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Excess Fructose Intake Activates Hyperinsulinemia and Mitogenic MAPK Pathways in Association With Cellular Stress, Inflammation, and Apoptosis in the Pancreas of Rats

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ABSTRACT

The increase in sugar consumption has been associated with current metabolic disease epidemics. This study aimed to investigate the pancreatic molecular mechanisms involved in cellular stress, inflammation, mitogenesis, and apoptosis in metabolic disease induced by high-fructose diet. Here, we used biochemical, histopathological, Western blot, and immunohistochemistry methods to determine the metabolic and pancreatic alterations in male *Wistar* rats fed 20% fructose in drinking water for 15 weeks. High-fructose consumption in rats increased the immunopositivity and protein expression of glucose transporter 2 (GLUT2) and insulin in the pancreatic tissue, in association with abdominal adiposity, hyperglycemia, and hypertriglyceridemia. The expressions of cellular stress markers, glucose-regulated protein-78 (GRP78) and PTEN-induced putative kinase 1 (PINK1), were increased in the pancreas. The levels of interleukin (IL)-6, nuclear factor kappa B (NFκB), tumor necrosis factor α (TNFα), and IL-1β and components of the Nod-like receptor protein 3 (NLRP3) inflammasome were elevated. Excess fructose intake stimulated the activation of mitogenic extracellular signal-regulated kinases 1/2 (ERK1/2), p38, and c-Jun N-terminal kinase (JNK)1 as well as the apoptotic p53 and Fas pathways in the pancreas of rats. There was also an increase in caspase-8 and caspase-3 cleavage. Our findings revealed that dietary high-fructose in the pancreas causes hyperinsulinemia due to the upregulation of GLUT2 together with cellular stress and inflammatory markers, thereby stimulates mitogenic mitogen-activated protein kinase (MAPK) and apoptosis pathways, resulting in a complex pathological situation.

1 | Introduction

Metabolic diseases such as obesity, metabolic syndrome, and Type 2 diabetes are considered severe health problems worldwide. The main causes of metabolic diseases include sedentary lifestyles, genetic factors, environmental exposure, and soft drink consumption in all age groups, particularly children and adolescents [1]. Numerous studies have indicated a strong relationship between high-fructose beverage consumption and metabolic disease occurrence [2–5]. Previously, we showed that the excess fructose intake suppressed insulin signaling pathways in different tissues of rats, such as blood vessels, liver, kidney,

Abbreviations: ASC, apoptosis-associated speck-like protein; ERK1/2, extracellular signal-regulated kinases 1/2; GLUT2, glucose transporter 2; H&E, hematoxylin and eosin; IL, interleukin; JNK, c-Jun N-terminal kinase; MAPK, mitogen-activated protein kinase; NFκB, nuclear factor kappa B; NLRP3, Nod-like receptor protein 3; PAS, periodic acid-Schiff; TNFα, tumor necrosis factor α.

testis, and adipose tissue, thereby causing systemic and tissue insulin resistance [5, 6]. High-fructose-induced hyperglycemia, hyperinsulinemia, and insulin resistance may lead to structural and functional changes in the pancreas. However, research on pancreatic irregularities caused by fructose-rich diet is limited. Various changes to pancreatic morphology resulting from dietary fructose have been observed [7], but the underlying molecular mechanisms remain unclear.

In a hyperinsulinemic state, increased secretory activity and cellular energy utilization in the pancreas cause an overload in organelles such as endoplasmic reticulum (ER) and mitochondria [8]. Elevation of ER workload induces ER stress, which is also predicted to play a role in diabetes pathogenesis [9]. Stress in cellular organelles with critical functions, such as the ER and mitochondria, can activate inflammatory and apoptotic pathways as a pathological condition [8, 10]. A chronic low-grade inflammatory state is one of the components of metabolic syndrome, which can lead to tissue damage and contribute to the pathogenesis of metabolic diseases [11]. Previous studies have reported an association between diabetes pathogenesis and inflammatory cytokines such as interleukin (IL)-6, IL-1β, and tumor necrosis factor α (TNFα). Elevated levels of these cytokines have been determined in the plasma and pancreatic tissue of Type 2 diabetic patients [12, 13]. Specifically, IL-1β-mediated pancreatic islet destruction plays a role in the pathogenesis of diabetes [14]. Another component involved in inflammatory conditions is the Nod-like receptor protein 3 (NLRP3) inflammasome, an inflammatory complex composed of NLRP3, apoptosis-associated speck-like proteins (ASCs), and caspase-1 proteins. Various stress factors, such as intracellular damage and stimulation of nuclear factor kappa B (NFκB) pathway, trigger the formation of this inflammasome complex and lead to the activation of IL-1β [15]. Furthermore, studies show that NLRP3 inflammasome production induces islet dysfunction [16, 17]. These findings indicate that several inflammatory factors play a role in pancreatic abnormalities associated with diabetes pathogenesis. A detailed investigation of the alterations in pancreatic inflammatory mediators of high-fructose-induced insulin-resistant conditions may provide a better understanding of diabetes and metabolic disturbances.

Mitogen-activated protein kinases (MAPKs) are involved in regulating various cellular events, including proliferation, differentiation, survival, and death. Because of these critical cellular functions, alterations in this pathway have been implicated in the pathogenesis of numerous diseases, such as cancer and metabolic diseases, including obesity and Type 2 diabetes [18]. The extracellular signal-regulated kinases 1/2 (ERK1/2) MAPK is a major signaling pathway involved in the regulation of cell growth and development. In addition, this pathway plays a positive regulatory role in glucose-stimulated insulin secretion and pancreatic β-cell survival [18]. The activation of p38 and c-Jun N-terminal kinase (JNK) MAPKs under different stress conditions, including inflammation and oxidative stress, may contribute to the dysregulation of glucose and lipid metabolism [19]. Studies have shown that the inhibition of p38 and JNK1 reduces adiposity and improves insulin sensitivity in diabetic or obese mice [20, 21]. These findings suggest that MAPK pathways are essential for maintaining the physiological function of the endocrine pancreas. Alterations in the expression of molecules involved in these pathways play an influential role in the pathophysiology of diabetes. Therefore, it is crucial to elucidate possible changes in the pancreatic ERK1/2, p38, and JNK1 MAPK pathways caused by excessive fructose intake, which is a known contributor to metabolic disturbances.

Another pathophysiological process implicated in the development of diabetes is controlled cell death, also known as apoptosis, which is activated by cellular stress, inflammatory cytokines, and mitogenic factors [22]. Evidence suggests that both mitochondrial (intrinsic) and receptor-mediated (extrinsic) apoptotic pathways play a role in inadequate insulin secretion and islet β-cell failure, which occur in diabetes [23]. A previous study involving diabetic rat with a high-fructose/high-fat diet showed an increase in apoptosis-related molecules, including JNK1 and caspase-3, in the pancreatic tissue [24]. However, the apoptotic pathways in the pancreas in relation to fructose-rich diets remain unclear. This study aimed to investigate the changes in the mitogenic ERK1/2, p38, and JNK1 pathways and several interrelated parameters such as cellular stress, inflammation, and apoptosis in the pancreatic tissue of rats with high-fructose-induced metabolic disorder. Ultimately, this study demonstrates how excess dietary fructose leads to pancreatic dysfunction and diabetes development using the molecular mechanisms driven by this metabolic stress. These findings may be critical for the development of novel preventive and curative strategies for the global metabolic burden attributable to high sugar/fructose intake.

2 | Experimental Section

2.1 | Animals and Diets

The experimental protocol of this study was approved by the Animal Experiments Local Ethics Committee of Gazi University (G.Ü.ET-21.054). Three-week-old male *Wistar* rats were housed in temperature- and humidity-controlled rooms (20–22°C and 45%–55% relative humidity) with a 12-h light–dark cycle. The animals were fed a standard rodent chow diet containing 62% starch, 23% protein, 4% fat, 7% cellulose, standard vitamins, and a salt mixture. After 1 week of acclimatization, the rats were randomly divided into two groups: control ($n = 6$) and high-fructose ($n = 9$). Rats in the high-fructose group were fed ad libitum drinking water containing 20% (w/v) sugar (fructose; Danisco Sweeteners OY, Kotka, Finland) and regular chow for 15 weeks. Body weight and food and liquid intakes were recorded weekly during the experimental period of all rats. At the end of the experimental period, rats were anesthetized with an intraperitoneal injection containing a ketamine (100 mg/kg) and xylazine (10 mg/kg) mixture. Subsequently, blood samples were rapidly collected via cardiac puncture. Pancreatic tissues were immediately isolated, weighed, frozen in liquid nitrogen, and stored at −85°C for further analysis. Abdominal and epididymal adipose tissues were isolated and weighed.

2.2 | Biochemical and Metabolic Measurements in the Plasma and Pancreas

The plasma of the blood samples was immediately separated by centrifugation at 1500 × *g* for 15 min at 4°C. The pancreatic samples were homogenized with 0.1 M phosphate buffer (1:10

w/v), pH 7.4, using a homogenizer (MICCRa D1). The pancreatic homogenates were centrifuged at 10 000 × *g* for 10 min at 4°C, and supernatant fractions were collected. The plasma and pancreatic supernatants were immediately stored at −85°C until analysis. Plasma glucose and lipid parameters, including triglyceride, very-low-density lipoprotein (VLDL), low-density lipoprotein (LDL), high-density lipoprotein (HDL), and total cholesterol levels, were measured using an automatic biochemical analyzer (Otto Scientific, Mindray-BS400). According to the manufacturer's instructions, insulin, glucagon, IL-6, NFκB, TNFα, and IL-1β levels in the plasma or pancreatic tissue were measured using rat-specific ELISA kits (Bioassay Technology Laboratory or ELK Biotechnology).

2.3 | Histopathological and Immunohistochemical Examinations in the Pancreas

Pancreatic tissues were fixed in buffered formalin and embedded in paraffin. The paraffin tissue blocks were then sectioned at 5-μm thickness. Pancreatic tissue samples were deparaffinized in xylene and rehydrated using a graded alcohol series (5 min each; 100%, 95%, 90%, 80%, and 70%). Tissue sections were stained with hematoxylin and eosin (H&E) [25] and periodic acid-Schiff (PAS) using standard protocols according to Luna [25] and the PAS kit (Bioptica, Italy). For immunohistochemistry, the tissue sections were deparaffinized and rehydrated in phosphate buffered saline (PBS) solution. Subsequently, the sections were treated with a citrate buffer-EDTA mix (pH 6.0) and used to reveal antigenic determinants in a microwave oven at 800 W for 25 min. Pancreatic sections were kept in 3% hydrogen peroxide (H_2O_2)-methanol solution for 20 min to eliminate endogenous peroxidase activity. Tissue sections were blocked with bovine serum albumin (BSA) to prevent nonspecific protein binding. The samples were incubated with glucose transporter 2 (GLUT2; 1:200, Bioss), insulin (1:200, Abcam), and NFκB (1:200, BioLegend), antibodies at 37°C for 60 min. Sections were incubated with avidin/biotin complex peroxidase (ABC-P) as a secondary antibody (HRP/DAB Detection IHC kit, Abcam). The following steps were performed according to the ABC-P staining method, following the manufacturer's instructions. The tissue sections were washed twice with PBS for 5 min at the end of each step, except for the protein-blocking step. As a negative control, tissue sections were incubated with PBS instead of the primary antibody. The sections were stained with diaminobenzidine (DAB) as a chromogen and counterstained with Gill's hematoxylin. All results were semi-quantitatively determined using a light microscope at 40, 100, and 250 × magnifications. Field counting was performed 10 times, and the average value was calculated (Olympus BX51, Olympus, Tokyo, Japan).

2.4 | Western Blot Analysis in the Pancreas

Pancreatic tissue samples (75 mg) were homogenized in RIPA-homogenization buffer (four-fold volume) containing 50 mM Tris, 150 mM sodium chloride, 5 mM EDTA, 1% (w/w) Triton X-100, 0.26% (w/v) sodium deoxycholate, 50 mM sodium fluoride, 0.1 mM sodium orthovanadate, and 0.2 mM PMSF. Tissue homogenates were centrifuged at 1500 × *g* for 10 min at 4°C, and the supernatants were collected. The total protein concentration was determined using the Lowry method [26]. An equal amount of total proteins (40 μg) for each group were separated using 12% polyacrylamide gel by electrophoresis and transferred to a polyvinylidene fluoride membrane using a semi-dry blotting apparatus (TransBlot Turbo, Bio-Rad Laboratories). Subsequently, the membranes were then blocked in 3% BSA for 1 h at room temperature and incubated with the appropriate primary antibodies, including GLUT2 (1:1000, Bioss), glucose-regulated protein-78 (GRP78) (1:1000, Santa Cruz), PTEN-induced putative kinase 1 (PINK1) (1:500, Santa Cruz), NFκB (1:500, BioLegend), phospho NFκB (pNFκB; 1:500, Santa Cruz), TNFα (1:500, Abcam), NLRP3 (1:500, Boster), ASC (1:500, Santa Cruz), caspase-1 (1:500, Santa Cruz), IL-1β (1:1000, Abcam), Raf1 (1:1000, Abcam), ERK1/2 (1:1000, Abcam), phospho ERK1/2 (pERK1/2; 1:1000, Abcam), p38 (1:2000, Abcam), phospho p38 (pp38; 1:1000, Abcam), JNK1 (1:1000, Abcam), phospho JNK1 (pJNK1; 1:500, Abcam), Fas (1:1000, Abcam), p53 (1:1000, Abcam), caspase-8 (1:1000, Abcam), caspase-3 (1:1000, Abcam), and actin (1:2000, Abcam) at 4°C overnight. After washing with Tris-buffered saline, the membranes were incubated with horseradish peroxidase-conjugated rabbit or mouse secondary antibodies (1:10 000, Advansta or Santa Cruz) for 1 h at room temperature. Subsequently, the blots were detected with enhanced chemiluminescence Western Blotting substrate (WesternBright, Advansta) for 5 min. Blot images were captured using a chemiluminescence detection system (c300, Azure). Protein band intensities were quantified using the ImageLab 6.1 software (Bio-Rad Laboratories). Actin was used as the internal control. The catalog numbers of all antibodies used in this study are listed in Table S1.

2.5 | Statistical Analysis

Data is presented as mean ± standard error of the mean (SEM), where *n* represents the number of rats. The protein expression results were normalized to the mean of the control groups, and the relative changes were expressed as fold changes relative to the control. Data were assessed for normal distribution and homogeneity of variance between groups before further analysis. Statistical analyses were performed using an unpaired Student's *t* test. Data were evaluated with GraphPad Prism (version 8.0, GraphPad Software, La Jolla, CA, USA). Values were considered statistically significant when the *p* value was less than 0.05.

3 | Results

3.1 | The Changes in Metabolic and Biochemical Parameters

The initial and final body weights, in addition to the daily food, liquid, fructose, and calorie intake of the rats are shown in Table 1. Food and liquid intake were significantly decreased in high-fructose-fed rats, whereas total caloric intake was increased due to the additional calories from fructose. Dietary fructose caused a statistically significant increase in abdominal and epididymal adipose tissue weights, but not in final body weight. Pancreatic weight and body weight ratio did not change significantly between the groups. This dietary intervention elevated plasma triglyceride, VLDL, and total cholesterol levels, but not LDL levels in rats. The increase in plasma HDL levels was also considered.

TABLE 1 | Metabolic parameters of rats.

Parameters	Control	High-fructose
Initial body weight (g)	85.64 ± 3.47	87.64 ± 5.30
Final body weight (g)	349.4 ± 15.41	355.5 ± 14.90
Food intake (g/day)	19.87 ± 0.4	13.83 ± 0.28*
Liquid intake (mL/day)	43.21 ± 1.08	38.56 ± 0.39*
Fructose intake (g/day)	–	7.7 ± 0.1
Total caloric intake (kcal)	69.53 ± 1.41	77.72 ± 1.13*
Plasma triglyceride (mg/dL)	97.33 ± 4.34	269.8 ± 8.16*
Plasma VLDL (mg/dL)	19.47 ± 0.87	53.96 ± 1.63*
Plasma LDL (mg/dL)	23.50 ± 3.56	18.21 ± 0.83
Plasma HDL (mg/dL)	36.89 ± 2.70	50.86 ± 2.07*
Plasma total cholesterol (mg/dL)	31.25 ± 3.01	43.93 ± 2.05*
Abdominal adipose tissue weight (g)	2.4 ± 0.32	5.9 ± 0.47*
Abdominal adipose tissue weight to bw (g/100 g bw)	0.82 ± 0.11	1.77 ± 0.08*
Epididymal adipose tissue weight (g)	3.331 ± 0.20	6.302 ± 1.26*
Epididymal adipose tissue weight to bw (g/100 g bw)	1.105 ± 0.056	1.732 ± 0.21*
Pancreas weight (g)	0.962 ± 0.054	0.972 ± 0.037
Pancreas weight to body weight (g/100 g bw)	0.297 ± 0.015	0.300 ± 0.014

3.2 | The Findings of Histopathological Analysis

Routine histopathological examination of the pancreatic tissues of high-fructose-fed rats revealed intense degenerative changes in the nucleus, cytoplasm, and cell membrane. Dietary fructose was observed to cause the loss of cell boundaries, pale pink cytoplasm, and karyopyknosis. In addition, vascular changes such as hyperemia were observed in the high-fructose group but not in the control group. PAS staining was performed to detect glycogen deposits, showing a significant increase in the number of PAS-positive cells in the pancreatic tissue of fructose-fed rats. All histopathological findings are presented in Figure 1.

3.3 | The Changes in Plasma or Pancreatic Endocrine Parameters

Plasma glucose levels and endocrine parameters of the rats are shown in Figure 2. A high-fructose diet caused an elevation in plasma glucose and insulin levels as well as pancreatic insulin levels. Immunohistochemical examination demonstrated that excessive fructose intake leads to an increase in insulin expression in the pancreatic tissue, given a supply to plasma and pancreatic high levels of insulin. However, excessive fructose intake did not alter plasma glucagon levels.

3.4 | The Changes in GLUT2 Expression

High-fructose consumption markedly increased the immunostaining and protein expression of GLUT2 (glucose and fructose transporter) in the pancreas (Figure 3).

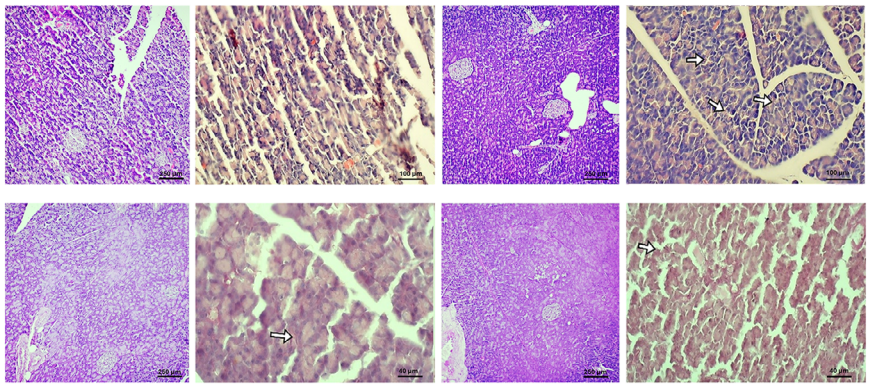
3.5 | The Changes in Cellular Stress Parameters

Cellular stress-associated parameters, such as GRP78, an ER stress marker, and PINK1, a mitophagy marker, were significantly increased in the pancreas of rats fed high-fructose diet (Figure 4).

3.6 | The Changes in Inflammatory Parameters

The changes in inflammatory parameters such as IL-6, NFκB, TNFα, IL-1β, and NLRP3 inflammasome components were determined in the pancreatic tissue of rats (Figure 5). In addition, systemic IL-6 and NFκB levels were measured in the plasma. Excessive fructose intake increased plasma levels of IL-6, but that of NFκB did not reach a statistical significance. Pancreatic IL-6, NFκB, TNFα, and IL-1β levels were elevated in high-fructose-fed rats. Dietary fructose caused an increase in NFκB and pNFκB protein expression in the pancreatic tissue but did not change the pNFκB/NFκB ratio. Supportively, excess fructose increased immunostaining of NFκB, an inflammatory factor and cellular stress marker, in the pancreas. High-fructose diet caused a significant enhancement in TNFα protein levels and expression in pancreatic tissue samples of rats. Regarding the NLRP3 inflammasome components, a high-fructose diet caused an upregulation of the protein expression of NLRP3, a sensor protein of the inflammasome, but did not alter the expression of ASC, an adaptor protein of the inflammasome. Additionally, this dietary intervention elevated the protein expression of pro-caspase-1 and cleaved-caspase-1, which are effector proteins of the inflammasome. In addition, high-fructose intake significantly increased levels of IL-1β and the protein expressions of pro-IL-1β and mature-IL-1β in the pancreatic tissue of rats.

a)



b)

Parameters		Control	High-Fructose
Degenerative changes	Membrane	2,77±2,77	42,84±6,14*
	Cytoplasm	2,77±2,77	42,84±4.95*
	Nucleus	0,0±0,0	26,18±4,95*
Vascular changes		0,0±0,0	12,21±5,28*
PAS-positivity		21,22±4,76	33,32±0,0*

FIGURE 1 | Effects of high-fructose diet on the pancreatic morphology of rats. H&E and PAS staining images (a) and histopathological degeneration scores (b) of the pancreas. Values are expressed as mean ± SEM, $n = 6–9$ rats. Statistical differences were analyzed using an unpaired Student's t test, with significance set at $^*p < 0.05$. Scale bar = 250, 100, and 40 μm. H&E, hematoxylin and eosin; PAS, periodic acid-Schiff; SEM, standard error of the mean.

3.7 | The Changes in the Mitogenic MAPK Pathway Elements

The changes in mitogenic pathway proteins, including ERK1/2, p38, JNK1, their phosphorylated forms, and Raf1, were examined following fructose exposure (Figure 6). Raf1, ERK1/2, and pERK1/2 expression levels in the pancreas were significant in fructose-fed rats. Additionally, the expression of other MAPKs, including p38, pp38, JNK1, and pJNK1, was significantly elevated in the pancreatic tissues. The ratios of pERK1/2 to ERK1/2 and pJNK1 to JNK1, but not pp38 to p38, were increased.

3.8 | The Changes in Apoptotic Parameters

The changes in apoptotic marker proteins such as Fas and p53, as well as caspase-8 and caspase-3 (initiator and effector caspases, respectively), were examined in fructose-fed rats (Figure 7). High dietary fructose intake caused a statistically significant increase in the protein expression of Fas and p53 in the pancreatic tissue of rats. In addition, this dietary intervention increased the expression of cleaved-caspase-8 and cleaved-caspase-3 (active proteins), but not that of pro-caspase-8 (an inactive form of caspase-8).

4 | Discussion

Excessive fructose intake, especially in children and young adults, has drawn attention as an important health issue. Currently, it has been confirmed that high-fructose consumption contributes to the global increase in the prevalence of metabolic disorders [27]. Numerous studies have demonstrated that a high-fructose diet causes various pathologies, such as abdominal obesity, insulin resistance, hypertriglyceridemia, and hepatic steatosis [2, 5, 28, 29]. However, there is a lack of research examining molecular mechanisms behind excess fructose-related changes in the pancreas, one of the most critical endocrine organs. This study on excess fructose intake displayed that hyperglycemia and hyperinsulinemia, due to increased expression of GLUT2 in association with cellular stress, inflammatory status, activates mitogenic ERK1/2, p38, and JNK1 MAPK pathways as well as apoptosis, thereby showing a metabolic adaptation to high-fructose conditions in the pancreas.

A fructose-rich diet caused abdominal and epididymal adiposity rather than general obesity, which is characteristic of this monosaccharide [5]. In similarity with previous study results, we have shown that high-fructose consumption of rats causes abdominal and epididymal adiposity together with hyperglycemia and hypertriglyceridemia, thus presenting the development of metabolic syndrome. Previously, it was reported that the metabolic disturbance due a high-fructose diet may lead to alterations in pancreatic morphology including the islet number and volume abnormalities [7]. We observed necrotic changes and membrane and cytoplasmic degeneration in the pancreas of rats fed high-fructose diet. In a further step, we investigated various cellular pathways at the molecular level to elucidate the mechanisms underlying these pancreatic morphological changes.

Pancreatic cells with high secretory activity possess large and well-developed ER. However, in hyperinsulinemia, increased secretory activity can overload the ER and lead to ER stress [8]. Accumulating evidence suggests that impaired ER homeostasis plays a role in the pathogenesis of Type 2 diabetes [8, 9]. Studies have shown that a high-fat, high-fructose diet increases the

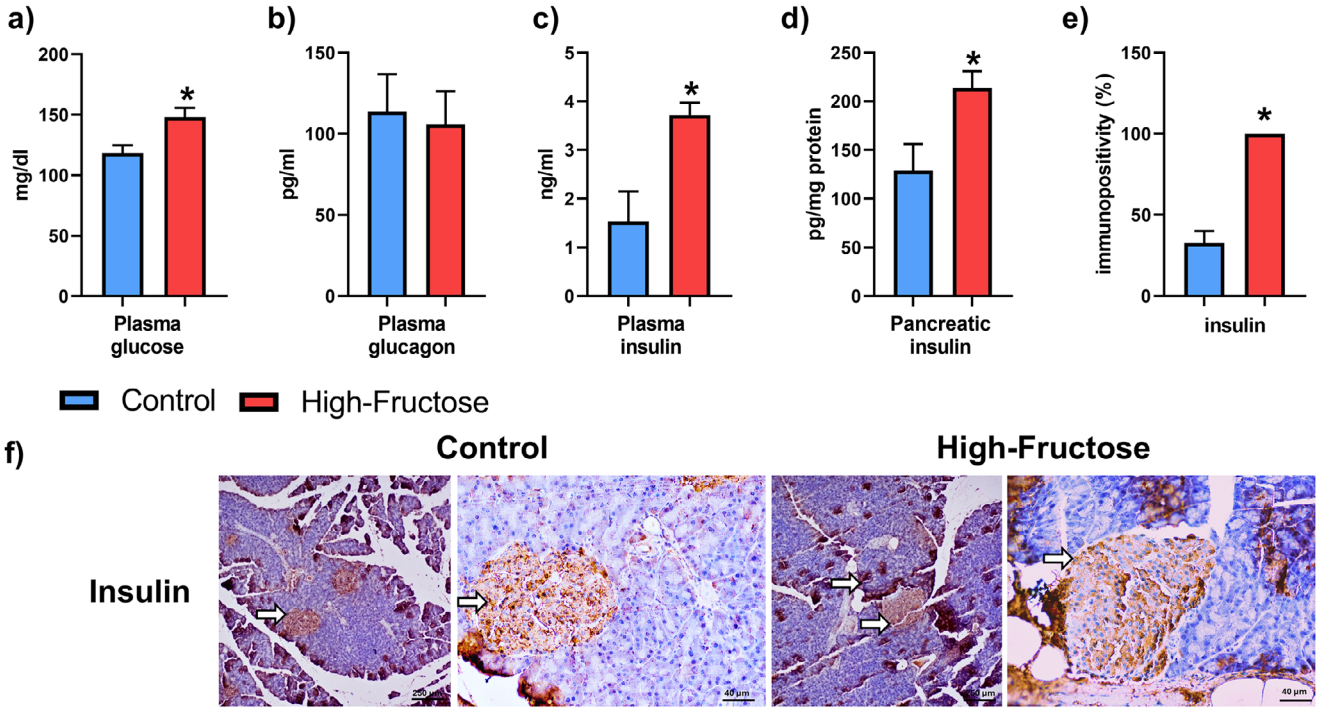


FIGURE 2 | Plasma levels of glucose (a), glucagon (b), and insulin (c) in rats. Pancreatic insulin levels (d), relative immunopositivity (e), and immunohistochemical staining (f) for insulin in rats. Values are expressed as mean ± SEM, $n = 6–9$ rats. Statistical differences were analyzed using an unpaired Student's t test, with significance set at $^*p < 0.05$. Scale bar = 250 and 40 μm. SEM, standard error of the mean.

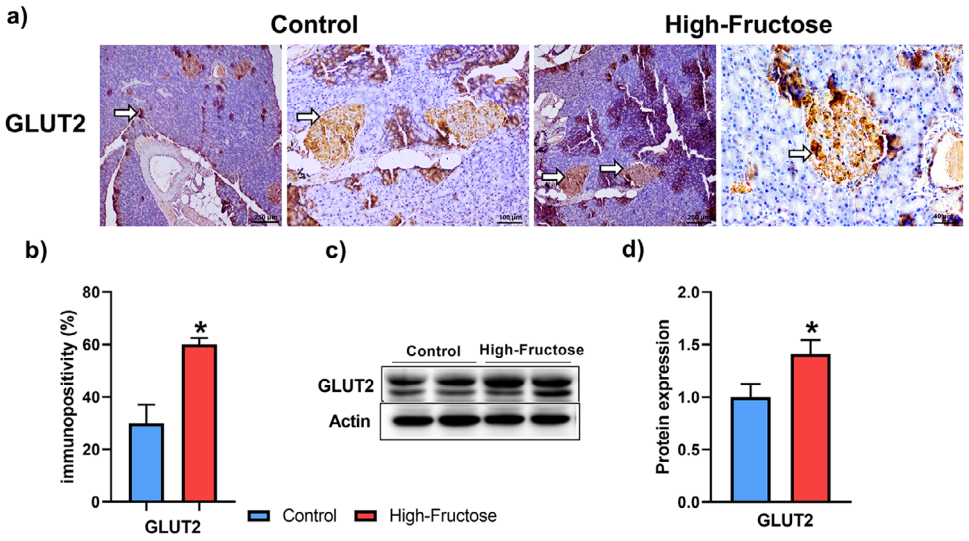


FIGURE 3 | Effects of high-fructose diet on GLUT2 expressions in the pancreas. Immunohistochemical staining (a), relative immunopositivity (b), representative Western blot bands (c), and relative protein expression (d) of GLUT2. Values are expressed as mean ± SEM, $n = 6–9$ rats. Statistical differences were analyzed using an unpaired Student's t test, with significance set at $^*p < 0.05$. Scale bar = 250, 100, and 40 μm. GLUT2, glucose transporter 2; SEM, standard error of the mean.

expression of GRP78 and other proteins associated with ER stress in the rodents pancreas [24, 30, 31]. In present study, we found that the expression of GRP78, which acts as an ER stress sensor, was elevated in high-fructose-induced pancreatic hyperinsulinemia. ER stress triggers the unfolded protein response (UPR), which aims to restore cellular homeostasis. The UPR pathway can activate several cellular prosses, including the NLRP3 inflammasome, IL-6, TNFα, NFκB, and mitogenic ERK1/2, p38, and JNK pathways [32–34]. Moreover, high secretory activity in pancreatic β-cells under hyperinsulinemic conditions leads to increased cellular energy utilization, thereby causing mitochondrial damage and mitochondrial dysfunction. PINK1/Parkin signaling in the cells controls mitochondrial function and repairs organelle damage [35]. Previous studies have shown that dietary fructose may alter mitochondrial energy balance [36–38]. In these studies, it was determined an impaired mitochondrial function; how-

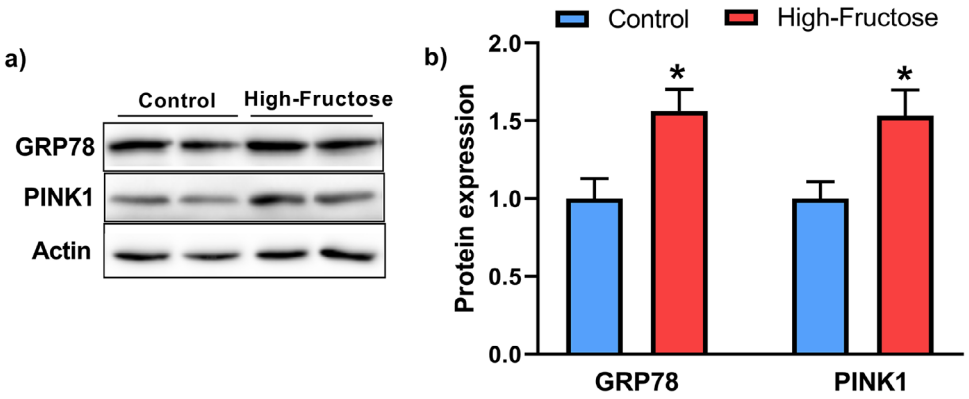


FIGURE 4 | Effects of high-fructose diet on cellular stress parameters in the pancreas. Representative Western blot bands (a) and relative protein expressions (b) of GRP78 and PINK1. Values are expressed as mean ± SEM, $n = 6–9$ rats. Statistical differences were analyzed using an unpaired Student's t test, with significance set at $^*p < 0.05$. GRP78, glucose-regulated protein-78; PINK1, PTEN-induced putative kinase 1; SEM, standard error of the mean.

ever, pancreatic mitophagy has not been investigated. Here, we demonstrated that the protein expression of PINK1, a mitophagy marker, was increased in the pancreatic tissue of fructose-fed rats. Thus, high-fructose diet may disrupt pancreatic cellular energy utilization and function by upregulating ER stress and mitophagy. This stressful condition may also provoke mitogenic and apoptotic processes in the pancreas.

A chronic inflammatory state is critical in the pathogenesis of metabolic diseases and diabetes. In this line, high levels of inflammatory factors such as IL-6, IL-1β, and TNFα were determined in the plasma of Type 2 diabetic patients [12]. Additionally, an increase in inflammatory cytokines and macrophages in pancreatic islets has been reported to contribute to β-cell destruction and dysfunction [39–41]. Evidence indicates that a high-fructose diet induces a systemic inflammatory state extending to various organs such as the liver, adipose tissue, testes, kidney, ileum, and heart [29, 42–49]. Histopathological examinations have demonstrated that high-fructose consumption causes inflammatory cell infiltration and tissue damage in the pancreas of rodents [24, 50]. Further, a high-fructose/high-fat diet has been shown to increase plasma IL-6, accompanied by decreased insulin secretion and impaired β-cell morphology in rats [51]. NFκB, an inflammatory factor, plays a key role in cellular response to various stimuli such as oxidative stress, inflammatory cytokines, and free radicals. NFκB activation is important in the pathogenesis of inflammation-related diseases, including diabetes [52]. Studies have shown that the protein expression of proinflammatory factors such as TNFα, IL-6, and NFκB is enhanced in the pancreatic tissue of diabetic rats [53, 54], and treatment with the oral antidiabetic drug metformin decreases these inflammatory factors in fructose- and streptozotocin-induced diabetic rats [54]. Consistent with these findings, we observed an elevation of pancreatic IL-6, TNFα, NFκB, pNFκB, and circulatory IL-6 in high-fructose-fed rats, thereby indicating an inflammatory burden on this endocrine organ. On the other hand, the activation of the NLRP3 inflammasome, a critical component of the immune system, can be triggered with various harmful conditions such as hyperglycemia, hypertriglyceridemia, and ER stress [9, 55]. The high-fructose-induced metabolic disturbance was also shown to induce NLRP3 inflammasome activation in metabolic organs, including the liver and adipose tissue [28, 56–58]. In this study, we identified an increase in NLRP3 as well as pro-caspase-1, cleaved-caspase-1, pro-IL-1β, and mature-IL-1β in the pancreas of high-fructose-fed rats. The results of our study suggest that excessive fructose intake triggers a complex inflammatory state via IL-6, NFκB, TNFα, and NLRP3 inflammasome components in the pancreas. Previous studies showed that inflammation accompanies the increase in ERK1/2, p38, and JNK expressions in different tissues such as kidney, liver, heart, and testis in high-fructose-fed rats [5]. Hence, the collective increase in these inflammatory parameters may trigger the activation of MAPK pathways in the pancreatic tissue of high-fructose-fed rats.

The MAPK pathways have been shown to be involved in numerous physiological and pathophysiological cellular processes. Besides, ERK1/2 is the major regulatory pathway in cell growth and development [18]. It has been shown that high glucose-induced activation of ERK1/2 via Raf1 leads to mitogenesis in pancreatic β-cells [59], while inhibition of the ERK signaling pathway reduces β-cell proliferation and mass [60]. Furthermore, studies have revealed that increased ERK1/2 activity causes systemic insulin resistance, because ERK1/2 knockdown in the liver of obese mice improves insulin resistance and glucose intolerance [61]. Similarly, we observed that Raf1, ERK1/2, and pERK1/2 protein expression levels increased in the pancreas of rats with high-fructose-induced hyperinsulinemia. The onset of insulin resistance, a sign of metabolic syndrome, leads to increased insulin production in response to decreased insulin utilization and efficiency in tissues [62]. Accordingly, pancreatic β-cells exhibit enhanced secretory activity, along with increased cell size and number [9]. In insulin resistance, glucose levels are high in the blood despite hyperinsulinemia. Concurrently, hyperglycemia upregulates the expression of glucose and fructose transporter GLUT2, thereby stimulating insulin secretion in β-cells [63]. In the present study, we found that pancreatic immunopositivity and protein expression of GLUT2 together with plasma levels of glucose and insulin are increased in response to a high-fructose intake, showing a consecutive status. In connection with these, pancreatic insulin expression and levels were also enhanced by this dietary intervention. However, the plasma glucagon level was not changed, which is consistent with previous studies [64–67]. Alternative studies have indicated that dietary interventions with high fat and/or fructose may alter

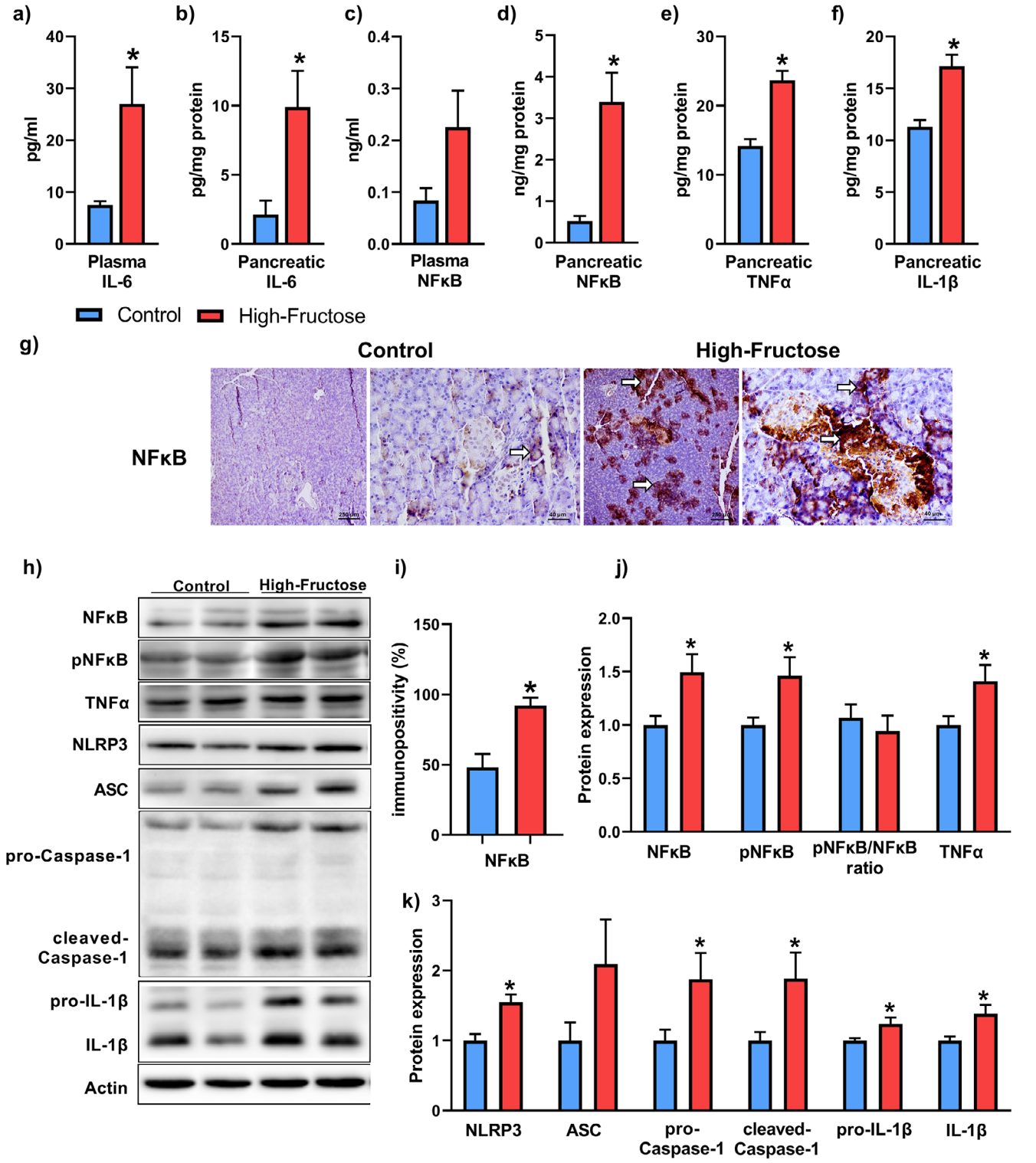


FIGURE 5 | Effects of high-fructose diet on inflammatory parameters. Plasma or pancreatic tissue levels of IL-6 (a, b), NFκB (c, d), TNFα (e), and IL-1β (f). Immunohistochemical staining (g) and relative immunopositivity (i) of NFκB in the pancreas. Representative Western blot bands (h) and relative protein expressions (j, k) of NFκB, pNFκB, TNFα, NLRP3, ASC, pro-caspase-1, claved-caspase-1, pro-IL-1β, and IL1-β. Values are expressed as mean ± SEM, $n = 6–9$ rats. Statistical differences were analyzed using an unpaired Student's t test, with significance set at $^*p < 0.05$. Scale bar = 250 and 40 μm. ASC, apoptosis-associated speck-like protein; IL, interleukin; NFκB, nuclear factor kappa B; NLRP3, Nod-like receptor protein 3; SEM, standard error of the mean; TNFα, tumor necrosis factor α.

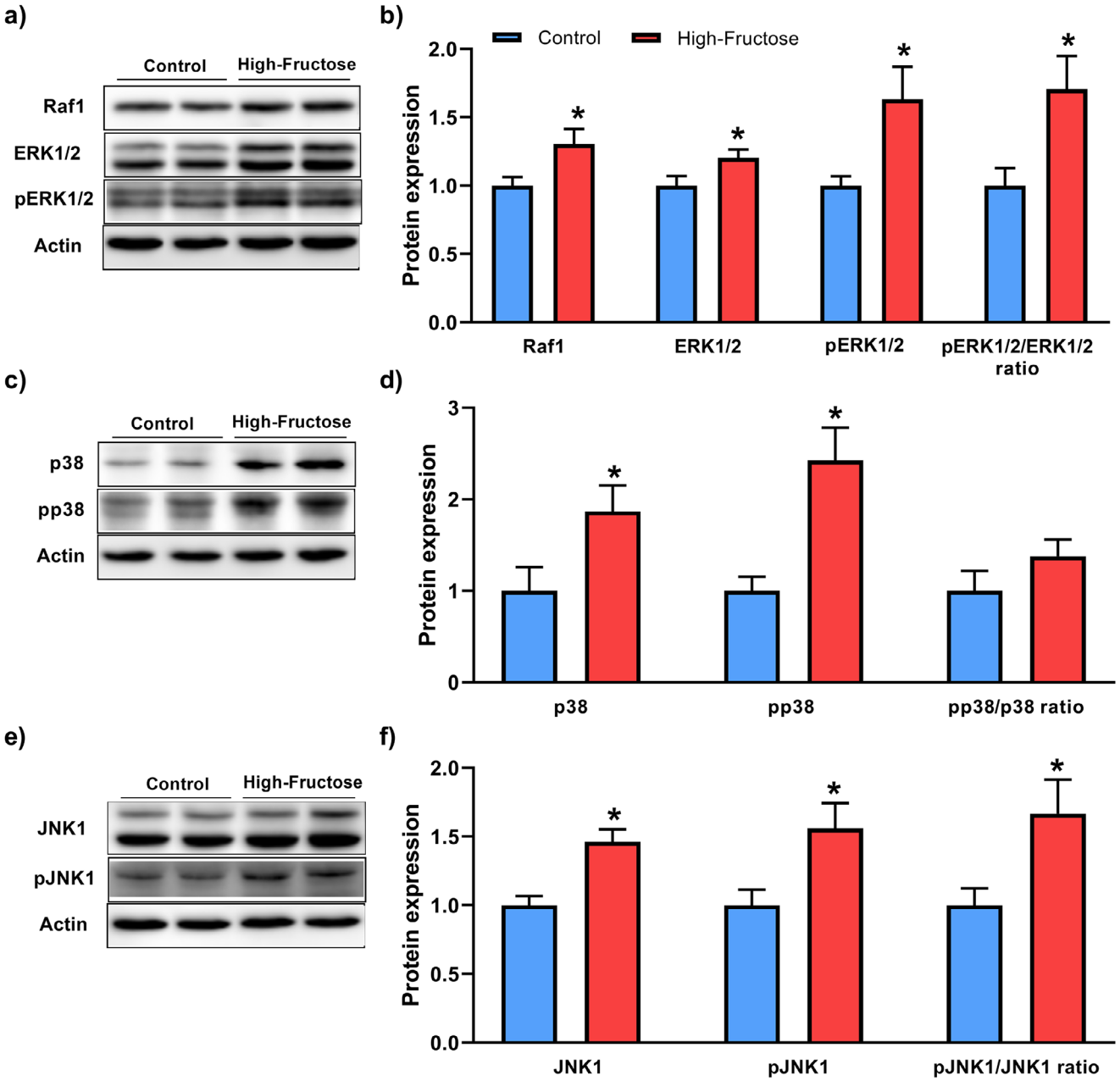


FIGURE 6 | Effects of high-fructose diet on mitogenic ERK1/2, p38, and JNK1 protein expressions in the pancreas. Representative Western blot bands (a, c, e) and relative protein expressions (b, d, f) of pancreatic Raf1, ERK1/2, pERK1/2, p38, pp38, JNK1, and pJNK1. Values are expressed as mean ± SEM, $n = 6–9$ rats. Statistical differences were analyzed using an unpaired Student's t test, with significance set at $^*p < 0.05$. ERK1/2, extracellular signal-regulated kinases 1/2; JNK, c-Jun N-terminal kinase; SEM, standard error of the mean.

pancreatic glucagon secretion implying its effects on glucose homeostasis and lipid metabolism [68, 69]. Further research is needed to elucidate the role of glucagon, a key endocrine hormone, in high-fructose-induced metabolic and pancreatic disturbances. Taken together, the present results demonstrate that high-fructose diet-induced hyperinsulinemia due to increased GLUT2 expression is associated with the upregulation of the Raf1, ERK1/2, and pERK1/2 pathways in pancreatic tissue showing an organ-related metabolic adaptation.

Stress-associated MAPKs, including p38 and JNK, play a role in cellular responses to metabolic stress, including inflammation, oxidative stress, and apoptosis, thus contributing to the pathogenesis of several diseases [18]. Activation of the p38 pathway has been implicated in the process of pancreatic β-cell destruction and inflammation in the diabetic state [18, 70]. Treatment with p38 inhibitors was shown to improve blood glucose levels and insulin resistance and also reduce pancreatic expression of ER stress and apoptosis-related factors in diabetic *db/db* mice [20]. In high-fat diet and streptozotocin-induced diabetic rats, inflammatory parameters and mitogenic p38 and JNK protein expressions were reported to increase in pancreatic tissue [71]. Furthermore, activation of the JNK pathway in diabetes was shown to induce an inflammatory state via IL-1β, NFκB, and other inflammatory factors, mediating functional impairments in β-cells [14]. In this study, we demonstrated that excess fructose intake caused

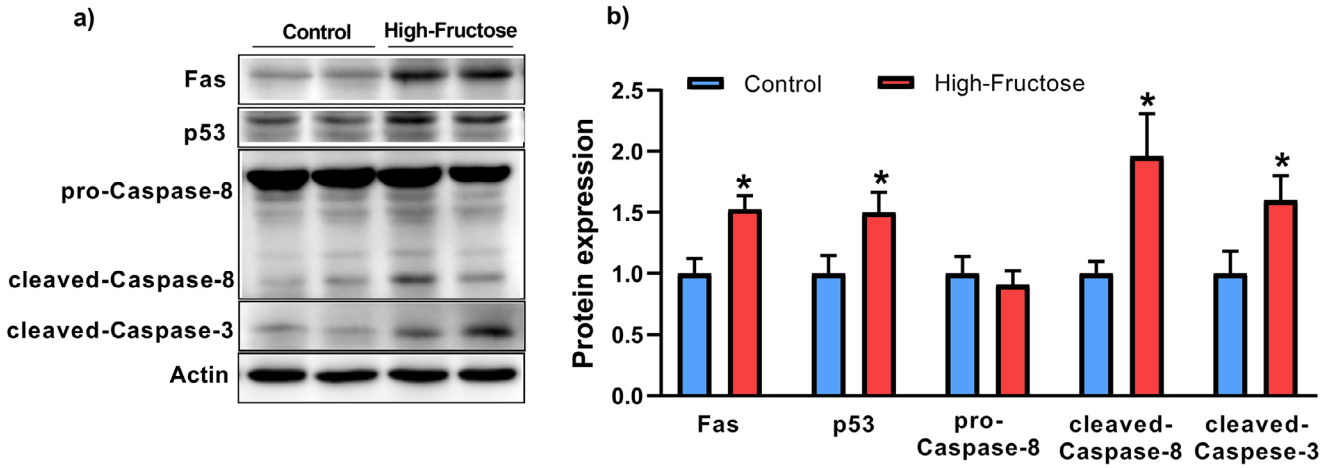


FIGURE 7 | Effects of high-fructose diet on apoptotic protein expressions in the pancreas. Representative Western blot bands (a) and relative protein expressions (b) of pancreatic Fas, p53, pro-caspase-8, cleaved-caspase-8, and cleaved-caspase-3. Values are expressed as mean ± SEM, $n = 6–9$ rats. Statistical differences were analyzed using an unpaired Student's t test, with significance set at $^*p < 0.05$. SEM, standard error of the mean.

an elevation of p38, pp38, JNK1, and pJNK1 expression in the pancreas in conjunction with ERK pathway activation and hyperinsulinemia. This upregulation of p38 and JNK signaling pathways in the pancreas may result from a complex stress response involving activation of GRP78, PINK1, IL-1β, IL-6, TNFα, NF-κB, and the NLRP3 inflammasome expression, ultimately contributing to β-cell damage and insulin resistance associated with high-fructose exposure.

Another mechanism involved in diabetes pathogenesis is β-cell failure due to increased apoptotic activity. An IL-1β-mediated increase in NFκB and Fas activation has been shown to induce the extrinsic apoptotic pathway and contribute to β-cell dysfunction [72]. Similarly, pancreatic islets isolated from patients with Type 2 diabetes exhibit increased apoptotic caspase-8 and caspase-3 activity, along with reduced insulin content [73]. In rodents fed a high-fat diet and streptozotocin, p53-mediated apoptotic activity and caspase-3 levels are elevated in the pancreas during late stages [74]. Moreover, it has been reported that the increased expression of JNK leads to the activation of apoptotic caspase-3 and destruction of pancreatic islets of rats fed high-fructose/high-fat diet [24]. In this study, we demonstrated that the expression of apoptotic response elements consisting of p53, Fas, caspase-8, and caspase-3 was increased in the pancreas of high-fructose diet-fed rats. Thus, excessive fructose intake appears to provoke apoptotic pathway in the pancreas. Herein, despite ongoing increased apoptotic activity, presentation of hyperinsulinemia and upregulation of the mitogenic pathway should be considered as an outcome. Prolonged excess fructose intake could potentially lead to a reduction in insulin content owing to increased apoptotic activity, as a hyperinsulinemic state was observed in the 15-week fructose diet model.

5 | Conclusion

The present study demonstrated that a high-fructose diet activated the pancreatic mitogenic ERK1/2, p38, and JNK1 pathways in association with metabolic disturbances, comprising hyperglycemia, hyperinsulinemia, hypertriglyceridemia, and abdominal and epididymal adiposity. Herein, excessive fructose-induced hyperglycemia and hyperinsulinemia due to the upregulation of GLUT2 together with cellular stress and inflammatory markers may promote the activation of MAPK and apoptotic pathway. Increased mitogenesis in the pancreas, despite ongoing structural destruction, is reflected by the overproduction of insulin, indicating the early stage of Type 2 diabetes. Pancreatic tissue may be the first hit site by dietary high-fructose, and other pathologies may be succeeded by hyperinsulinemia. Our study presents notable results regarding the molecular mechanisms of fructose-rich diet-induced changes in the pancreas, which can be extended to treatment strategies. Moreover, these findings are valuable in addressing high-fructose intake as the cause of diabetic metabolic diseases in the development of public health policy.

Author Contributions

CG: Investigation, visualization, formal analysis, drafting of manuscript, editing and revising of manuscript; **MEA**: Investigation, visualization, formal analysis; **FA**: Supervision, project administration, methodology, editing and revising of manuscript. All authors read and approved of the final manuscript.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Peer Review

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References

1. S. Weihrauch-Bluher, P. Schwarz, and J. H. Klusmann, "Childhood Obesity: Increased Risk for Cardiometabolic Disease and Cancer in Adulthood," *Metabolism* 92 (2019): 147–152.

2. S. Jung, H. Bae, W. S. Song, and C. Jang, "Dietary Fructose and Fructose-Induced Pathologies," *Annual Review of Nutrition* 42 (2022): 45–66.

3. J. K. DiStefano and G. Q. Shaibi, "The Relationship Between Excessive Dietary Fructose Consumption and Paediatric Fatty Liver Disease," *Pediatric Obesity* 16 (2021): 12759.

4. D. F. Lelis, J. M. O. Andrade, C. C. P. Almenara, G. B. Broseguini-Filho, J. G. Mill, and M. P. Baldo, "High Fructose Intake and the Route Towards Cardiometabolic Diseases," *Life Sciences* 259 (2020): 118235.

5. C. Guney, N. B. Bal, and F. Akar, "The Impact of Dietary Fructose on Gut Permeability, Microbiota, Abdominal Adiposity, Insulin Signaling and Reproductive Function," *Heliyon* 9 (2023): 18896.

6. S. Softic, K. L. Stanhope, J. Boucher, et al., "Fructose and Hepatic Insulin Resistance," *Critical Reviews in Clinical Laboratory Sciences* 57 (2020): 308–322.

7. C. Guney and F. Akar, "The Possible Mechanisms of High-Fructose Diet-Induced Pancreatic Disturbances," *Journal of Research in Pharmacy* 27 (2023): 753–761.

8. E. Burgos-Moron, Z. Abad-Jimenez, A. M. Maranon, et al., "Relationship Between Oxidative Stress, ER Stress, and Inflammation in Type 2 Diabetes: The Battle Continues," *Journal of Clinical Medicine* (2019): 8.

9. J. Yong, J. D. Johnson, P. Arvan, J. Han, and R. J. Kaufman, "Therapeutic Opportunities for Pancreatic β-Cell ER Stress in Diabetes Mellitus," *Nature Reviews Endocrinology* 17 (2021): 455–467.

10. D. R. Laybutt, A. M. Preston, M. C. Akerfeldt, et al., "Endoplasmic Reticulum Stress Contributes to Beta Cell Apoptosis in Type 2 Diabetes," *Diabetologia* 50 (2007): 752–763.

11. G. S. Hotamisligil, "Inflammation and Metabolic Disorders," *Nature* 444 (2006): 860–867.

12. J. Spranger, A. Kroke, M. Mohlig, et al., "Inflammatory Cytokines and the Risk to Develop Type 2 Diabetes," *Diabetes* 52 (2003): 812–817.

13. M. Boni-Schnetzler, J. Thorne, G. Parnaud, et al., "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," *Journal of Clinical Endocrinology and Metabolism* 93 (2008): 4065–4074.

14. M. Banerjee and M. Saxena, "Interleukin-1 (IL-1) Family of Cytokines: Role in Type 2 Diabetes," *Clinica Chimica Acta* 413 (2012): 1163–1170.

15. D. Sharma and T. D. Kanneganti, "The Cell Biology of Inflammasomes: Mechanisms of Inflammasome Activation and Regulation," *Journal of Cell Biology* 213 (2016): 617–629.

16. M. Sokolova, A. Sahraoui, M. Hoyem, et al., "NLRP3 Inflammasome Mediates Oxidative Stress-Induced Pancreatic Islet Dysfunction," *American Journal of Physiology. Endocrinology and Metabolism* 315 (2018): E912–E923.

17. C. Westwell-Roper, D. Nackiewicz, M. Dan, and J. A. Ehses, "Toll-Like Receptors and NLRP3 as Central Regulators of Pancreatic Islet Inflammation in Type 2 Diabetes," *Immunology and Cell Biology* 92 (2014): 314–323.

18. T. Kassouf and G. Sumara, "Impact of Conventional and Atypical MAPKs on the Development of Metabolic Diseases" *Biomolecules* (2020): 10.

19. E. Manieri and G. Sabio, "Stress Kinases in the Modulation of Metabolism and Energy Balance," *Journal of Molecular Endocrinology* 55 (2015): R11–22.

20. X. Wei, N. Gu, N. Feng, X. Guo, and X. Ma, "Inhibition of p38 Mitogen-Activated Protein Kinase Exerts a Hypoglycemic Effect by Improving β Cell Function via Inhibition of β Cell Apoptosis in db/db Mice," *Journal of Enzyme Inhibition and Medicinal Chemistry* 33 (2018): 1494–1500.

21. J. Hirosumi, G. Tuncman, L. Chang, et al., "A Central Role for JNK in Obesity and Insulin Resistance," *Nature* 420 (2002): 333–336.

22. L. Demirtas, A. Guclu, F. M. Erdur, et al., "Apoptosis, Autophagy & Endoplasmic Reticulum Stress in Diabetes Mellitus," *Indian Journal of Medical Research* 144 (2016): 515–524.

23. S. You, J. Zheng, Y. Chen, and H. Huang, "Research Progress on the Mechanism of Beta-Cell Apoptosis in Type 2 Diabetes Mellitus," *Frontiers in Endocrinology* 13 (2022): 976465.

24. M. Balakumar, L. Raji, D. Prabhu, et al., "High-Fructose Diet Is as Detrimental as High-Fat Diet in the Induction of Insulin Resistance and Diabetes Mediated by Hepatic/Pancreatic Endoplasmic Reticulum (ER) Stress," *Molecular and Cellular Biochemistry* 423 (2016): 93–104.

25. L. G. Luna, *Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology*, 3rd ed. (Blakiston Division, 1968).

26. O. H. Lowry, N. J. Rosebrough, A. L. Farr, and R. J. Randall, "Protein Measurement With the Folin Phenol Reagent," *Journal of Biological Chemistry* 193 (1951): 265–275.

27. D. M. Sigala, B. Hieronimus, V. Medici, et al., "Consuming Sucrose- or HFCS-Sweetened Beverages Increases Hepatic Lipid and Decreases Insulin Sensitivity in Adults," *The Journal of Clinical Endocrinology and Metabolism* 106 (2021): 3248–3264.

28. S. Singh, A. Sharma, S. Ahmad, et al., "Convergence of Fructose-Induced NLRP3 Activation With Oxidative Stress and ER Stress Leading to Hepatic Steatosis," *Inflammation* 46 (2023): 217–233.

29. F. Akar, E. Sumlu, M. E. Alcigir, A. Bostanci, and G. Sadi, "Potential Mechanistic Pathways Underlying Intestinal and Hepatic Effects of Kefir in High-Fructose-Fed Rats," *Food Research International* 143 (2021): 110287.

30. K. Kalpana, E. Priyadarshini, S. Sreeja, K. Jagan, and C. V. Anuradha, "Scopoletin Intervention in Pancreatic Endoplasmic Reticulum Stress Induced by Lipotoxicity," *Cell Stress & Chaperones* 23 (2018): 857–869.

31. M. S. Izadi, F. Eskandari, F. Binayi, et al., "Oxidative and Endoplasmic Reticulum Stress Develop Adverse Metabolic Effects Due to the High-Fat High-Fructose Diet Consumption From Birth to Young Adulthood," *Life Sciences* 309 (2022): 120924.

32. A. Fernandes-da-Silva, C. S. Miranda, D. A. Santana-Oliveira, et al., "Endoplasmic Reticulum Stress as the Basis of Obesity and Metabolic Diseases: Focus on Adipose Tissue, Liver, and Pancreas," *European Journal of Nutrition* 60 (2021): 2949–2960.

33. K. Zhang and R. J. Kaufman, "From Endoplasmic-Reticulum Stress to the Inflammatory Response," *Nature* 454 (2008): 455–462.

34. W. Li, T. Cao, C. Luo, et al., "Crosstalk Between ER Stress, NLRP3 Inflammasome, and Inflammation," *Applied Microbiology and Biotechnology* 104 (2020): 6129–6140.

35. W. X. Ding and X. M. Yin, "Mitophagy: Mechanisms, Pathophysiological Roles, and Analysis," *Biological Chemistry* 393 (2012): 547–564.

36. M. M. Dethlefsen, C. M. Kristensen, A. S. Tondering, S. B. Lassen, S. Ringholm, and H. Pilegaard, "Impact of Liver PGC-1α on Exercise and Exercise Training-Induced Regulation of Hepatic Autophagy and Mitophagy in Mice on HFF," *Physiological Reports* 6 (2018): 13731.

37. S. Softic, J. G. Meyer, G. X. Wang, et al., "Dietary Sugars Alter Hepatic Fatty Acid Oxidation via Transcriptional and Post-Translational Modifications of Mitochondrial Proteins," *Cell Metabolism* 30 (2019): 735–753.e4.

38. E. Grasselli, F. Baldini, G. Vecchione, et al., "Excess Fructose and Fatty Acids Trigger a Model of Non-Alcoholic Fatty Liver Disease Progression In Vitro: Protective Effect of the Flavonoid Silybin" *International Journal of Molecular Medicine* 44 (2019): 705–712.

39. W. Ying, Y. S. Lee, Y. Dong, et al., "Expansion of Islet-Resident Macrophages Leads to Inflammation Affecting β Cell Proliferation and Function in Obesity," *Cell Metabolism* 29 (2019): 457–474.e5.

40. S. J. Richardson, A. Willcox, A. J. Bone, A. K. Foulis, and N. G. Morgan, "Islet-Associated Macrophages in Type 2 Diabetes," *Diabetologia* 52 (2009): 1686–1688.

41. M. J. Butcher, D. Hallinger, E. Garcia, et al., "Association of Proinflammatory Cytokines and Islet Resident Leucocytes With Islet Dysfunction in Type 2 Diabetes," *Diabetologia* 57 (2014): 491–501.

42. O. A. Korkmaz, E. Sumlu, H. B. Koca, et al., "Effects of Lactobacillus Plantarum and Lactobacillus Helveticus on Renal Insulin Signaling, Inflammatory Markers, and Glucose Transporters in High-Fructose-Fed Rats" *Medicina (Kaunas, Lithuania)* (2019): 55.

43. S. Kovacevic, J. Brkljacic, D. Vojnovic Milutinovic, et al., "Fructose Induces Visceral Adipose Tissue Inflammation and Insulin Resistance Even Without Development of Obesity in Adult Female but Not in Male Rats," *Frontiers in Nutrition* 8 (2021): 749328.

44. N. Maithilikarpagaselvi, M. G. Sridhar, R. P. Swaminathan, and B. Zachariah, "Curcumin Prevents Inflammatory Response, Oxidative Stress and Insulin Resistance in High Fructose Fed Male Wistar Rats: Potential Role of Serine Kinases," *Chemico-Biological Interactions* 244 (2016): 187–194.

45. M. B. Pektas, H. B. Koca, G. Sadi, and F. Akar, "Dietary Fructose Activates Insulin Signaling and Inflammation in Adipose Tissue: Modulatory Role of Resveratrol," *BioMed Research International* 2016 (2016): 8014252.

46. M. B. Pektas, G. Yucel, H. B. Koca, et al., "Dietary Fructose-Induced Hepatic Injury in Male and Female Rats: Influence of Resveratrol" *Drug Research (Stuttgart, Ger)* 67 (2017): 103–110.

47. O. G. Yildirim, E. Sumlu, E. Aslan, et al., "High-Fructose in Drinking Water Initiates Activation of Inflammatory Cytokines and Testicular Degeneration in Rat," *Toxicology Mechanisms and Methods* 29 (2019): 224–232.

48. D. M. Zhang, R. Q. Jiao, and L. D. Kong, "High Dietary Fructose: Direct or Indirect Dangerous Factors Disturbing Tissue and Organ Functions" *Nutrients* (2017): 9.

49. N. B. Bal, C. Guney, O. G. Yildirim, F. Akar, and E. Demirel-Yilmaz, "Myricetin May Improve Cardiac Dysfunction Possibly Through Regulating Blood Pressure and Cellular Stress Molecules in High-Fructose-Fed Rats" *Anatolian Journal of Cardiology* 28 (2024): 55–64.

50. Y. Wang, W. Qi, G. Song, et al., "High-Fructose Diet Increases Inflammatory Cytokines and Alters Gut Microbiota Composition in Rats" *Mediators Inflammation* 2020 (2020): 6672636.

51. Y. Zhao, Q. Y. Wang, L. T. Zeng, et al., "Long-Term High-Fat High-Fructose Diet Induces Type 2 Diabetes in Rats through Oxidative Stress" *Nutrients* (2022): 14.

52. S. Patel and D. Santani, "Role of NF-κB in the Pathogenesis of Diabetes and Its Associated Complications," *Pharmacological Reports* 61 (2009): 595–603.

53. M. Jayachandran, R. Vinayagam, R. R. Ambati, B. Xu, and S. S. M. Chung, "Guava Leaf Extract Diminishes Hyperglycemia and Oxidative Stress, Prevents β-Cell Death, Inhibits Inflammation, and Regulates NF-kB Signaling Pathway in STZ Induced Diabetic Rats," *BioMed Research International* 2018 (2018): 4601649.

54. T. O. Obafemi, K. F. Jaiyesimi, A. A. Olomola, et al., "Combined Effect of Metformin and Gallic Acid on Inflammation, Antioxidant Status, Endoplasmic Reticulum (ER) Stress and Glucose Metabolism in Fructose-Fed Streptozotocin-Induced Diabetic Rats," *Toxicology Reports* 8 (2021): 1419–1427.

55. I. M. Nitulescu, G. Ciulei, A. Cozma, L. M. Procopciuc, and O. H. Orasan, "From Innate Immunity to Metabolic Disorder: A Review of the NLRP3 Inflammasome in Diabetes Mellitus" *Journal of Clinical Medicine* (2023): 12.

56. S. Deng, Y. Ge, Z. Zhai, et al., "Fructose Induces Hepatic Steatosis in Adolescent Mice Linked to the Disorders of Lipid Metabolism, Bile Acid Metabolism, and Autophagy," *Journal of Nutritional Biochemistry* 129 (2024): 109635.

57. R. C. Rai, P. K. Bagul, and S. K. Banerjee, "NLRP3 Inflammasome Drives Inflammation in High Fructose Fed Diabetic Rat Liver: Effect of Resveratrol and Metformin," *Life Sciences* 253 (2020): 117727.

58. S. Singh, A. Sharma, B. Guru, et al., "Fructose-Mediated NLRP3 Activation Induces Inflammation and Lipogenesis in Adipose Tissue," *Journal of Nutritional Biochemistry* 107 (2022): 109080.

59. I. Briaud, M. K. Lingohr, L. M. Dickson, C. E. Wrede, and C. J. Rhodes, "Differential Activation Mechanisms of Erk-1/2 and p70S6K by Glucose in Pancreatic β-Cells," *Diabetes* 52 (2003): 974–983.

60. Y. M. Ikushima, M. Awazawa, N. Kobayashi, et al., "MEK/ERK Signaling in β-Cells Bifunctionally Regulates β-Cell Mass and Glucose-Stimulated Insulin Secretion Response to Maintain Glucose Homeostasis," *Diabetes* 70 (2021): 1519–1535.

61. P. Jiao, B. Feng, Y. Li, Q. He, and H. Xu, "Hepatic ERK Activity Plays a Role in Energy Metabolism," *Molecular and Cellular Endocrinology* 375 (2013): 157–166.

62. M. C. Petersen and G. I. Shulman, "Mechanisms of Insulin Action and Insulin Resistance," *Physiological Reviews* 98 (2018): 2133–2223.

63. K. S. Polonsky, B. D. Given, and E. Van Cauter, "Twenty-Four-Hour Profiles and Pulsatile Patterns of Insulin Secretion in Normal and Obese Subjects," *Journal of Clinical Investigation* 81 (1988): 442–448.

64. F. G. Debray, K. Seyssel, M. Fadeur, L. Tappy, N. Paquot, and C. Tran, "Effect of a High Fructose Diet on Metabolic Parameters in Carriers for Hereditary Fructose Intolerance," *Clinical Nutrition* 40 (2021): 4246–4254.

65. K. A. Le, D. Faeh, R. Stettler, et al., "A 4-wk High-Fructose Diet Alters Lipid Metabolism Without Affecting Insulin Sensitivity or Ectopic Lipids in Healthy Humans," *American Journal of Clinical Nutrition* 84 (2006): 1374–1379.

66. K. C. Coate, M. Scott, B. Farmer, et al., "Chronic Consumption of a High-Fat/High-Fructose Diet Renders the Liver Incapable of Net Hepatic Glucose Uptake," *American Journal of Physiology. Endocrinology and Metabolism* 299 (2010): E887–898.

67. K. C. Coate, G. Kraft, M. Lautz, M. Smith, D. W. Neal, and A. D. Cherrington, "A High-Fat, High-Fructose Diet Accelerates Nutrient Absorption and Impairs Net Hepatic Glucose Uptake in Response to a Mixed Meal in Partially Pancreatectomized Dogs," *Journal of Nutrition* 141 (2011): 1643–1651.

68. Z. A. Asghar, A. Cusumano, Z. Yan, M. S. Remedi, and K. H. Moley, "Reduced Islet Function Contributes to Impaired Glucose Homeostasis in Fructose-Fed Mice," *American Journal of Physiology. Endocrinology and Metabolism* 312 (2017): E109–E116.

69. M. Paunovic, M. Milosevic, O. Mitrovic-Ajtic, et al., "Polyphenol-Rich Black Currant and Cornelian Cherry Juices Ameliorate Metabolic Syndrome Induced by a High-Fat High-Fructose Diet in Wistar Rats," *Heliyon* 10 (2024): 27709.

70. X. Yu, L. Cui, F. Hou, et al., "Angiotensin-Converting Enzyme 2-Angiotensin (1-7)-Mas Axis Prevents Pancreatic Acinar Cell Inflammatory Response Via Inhibition of the p38 Mitogen-Activated Protein Kinase/Nuclear Factor-κB Pathway" *International Journal of Molecular Medicine* 41 (2018): 409–420.

71. J. Govindaraj and S. Sorimuthu Pillai, "Rosmarinic Acid Modulates the Antioxidant Status and Protects Pancreatic Tissues From Glucolipotoxicity Mediated Oxidative Stress in High-Fat Diet: Streptozotocin-Induced Diabetic Rats," *Molecular and Cellular Biochemistry* 404 (2015): 143–159.

72. K. Maedler, P. Sergeev, F. Ris, et al., "Glucose-Induced β Cell Production of IL-1β Contributes to Glucotoxicity in Human Pancreatic Islets," *Journal of Clinical Investigation* 110 (2002): 851–860.

73. P. Marchetti, S. Del Guerra, L. Marselli, et al., "Pancreatic Islets From Type 2 Diabetic Patients Have Functional Defects and Increased Apoptosis That Are Ameliorated by Metformin," *Journal of Clinical Endocrinology and Metabolism* 89 (2004): 5535–5541.

74. M. R. Alipour, R. Naderi, A. Alihemmati, R. Sheervalilou, and R. Ghiasi, "Swimming Training Attenuates Pancreatic Apoptosis Through miR-34a/Sirtu in1/P53 Axis in High-Fat Diet and Streptozotocin-Induced Type-2 Diabetic Rats," *Journal of Diabetes & Metabolic Disorders* 19 (2020): 1439–1446.

Supporting Information

Additional supporting information can be found online in the Supporting Information section.