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Original Research

Ameliorative Effects of Oral Glucosamine on Insulin Resistance and Pancreatic Tissue Damage in Experimental Wistar rats on a High-fat Diet

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Hyperlipidemia due to a high-fat diet (HFD) is a risk factor for inducing insulin resistance (IR) and adverse effects on pancreatic β-cells in obesity and type 2 diabetes mellitus. This relationship may be due to activation of the hexosamine-biosynthesis pathway. Administration of exogenous glucosamine (GlcN) can increase the end product of this pathway (uridine-5′-diphosphate-N-acetyl-glucosamine), which can mediate IR and protein glycosylation. The objective of this study was to evaluate the effects of oral GlcN and HFD on IR and pancreatic histologic damage in a 22 wk study of 4 groups of male Wistar rats: control group with normal chow diet, HFD group (24%. g/g lard), GlcN group (500 mg/kg^{-1} per day of glucosamine hydrochloride in drinking water) and HFD plus oral GlcN. Metabolic variables related to IR that were measured included triglycerides (TG), free fatty acids (FFAs) and malondialdehyde (MDA). Histopathologic evaluation of the pancreas was also performed. The results showed IR in the HFD group, which had increased pancreatic nuclear pyknosis and vacuolization, with fatty infiltration and structural alteration of the islets of Langerhans. TG, FFAs and MDA were higher in serum and pancreatic tissue as compared with the control group. The GlcN group did not develop IR and had only mild nuclear pyknosis with no significant change in the pancreatic content of TG, FFAs and MDA. However, the combined administration of GlcN and HFD attenuated IR and improved TG, FFAs and MDA levels in serum and pancreatic tissue and the pancreatic histopathologic changes, with no significant differences as compared with the control group. These findings suggest that the oral GlcN at a dose of 500 mg/kg^{-1} is protective against IR and the pancreatic histologic damage caused by HFD.

Abbreviations: AMPK, protein kinase; ANOVA, analysis of variance; C, control; CPT1, carnitine palmitoyl transferase-1; ELISA, enzyme-linked immunosorbent assay; FFAs, free fatty acids; GFAT, glutamine:fructose-6-phosphate amido transferase; GlcN, glucosamine; HBP, hexosamine-biosynthesis pathway; HFD, high-fat diet; HFD + GlcN, high-fat diet + glucosamine; HOMA-IR, homeostasis model assessment of insulin resistance; IR, insulin resistance; MDA, malondialdehyde; NEFA, non-esterified fatty acid; O-GlcNAc, O-Linked β-N-acetyl glucosamine; ROS, reactive oxygen species; TBARS, thiobarbituric acid reactive substances; TG, triglycerides; UDP-GlcNAc, uridine-5′-diphosphate-N-acetyl-glucosamine

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Obesity, which is an insulin resistance (IR) factor, occurs because of excess caloric intake. Clinically, obesity is associated with high levels of free fatty acids (FFAs) in plasma due to the reduced suppression of lipolysis, is associated with conditions such as diabetes type 2 mellitus, hypertension, atherosclerosis, and metabolic syndrome,[2] and is an important factor in the pathogenesis of long-term organic damage.[36] A previous study[49] reported that an alteration in the ability of adipocytes to store excess calories as triglycerides (TG) contributes to a greater accumulation of lipids and their metabolites in other tissues. These tissues are not necessarily adapted to their storage, resulting in cellular abnormalities such as apoptosis, oxidative stress, and endoplasmic reticulum stress, which alter cell function. However, both hyperlipidemia and hyperglycemia can have harmful effects on cell function, termed lipotoxicity and glucotoxicity, respectively.[1,36] These effects can lead to desensitization of the target peripheral tissues to the biologic actions of insulin and can also induce an insufficient response of the β cells of the pancreas by glucose stimulation.

High-fat diets (HFD) have been associated with hyperlipidemia,[32] which in turn leads to IR and pathologic consequences in the pancreas. Hyperlipidemia causes overactivation of the hexosamine biosynthesis pathway (HBP) and overexpression of glutamine:fructose-6-phosphate amidotransferase (GFAT); these give rise to uridine-5′-diphosphate-N-acetyl-glucosamine (UDP-GlcNAc), which causes both IR and alteration of protein glycosylation, leading to selective pancreatic cell destruction.[10,25,44] This pathway can also be activated by administration of exogenous glucosamine (GlcN), suggesting that GlcN in relatively high doses can lead to IR both in vitro and in vivo[41] through an inhibitory effect on early insulin signal transduction,[15] The diabetogenic effect, which is caused in part by interference with glucose utilization in pancreatic cells, reduces insulin release. However, other work[20] has argued that exogenous GlcN

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promotes the development of embryonic pancreatic cells, but did not study pancreatic damage due to subchronic infusion of GlcN in vivo.

Although exogenous GlcN is widely used for the treatment of osteoarthritis,[4,40,51] the combination of GlcN with a HFD increases plasma FFAs that can induce IR and affect pancreatic tissue. The objective of the present study was to evaluate the long-term effect of oral GlcN on IR and on pancreatic histopathologic changes produced by a HFD in rats.

Materials and Methods

Animals preparation. Male Wistar rats (*Rattus norvegicus albinus*) (n = 28, 150 to 200 g) were housed individually in open-bottom metal cages (20 × 30 × 18 cm, KT Fammai, Mexico, City), with food and tap water provided ad libitum. Racks permitted auditory and olfactory contact. Rooms maintained temperatures of 22 ± 2 °C and a 12:12 h light:dark cycle (lights on from 0700 to 1900). After 2 wk of adaptation, the rats were randomly assigned to a commercial rodent chow diet (Rodent Laboratory Chow 5001. PMI, Richmond, IN) or HFD (enriched with fat from a lard mixture (20% w / w) and 80% w /w of the same commercial diet). All the experimental procedures described in this study are in accordance with the guidelines of the Laws and Codes of Mexico in the Seventh Title of the Regulation of the General Health Law on Health Research and the Official Mexican Standard NOM-062-ZOO-1999 detailing the technical aspects and specifications for the production, care and use of laboratory animals. The minimal number of animals needed to achieve the objectives of this study was used and approved by the Ethics and Biosafety Committee of the National School of Biologic Sciences.

Experimental design. The rats were divided in the following 4 groups: control (C), HFD, GlcN, and the combination of HFD and GlcN (HFD + GlcN), with 7 rats per group. The C group received tap water and a normal diet with purine pellets (Rodent Laboratory Chow 5001. PMI, Richmond, IN) and was comprised of (wt/wt) 4.5% fat, 23% protein, and 49% carbohydrate with an energy content of 3.27 Cal/g and tap water. The HFD group received tap water and a diet enriched with fats from a mixture of lard (20% wt/wt) and 80% wt/wt of the same commercial diet, resulting in 24% fat, 18% protein, and 39% carbohydrate, with an energy content of 4.41 Cal/g (48% of energy was derived from saturated fat of pork lard).[9,50] GlcN group received purine pellets and 500 mg/kg^{-1}day^{-1} of glucosamine (D $^{(+)}$ glucosamine hydrochloride; Sigma–Aldrich, St Louis MO) in the drinking water. The dosage of GlcN given to rats was not markedly higher than the daily dosage recommended for humans with osteoarthritis (1500 mg, or 20 mg/kg for a person weighing 75 kg), considering that the metabolic rate per kilogram of body weight of the rat is approximately 10 times that of humans. The mean weight of our rats was 150 g at the start of the experiment and reached 350 to 400 g at euthanasia. Therefore, the dosage the rats received varied from 75 to 200 mg/d of glucosamine. The HFD + GlcN group received 24% (48% of energy) of the derivative of saturated fat in addition to 500 mg kg^{-1}day^{-1} glucosamine in drinking water. The rats were treated for 22 wk.

Analytical determinations. All rats were weighed once a week throughout the study. Blood samples were obtained from the tip of the tail at 0800 after an overnight fast of 12 h. Glucose and insulin concentrations were measured after 20 wk of treatment. The samples were centrifuged (centrifugal force of 1,957 × g, 10 min, 4 °C), and serum was immediately stored at -70 °C. Blood glucose levels were analyzed using a glucometer (OPTIUM XCEED, Optium MediSense, Abbott). The quantitative levels of serum triglycerides (TG; Randox Laboratories, Crumlin, United Kingdom) and serum FFAs, as non-esterified fatty acids (NEFA) (Wako Chemicals, Neuss, Germany), were measured using enzymatic-colorimetric kits. Insulin was measured using an immune competitive ELISA (ELISA kit 48-GLUHU-E01; Alpco, Salem, NH). IR was evaluated by the homeostasis model assessment, (HOMA-IR) calculated as the product of fasting serum insulin in microunits per milliliter and fasting blood glucose in milligrams per deciliter divided by 2430.[13]

Pancreas determinations. Rats were euthanized after 22 wk of treatment (after collection of blood for measurement of plasma insulin and glucose) by intraperitoneal injection of pentobarbital sodium (63 mg/kg^{-1}). The pancreas was quickly removed and dissected into 2 portions. One portion was used for histology, and the other for biochemical assays. After removal, the caudal pancreatic tissue was powdered on dry ice by using a mortar and pestle and was immediately stored at -70 °C for biochemical assays while the head of pancreas was fixed in 40 g/L paraformaldehyde for histologic analysis.

Thiobarbituric acid reactive substances (TBARS), TG, and FFAs concentrations were measured in pancreatic tissue; 0.3 g of pancreas tissue was homogenized in 3 mL of 100 mM phosphate buffer pH 7.3.

TBARS was assessed by measuring pancreatic malondialdehyde (MDA). A modified version of a previously described technique was used,[16] with results expressed as nmol MDA/mg of proteins. Protein was measured using the Bradford assay.[12] Briefly, for TBARS assay, 500 µL homogenized tissue was added to 1 mL of trichloroacetic acid (TCA)–thiobarbituric acid (TBA)–HCl [15%, w/v TCA; 0.375%, w/v TBA; 0.25N HCl]. The solution was heated for 10 min in a boiling water bath. After cooling, the flocculent was removed by centrifugation at 1000×g for 10 min. The absorbance of the sample was determined at 535 nm using a reagent only blank. The MDA content of the sample was calculated as an extinction coefficient of 1.56 × 105 M^{-1}cm^{-1}. For the Bradford assay, 2 mg/mL of bovine serum albumin solution was diluted with 0.05 N NaOH to prepare a series of concentrations in the range 0.125^{-1} mg/mL to plot the calibration curve. The tissue was dissolved in 500 µL of 0.05 N NaOH. 5 µL of BSA standard, resuspended protein pellets or 0.05 N NaOH blank was added to microtiter plate. 250 µL of Bradford 1×dye reagent was then added to the solution in the plate. The plate was then read at 595 nm after 15 min of incubation at room temperature. The protein concentration was then determined from BSA standard calibration curve.

Pancreatic TG and FFAs (as NEFA) were measured using the same colorimetric commercial kits that were used for the blood analysis (Randox Laboratories, Crumlin, United Kingdom and Wako Chemicals, Neuss, Germany, respectively).[33]

Pancreatic Histopathology. The histopathologic analysis of the pancreatic was based on methodology described previously.[37] Pancreatic tissue samples were fixed in 40 g/L paraformaldehyde and embedded in paraffin. Five micrometer thick sections were stained with hematoxylin/eosin, dehydrated, and mounted with resin. An individual who was blind to the experimental groups examined approximately 10 sections and quantified almost 30 Langerhans islets for each rat, using AXIOVISION ^{MR} USA program. Langerhans islets were considered normal if the cytoarchitecture was preserved and its form was round or oval with a clear limit. Abnormal islets had polycyclic or starry contours, or insular disintegration. The severity of pyknosis in Langerhans islets cells was recorded using a graded scale (1 to 3) as follows: 1) mild pyknosis (0%

Table 1. Body weight and serum triglycerides and fatty acids (NEFA) levels, of rats for 20 wk with high-fat diet with or without GlcN

Variable	Control (n = 7)	High-fat diet (n = 7)	Glucosamine (n = 7)	High-fat + Glucosamine (n = 7)
Body weight (g)	384 ± 12	442 ± 12*,$^{\alpha}$	386 ± 9	404 ± 11
NEFA (mmol/L)	1.08 ± 0.07	1.43 ± 0.11	1.14 ± 0.02	1.18 ± 0.12
TG (mmol/L)	0.45 ± 0.03	0.77 ± 0.10*,$^{\alpha}$	0.43 ± 0.02	0.75 ± 0.07*,$^{\alpha}$

Values are expressed as mean + SEM. * $P < 0.05$ *vs* control group, $^{\alpha}$ $P < 0.05$ *vs* glucosamine group.

to 33%), 2) moderate pyknosis (>33% to 66%) and 3) severe pyknosis (>66%).

Statistical analysis. Glucose, insulin, HOMA-IR, TBARS, TG and NEFA are expressed as mean ± SEM; Islet of Langerhans cell pyknotic scores are expressed as median ± percentile ranges. Glucose, insulin, HOMA-IR, TBARS, TG, and NEFA were analyzed using one-way ANOVA with Tukey posthoc testing. Pyknosis scores were analyzed using ANOVA by the Kruskal–Wallis method. Pearson correlation analysis was performed to determine the relationship between body weight, serum TG and NEFA as compared with HOMA-IR and TBARS. Factors evaluated were diet and glucosamine. $P < 0.05$ was considered statistically significant. SigmaStat 3.5 software (Systat, 252 Richmond, CA) was used.

Results

Body weight, serum triglyceride and fatty acids levels. After 20 wk of treatment (Table 1), the HFD group was significantly heavier than the C and GlcN groups ($P < 0.01$). Serum TG levels were not significantly different among any of the groups. The NEFA levels were significantly higher in both the HFD and HFD + GlcN groups as compared with groups C ($P < 0.05$) and GlcN ($P < 0.01$, $P < 0.012$, respectively).

Blood glucose and serum insulin levels. Rats on the HFD had significantly ($P < 0.05$) higher blood glucose and serum insulin concentrations and HOMA-IR as compared with values from rats on the commercial rodent chow diet. Also, GlcN attenuated the increases of blood glucose and serum insulin levels and HOMA-IR index in the HFD rats (Figure 1). After 20 wks of treatment, glycemia was statistical different in HFD rats as compared with the C group ($P < 0.001$; 6.3 ± 0.3 *vs* 4.8 ± 0.2 mmol/L), respectively, and with the GlcN group ($P < 0.05$; 5.3 ± 0.2 mmol/L). The group that received only the GlcN showed no significant change in glycemia (5.3 ± 0.2 mmol/L) as compared with the C group ($P > 0.05$). The HFD + GlcN group (5.5 ± 0.2 mmol/L) showed no significant change at the end of the treatments as compared with HFD rats or with the group ($P > 0.05$) (Figure 1 A).

The serum insulin concentration was higher in the HFD group than in the C group ($P < 0.01$; 32 ± 2 and 16 ± 1 µU/mL, respectively) and GlcN group ($P < 0.01$). GlcN (16 ± 3 µU/mL) and HFD + GlcN (23 ± 4 µU/mL) groups showed no significant increase relative to the C group ($P > 0.05$ for both) (Figure 1 B).

The HOMA-IR index was significantly higher for the HFD group as compared with the other groups ($P < 0.01$; HFD, 1.54 ± 0.09; C: 0.57 ± 0.04: GlcN, 0.65 ± 0.12; HFD + GlcN, 0.87 ± 0.14). GlcN and HFD + GlcN groups showed no significant difference relative to group C ($P > 0.05$) (Figure 1 C).

Pancreas MDA, TG and NEFA. Rats on HFD had significantly ($P < 0.05$) higher levels of TBARS (as MDA), TG and NEFA in pancreatic tissue than did group C rats; however, with the addition of GlcN, values were not significantly different from controls ($P > 0.05$) HFD resulted in significantly ($P < 0.05$) higher TBARS (as MDA), TG and NEFA levels in pancreatic tissue as compared with C group; however, with the addition of GlcN to the HFD diet, values were not significantly different from control ($P > 0.05$) (Figure 2).

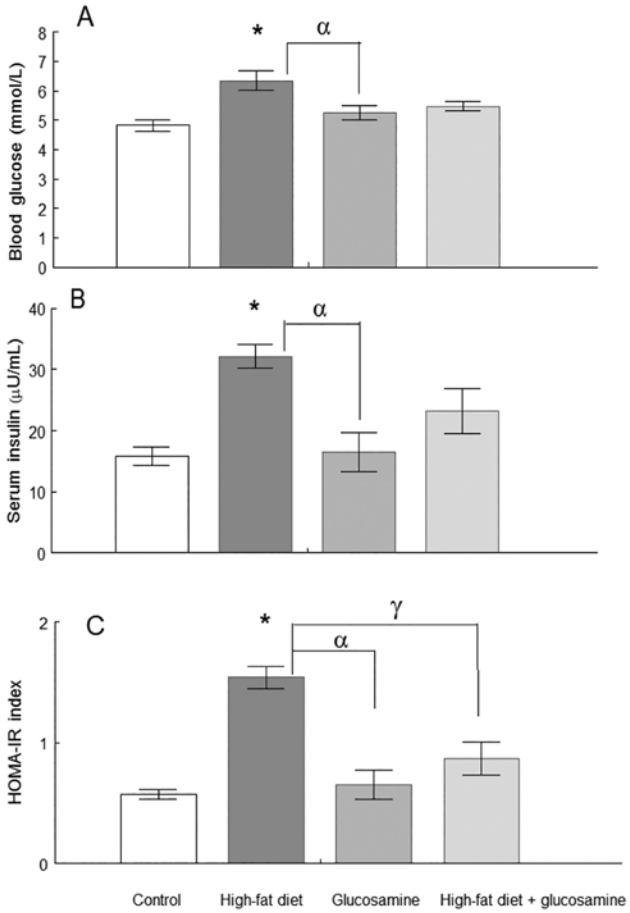

Figure 1. Blood glucose (A), serum insulin (B) and HOMA-IR index (C) of male rats fed a control or a high-fat diet without (Control and High-fat diet groups) or with (Glucosamine and High-fat diet + glucosamine groups) 500 mg/kg of GlcN hydrochloride in the drinking water for 20 wk. Values are means ± SE (n = 7). *$P < 0.05$ *compared with* control group, α $P < 0.05$ *compared with* glucosamine group, γ $P < 0.05$ *vs* high-fat + glucosamine group, by ANOVA test.

Values of MDA in pancreatic tissue as a direct indicator of cellular damage and lipid peroxidation was significantly higher in HFD group as compared with the C group ($P < 0.01$; 86 ± 17 and 28 ± 5 nmol/mg protein, respectively) (Figure 2 A). Values for GlcN (44 ± 9 nmol/mg protein) and HFD + GlcN (65 ± 11 nmol/mg of proteins) groups were not significantly different from the C group ($P > 0.05$ for both).

The pancreas TG content of the HFD group was significantly higher than that of the C group (0.65 ± 0.09 and 0.21 ± 0.05 mg/g of tissue, respectively; $P < 0.001$) and the GlcN group (0.37± 0.07 mg/g of tissue; $P < 0.05$). The GlcN (0.37 ± 0.07 mg/g of tissue) and HFD + GlcN (0.43 ± 0.07 mg/g of tissue) groups showed no significant increase relative to the C group ($P > 0.05$, for both) (Figure 2 B).

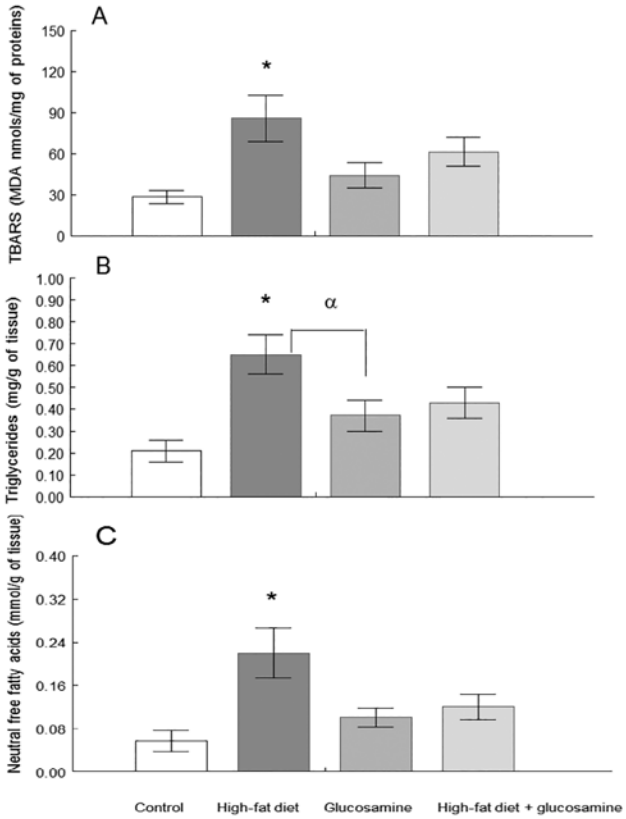

Figure 2. TBARS (as MDA) (A), Triglycerides (B) and NEFA (C) of male rats fed a control or a high-fat diet without (Control and High-fat diet groups) or with (Glucosamine and High-fat diet + glucosamine groups) 500 mg/kg of GlcN hydrochloride in the drinking water for 22 wk. Values are means ± SE ($n = 7$). *$P < 0.05$ compared with control group, α $P < 0.05$ *compared with* glucosamine group, by ANOVA test.

The pancreas NEFA content was significantly higher for the HFD group as compared with the C group (0.22 ± 0.05 as compared with 0.06 ± 0.02 mmol/g of tissue, respectively; $P < 0.01$). GlcN (0.10 ± 0.02) and HFD + GlcN (0.12 ± 0.02 mmol/g of tissue) groups were not significantly different from C group ($P > 0.05$, for both) (Figure 2 C).

Pancreas Histology. The histologic study revealed that a HFD is associated with polycyclic contours, pyknosis, vacuolization, and fat infiltration (panel B). However, GlcN alone, like the C group, did not have histologic alteration (panels C and A, respectively). Moreover, HFD + GlcN prevented the histologic alterations associated with the HFD (Figure 3 D).

The morphometric analysis (Figure 4) showed that the HFD group had significantly fewer morphologically normal Langerhans islets (A) than did the C and GlcN groups ($P < 0.01$). The pyknosis score (B) for the HFD group was significantly higher than that of group C ($P < 0.05$). However, GlcN prevented the changes associated with the HFD, as the pyknosis score was not significantly different the GlcN + HDF and the C groups ($P > 0.05$).

Correlations of body weight, serum triglyceride and fatty acids levels with pancreatic MDA and HOMA-IR. Because the data showed an increase in body weight, plasma FFAs, and TG levels for the HFD group as compared with the C and GlcN groups, we determined the correlations of these variables with MDA and HOMA-IR. Body weight was significantly correlated with HOMA-IR and MDA ($P < 0.01$), whereas FFAs had a significant correlation only with MDA (Table 2). TG was not significantly correlated with MDA or HOMA-IR ($P > 0.05$).

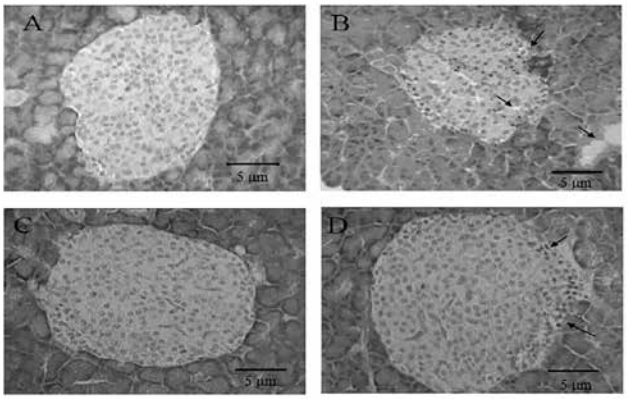

Figure 3. Photomicrographs of pancreatic islet. Panel A shows C group. Panel B shows HF diet group. Panel C shows GlcN group. Panel D shows HF + GlcN group, of male rats fed a control or a high-fat diet without (Control and High-fat diet groups) or with (Glucosamine and High-fat diet + glucosamine groups) 500 mg/kg of GlcN hydrochloride in the drinking water for 22 wk. The tissue was stained by hematoxylin-eosin 400×. High fat diet causes shape alteration as polycyclic contours, pyknosis, vacuolization, and fat infiltration (shows arrows). Meanwhile, high fat diet + glucosamine prevent histologic alterations (shows arrows).

Discussion

The first description of HFD's ability to induce obesity revealed that it promotes hyperglycemia and IR while also exerting effects on cell physiology and insulin signal transduction.[42] This work helped to validate the use of a HFD to generate a model of the metabolic syndrome with IR and compromised function of the pancreatic β–cells.

The pathophysiology of pancreatic β-cells is an extensive topic. Despite many studies that have been carried out in both humans and animal, the field has not yet achieved full understanding of the mechanisms that affect these cells and how external factors influence pancreatic functioning.[17] In the current study, the ingestion of a HFD increased blood glucose and serum insulin levels, indicating IR (Figure 1 A, B and C). This phenomenon has been previously described as a heterogeneous disorder that is associated with an initial hyperinsulinemia.[19] This hyperinsulinemia helps to maintain normal values of blood glucose, resulting eventually in a gradual decrease in this hormone and a failure in its action, indicating that HFD can induce hyperglycemia that compromises the function of β–cells[18] resulting in abnormal insulin secretion[8] that correlates with obesity.[11] Likewise, lipid infusions designed to raise plasma fatty acid levels, as occurred in this study in the HFD group, increase body weight and serum levels of TG and NEFA (Table 1). This elevation of plasma fatty acids alters the effects of insulin on glucose metabolism and can modulate insulin concentrations by stimulating its secretion under basal condition or after actue increases.[3,35,43] This report[26] showed that rats fed a HFD accumulate TG in the skeletal muscle; this accumulation is related to a loss of insulin sensitivity (that is, as body weight increases, insulin sensitivity decreases). Although the IR had been described in terms of glucose metabolism, the last decade has seen a shift from the "glucocentric" point of view to a new "lipocentric" point of view.[7,38]

The lipocentric hypothesis also supports that abnormalities in fatty acid metabolism can result in an accumulation of lipids in muscle, liver and pancreas,[45] a situation observed in the current study, which found an increase in TG and NEFA in pancreas in the HFD group (Figure 2 B and C); this explains the effects on IR

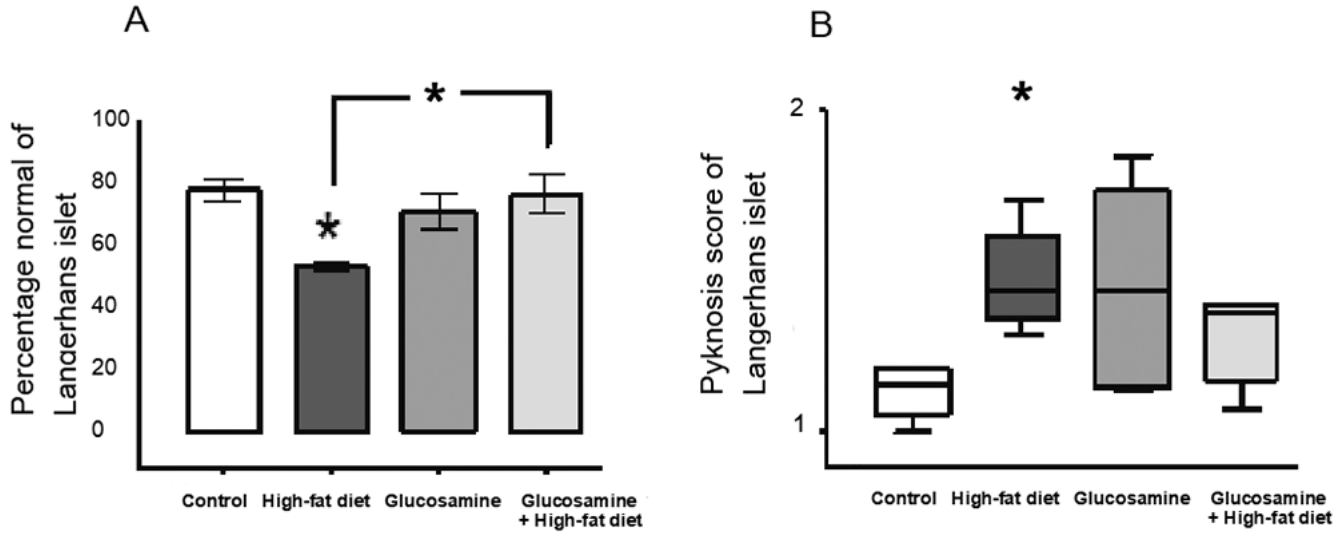

Figure 4. Effect of HF diet plus GlcN on percentage of normal Langerhans islets (A) and pycnotic score (B), of male rats fed a control or a high-fat diet without (Control and High-fat diet groups) or with (Glucosamine and High-fat diet + glucosamine groups) 500 mg/kg of GlcN hydrochloride in the drinking water for 22 wk. Values are expressed as mean + SEM * $P < 0.05$ *compared with* control, by the Kruskal–Wallis test.

Table 2. Pearson correlation coefficients between body weight, serum TG and NEFA levels *vs* TBARS and HOMA.

Variable	TBARS (as MDA) in pancreas	HOMA-IR index
Body weight	0.580†	0.664†
Serum TG	0.285	0.419
Serum NEFA	0.266	0.509*

* $P < 0.05$; † $P < 0.01$.

alterations in the HFD group, which agree with another report.[35] The current report also suggests that elevated NEFA increases pancreatic TG content in rodents. Another previous study found that in diabetic rats with an obese phenotype, pancreatic cells exhibit TG concentrations 50 times higher than normal values as a consequence of a higher esterification capacity, leading to significantly altered secretory function.[38] These alterations lead to increase oxidative damage and apoptosis of pancreatic β–cells,[38] which are extensive in obesity and type 2 diabetes mellitus. This finding is consistent with the results of the current study using analysis of MDA, which was also higher in the HFD group, as an indicator of oxidative stress (Figure 2 A). This finding signals the onset of cell damage, suggesting that chronic overnutrition can cause a transient increase in glucose and in circulating and intracellular lipids due to glycolipotoxicity, which contributes to IR and β–cell damage. The results in the current study found a positive correlation between weight gain with increased pancreatic MDA and the induction of IR. The results also show a positive correlation between NEFA levels and IR (Table 2), suggesting that the increased fatty acid flux in muscle and IR is due to hyperactivation of HBP,[26] and that the pathogenic effect of hyperglycemia is mediated by increased reactive oxygen species (ROS) and consequent oxidative stress. ROS indirectly induce tissue damage by activating stress-sensitive cellular pathways, such as HBP.[34] Another hypothesis proposed in previous research[15] involves the posttranslational modification of serine and threonine residues of proteins by N-acetylglucosamine, the final product of HBP. O-linked β-N-acetylglucosamine (O-GlcNAc) regulates various cellular processes and sustains the increases in HBP overactivity and O-GlcNAc levels that are implicated in the etiology of many chronic diseases and associated with glucose toxicity and complications in various organs.[25,27] Chronic hyperglycemia, chronic dyslipidemia, or a combination of both (glycolipotoxicity) have been postulated to contribute to worsening cell function over time.[39] This situation creates a vicious cycle whereby metabolic abnormalities continue to alter insulin secretion, further aggravating metabolic disturbances. In many experimental studies, elevated glucose or fatty acid levels have detrimental effects on β–cell function, indicating that an excess of both substances is synergistically harmful, leading to the concept of glycolipotoxicity.[38,48]

On the other hand, oral supplementation with GlcN at the usual doses negatively affects glucose metabolism.[27] However, previous work has reported that this is due to overactivity of HBP, which can inhibit the effect of insulin due to the irreversible action of GFAT.[26,41] In the current study, blood glucose, insulin secretion, (Figure 1 A and B) TG, and NEFA in the pancreas (Figure 2 B and C) were not significantly different for the GlcN group as compared with the C group, suggesting that the administration of oral GlcN does not affect these substances and does not induce damage such as that caused by a HFD. Further supporting this claim, previous data has also shown that oral GlcN minimizes blood glucose and serum insulin levels by attenuating the presence of IR when administered together with an HFD in rats,[9] which is similar to the findings of the current study (Figure 1). Furthermore, oral GlcN reduced the content of MDA, TG and NEFA in the pancreas when administered together with the HFD (Figure 2 A, B and C).

The lipotoxicity caused by HFD also explains the histologic results of the present study, which found a greater frequency of polycyclic contours in the analyzed islets and cells with moderate nuclear pyknosis and vacuolization accompanied by mild fatty infiltration, reducing the percentage of normal islets (Figure 3 B and 4 A and B). These data indicate possible cell damage, as do previous studies in which the a high-fat diet produced fat infiltration in the pancreatic acinar cells, related pancreatic fibrosis, acinar cell injury, and an increase in islet size.[6,31] Previous studies have also reported the appearance of vacuoles in pancreatic cell cytoplasm, which is also a criterion for pathologic change in rat pancreas.[22,23] Fat infiltration can also contribute to cellular dysfunction and the development of type 2 diabetes

mellitus.[46] In addition, the abnormally high and constant chronic lipid exposure produces apoptosis of β−cells, which leads to a long-term decrease in insulin secretion.[14,21] In contrast, the GlcN group showed round and oval contours throughout the analyzed islet (Figure 3 C), with mildly moderate nuclear pyknosis (Figure 4 B). These findings could indicate that cellular deterioration occurs, albeit to a lesser degree, maintaining the percentage of normal islets, without initiating the cellular damage caused by a HFD (Figure 4 A). When administered together, GlcN decreased the histologic pancreatic damage caused by HFD, with large oval-shaped islets and reduced cellular pyknosis (Figures 3 D and 4 B), maintaining the percentage of normal islets in relation to the C group (Figure 4 A).

This study supports that the administration of oral GlcN in drinking water as compared with HFD in rats does not induce IR or damage pancreatic tissue. Despite a reported analogy in the induction of hyperactivity in HBP,[32], the effects of exogenous GlcN reported here support a previous hypothesis[29] that glucosamine can promote the metabolism of cellular free fatty acids, thus minimizing lipotoxicity; this occurs due to the regulation of AMP-activated protein kinase (AMPK), which plays a role in altering malonyl-CoA levels[10,28,30] and inhibiting carnitine palmitoyltransferase-1 (CPT1). These processes regulate the transport of fatty acids to the mitochondria by increasing the oxidation of lipids from a HFD, for which AMPK activity is presumed to induce a lipooxidative effect favoring a greater use of glucose.[24,47] Activation of AMPK could be the means by which glucosamine minimizes the effects of a HFD on the metabolism of carbohydrates and lipids, delaying the induction of IR.[5] However, more studies are needed to better elucidate the effects of chronic oral GlcN in rats fed a HFD.

In conclusion, the current study indicates that a HFD can damage the pathophysiology of the pancreas and induce IR. The oral administration of GlcN at a dose of 500 $mg/kg^{-1}day^{-1}$ in rats fed a HFD attenuates IR induction and improves the serum lipid profile, presenting a possible protective effect against lipid accumulation and structural damage to pancreatic tissue.

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Author Contributions

Barrientos and Pérez, conceived, designed, and performed the experiments. Barrientos and Vazquez, analyzed the data. Barrientos, Perez, and Vazquez wrote the paper.

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