. These results imply that IFN may play a role in the pathogenesis of type 2 diabetes and its complications, such as hypertension. IFN, a cytokine released by NK and T lymphocytes, is crucial for activating macrophages and beginning inflammation [40]. Earlier studies concurred with our results. For example, Arababadi *et al.* discovered a connection between type 2 diabetes, increased serum concentrations of IFN is associated with

nephropathic effects. Notably, a number of studies have validated our conclusions about blood IFN concentration, involvement in the genesis of type 2 diabetes and its consequences [41]. Due to the positive and negative relationships between IFN and HDL and between IFN and age, the conclusion needs to be verified utilizing various in vivo experiments.

CRP production may be influenced by a variety of metabolic and inflammatory variables, including high blood sugar, adipokines, and levels of free fatty acids, which are all connected to the development of T2DM [42].

Furthermore, an elevated CRP level in diabetic patients serves as a trustworthy vascular abnormalities prognosis and the development of cardiovascular disease. Furthermore, numerous studies on humans and animals showed connections between elevated serum CRP levels and obesity as well as the development of IR into T2DM. These results support the idea that the pathogenesis of T2DM is heavily influenced by the inflammatory state indicated by higher CRP levels.

Numerous studies have found a connection between elevated CRP concentration and an elevated chanceof growing type 2 diabetes [43]. Endothelial dysfunction or injury can be roughly determined by detecting biomarkers released by endothelial cells, such as soluble intercellular adhesion molecule 1 (sICAM-1) and soluble vascular cell adhesion molecule 1 (sVCAM-1) [44].

Several cross-sectional investigations have revealed that individuals increased levels of when they develop diabetes of endothelial dysfunction markers. To better understand the part endothelial dysfunction plays prevent progression to type 2 diabetes to evaluate potential sex differences, we looked at the connection between 2 markers of endothelial dysfunction (V-CAM, ICAM-1) incident type 2 DM huge, community-based study of middle-aged men and women [45].

Likewise, Farsi *et al.* (2016) observed that after eight weeks of moderate and high-intensity endurance exercise, diabetic rats' serum levels of ICAM-1 had decreased [46]. Kargarfard *et al.* (2016) also claimed that eight weeks of high-intensity interval training and endurance training will cause decrease in ICAM-1 and VCAM1 in obese males. However, in males with normal weights, only endurance exercise might significantly reduce VCAM-147. Moreover, Rosety *et al.* (2016) found that older obese women who underwent resistance training for 12 weeks (3 weekly sessions) experienced significant drops in ICAM-1 and VCAM-1 levels [48]. In summary, Abd El-Kader *et al.* (2016) found that older obese women who engaged in three months of treadmill endurance training experienced significant reductions in ICAM-1, VCAM-1, and CRP 49. Khademi *et al.* (2016) found a decrease in ICAM-1 gene expression in the cardiac tissue of Wistar male rats [50].

CONCLUSION: Many experimental and clinical investigations suggest that the development of endothelial dysfunction is significantly influenced by vascular inflammation. Endothelium loses its physiological characteristics in endothelial dysfunction, nitric oxide bioavailability decreases, and the endothelium shifts to a vasoconstrictor, pro-thrombotic, and pro-inflammatory state. Perivascular adipose tissue is prominent to have plays a significant part inside the encouragement of vascular dysfunction through the pro-inflammatory and prooxidant milieu that interacts within the artery wall. The development of vascular disease associated with type 2 diabetes and obesity, as well as vascular dysfunction, are now recognized to be largely influenced by inflammation. The prevention of adipokines that cause inflammation, including adiponectin, from impairing the endothelial system. Knowing more about these pathways in detail would be extremely helpful in identifying new pharmaceutical targets for avoiding, and treating the inflammation, followed by endothelial dysfunction linked to obesity-related diabetes.

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