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Original Article

Long-term consumption of high-fat fructose diet increased the pancreatic-derived factor level and impaired glucose and lipid metabolisms in male rats

Mina Sadat Izadi[1, 2, 3], Farzaneh Eskandari[2, 3], Homeira Zardooz[2, 3*]

1. Student Research Committee, Department of physiology, Shahid Beheshti University of Medical Sciences, Tehran, Iran
2. Department of Physiology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran
3. Neurophysiology Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran

ABSTRACT

Introduction: High-fat fructose diet (HFFr) consumption leads to inflammatory response and adverse metabolic consequences. Evaluating the changes in pancreatic-derived factor (PANDER) as one of the inflammatory metabolites, due to its regulatory role in glucose metabolism, could provide insight into the glucose homeostasis mechanisms.

Methods: Dams and their pups were randomly assigned to a standard diet (StD) and HFFr groups. After weaning, the male Wistar offspring were allocated to StD, StD-DMSO (Vehicle: V), StD-4-phenyl butyric acid (Drug: D), HFFrD, HFFrD-V and HFFrD-D groups, while they were on their diet for five weeks and treated for ten days. At the end of the procedure, the plasma glucose, insulin and PANDER levels, atherogenic indices and pancreatic PANDER content were determined.

Results: HFFrD intake increased plasma glucose level and homeostasis model assessment of insulin resistance. Also, atherogenic indices were elevated through the increase of cholesterol and low-density lipoprotein and the decrease of high-density lipoprotein (HDL) in HFFrD group. An increase in both plasma and pancreatic PANDER levels was observed in the HFFrD group. The drug decreased the plasma and pancreatic PANDER levels along with plasma glucose, cholesterol and the ratio of cholesterol to HDL levels in the HFFrD group.

Conclusion: Since long-term HFFrD intake led to hyperglycemia, insulin resistance and dyslipidemia with an increase in plasma and pancreatic PANDER levels, and on the other hand, 4-phenyl butyric acid administration decreased PANDER levels as well as plasma glucose and cholesterol concentrations, PANDER increment may be the cause of glucose and lipid disturbances.

Keywords:
High-fat-fructose diet
Pancreatic-derived factor
Glucose homeostasis
4-Phenylbutyric acid

* Corresponding author: Homeira Zardooz, homeira_zardooz@sbmu.ac.ir
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Introduction

The nowadays pattern of food consumption is towards consuming high-calorie and palatable foods in advanced societies. Meanwhile, increasing the consumption of a high-fat-fructose diet (HFFrD) is prevalent (Feillet-Coudray et al., 2019). The inflammatory response is one of the consequences of HFFrD consumption, which could be the cause of various metabolic diseases (Wang et al., 2020). In this context, HFFrD consumption increases intestinal permeability to microbial products and increases intestinal secretion of pro-inflammatory cytokines, all of which are transported into the systemic circulation (Gupta et al., 2020). In addition, high-fructose consumption depletes hepatic ATP stores because it increases fructose-metabolizing enzymes, which causes abnormal production of inflammatory cytokines (Zhang et al., 2017). In this regard, high-fructose feeding for eight weeks activates nuclear factor-kappa beta signaling in the liver, which produces various inflammatory cytokines (Wang et al., 2013). In addition, a high-fat diet causes systemic inflammation by increasing inflammatory mediators and free fatty acids (FFAs) (Duan et al., 2018). In this context, pancreatic-derived factor (PANDER) is one of the cytokines of pancreatic β-cells (Cieślak et al., 2015; Wilson et al., 2011) that is secreted through consuming a high-fat diet (Robert-Cooperman et al., 2011). Studies have shown that PANDER is localized with insulin in granules and released when blood glucose levels increased (Wang et al., 2008; Wilson et al., 2011). Accordingly, it has been determined that blood glucose levels and high-fat dietary FFAs are regulatory factors that determine the plasma concentration of PANDER (Wilson et al., 2011). In this regard, PANDER knockout mice have decreased fasting insulin levels and in vivo insulin secretion in response to glucose injection (Robert-Cooperman et al., 2011). Notably, PANDER can participate in pancreatic islet apoptosis through the activation of cyclin-dependent kinase inhibitor 1A (p21) and the caspase-3 pathway (Wilson et al., 2011). As the modern lifestyle persuades people to consume high-calorie fast foods (i.e. HFFrD), understanding the function of PANDER, according to its glucose metabolism regulatory role, can provide insight into the mechanisms of glucose homeostasis and potentially be a way to treat diabetes and metabolic syndrome. Therefore, this study aimed to investigate the long-term HFFrD consumption effects on PANDER changes in plasma and pancreatic tissue. It is worth mentioning that 4-phenyl butyric acid (4-PBA) attenuates arthritis and joint swelling by reducing inflammatory cytokines (Choi et al., 2021). Therefore, the effect of 4-PBA in reducing the possible increase of PANDER, as an inflammatory cytokine after HFFrD consumption, was evaluated.

Material and methods

Animals

A total of four male and eight female Wistar rats, which were housed in a controlled environment with *ad libitum* access to a standard diet (StD) and water, were randomly mated. Then, the pregnant rats were kept separately. On the birthday, the dams with pups were randomly assigned to the StD and HFFrD groups (8 litters and 4-6 litter sizes were used in this study). At the end of the weaning, the male offspring were divided into: StD, StD- dimethyl sulfoxide (DMSO) (Vehicle: V), StD-4-phenyl butyric acid (#P21005, Sigma, Germany) (Drug: D), HFFrD, HFFrD-V and HFFrD-D groups (n=6 rats/group). The diet continued for five weeks. In the end, the drug (50mg/kg) was administrated intraperitoneally (IP) for ten days (Figure 1). The HFFrD was freshly elaborated in the laboratory (Binayi et al., 2023; Mamikutty et al., 2014) (Table 1). All experimental procedures were based on the guidelines for the care and use of laboratory animals (National Institutes Of Health Publication No. 80-23, revised 1996) and were approved by the Research and Ethics Committee of Shahid Beheshti University of Medical Sciences, Tehran, Iran (IR.SBMU.RETECH.REC.1400.963).

Blood sampling

At the end of the procedure (after 16h of fasting), rats were immediately (after isoflurane inhalation) decapitated and the trunk blood was collected in heparinized Eppendorf tube (10µl per 1ml of blood; 5000IU/ml, Iran), to survey the plasma glucose, insulin, triglyceride, cholesterol, low-density lipoprotein (LDL), high-density lipoprotein (HDL) and PANDER concentrations (6 rats/group, 6 litters/group).

Biochemical analyses

Plasma concentration of glucose was determined using a colorimetric enzymatic kit (Parsazmoon, Iran; sensitivity: 1mg/dl, intra-assay coefficient of variations: 1.74%). The rat enzyme-linked immunosorbent assay

TABLE 1: Compositions of diets (A) percentage and types of fatty acids in cow butter (B).

A

Compositions	Standard diet g% (w/w)	High-fat-fructose diet g% (w/w)
Protein	23	19.76
Carbohydrate	57.5	39.62
Soybean oil	4.5	1.3
Animal butter	-	31
Fiber	3	1.62
Ash	8	3.2
Total phosphate	0.59	0.24
Total calcium	0.95	0.8
Mineral mixture	2.46	2.46
+	g% (w/v)	g% (w/v)
Fructose	-	20

B

Acid type	Common name (acid)	Percentage of fatty acid
C4:0	Butyric	1.05
C6:0	Caproic	0.8
C8:0	Caprylic	0.6
C10:0	Capric	2.04
C12:0	Lauric	3.2
C14:0	Myristic	12.3
C14:1c n-5	Myristoleic	1.1
C16:0	Palmitic	38.3
C16:1c n-7	Palmitoleic	0.7
C18:0	Steric	6.8
C18:1c n-9	Oleic	30.5
C18:2c n-6	Linoleic	2.2
C20:0	Arachidonic	0.2

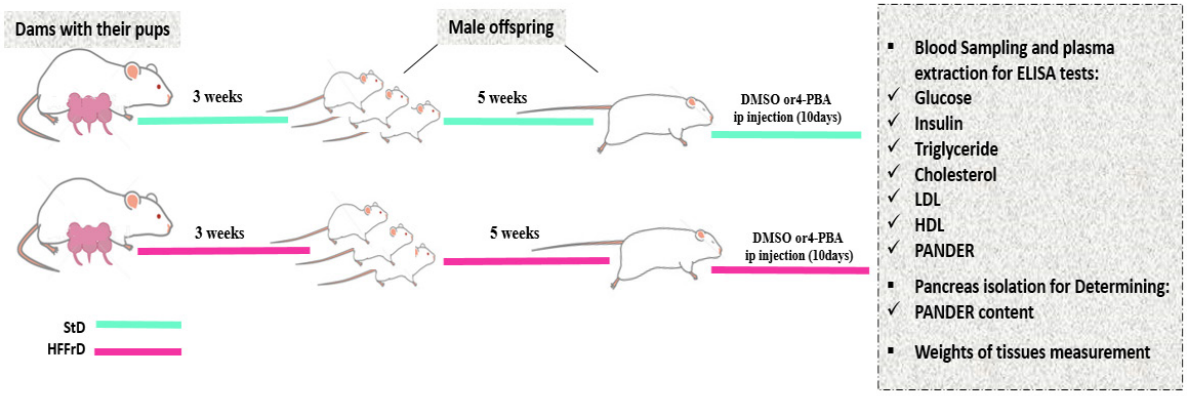

FIGURE 1. Schematic outline of the study.

(ELISA) kits were used for measuring insulin (ZellBio, Germany; sensitivity: 0.1mIU/l, intra-assay coefficient of variations: 5.1%) and PANDER (ZellBio, Germany; sensitivity: 6ng/ml, intra-assay coefficient of variations: 8.5%) concentrations. The Enzymatic photometric kits were used to measure the triglyceride (Parsazmoon, Iran; sensitivity: 1mg/dl, intra-assay coefficient of variations: 1.53%), cholesterol (Parsazmoon, Iran; sensitivity: 3mg/dl, intra-assay coefficient of variations: 1.62%), LDL (PishtazTeb, Iran; sensitivity: 1mg/dl, intra-assay coefficient of variations: 0.92%) and HDL (ZiestChem, Iran; sensitivity: 5mg/dl, intra-assay coefficient of variations: 1.1%) levels (6 rats/group, 6 litters/group). The Homeostasis model assessment of insulin resistance (HOMA-IR) and atherogenic indices (ARI) were determined using the following formulas (Chikezie et al., 2018):

HOMA-IR= fasting insulin (mIU/l)× fasting glucose (mmol/l)/22.5

ARI= log Triglyceride/ HDL; ARI= Cholesterol/ HDL; ARI= LDL/ HDL

Pancreas isolation

In the end, after the decapitation of anesthetized rats with isoflurane inhalation, the pancreas tissue was quickly isolated and froze (4 rats/group, 4 litters/group). The samples were homogenized in the ice-cold lysis buffer. After centrifugation, the supernatants were collected.

TABLE 2: The glucose, insulin, HOMA-IR, triglyceride, cholesterol, LDL, HDL and atherogenic indices alteration due to the HFFrD consumption and/or 4-PBA administration. Results are presented as mean±SEM (6 rats/group, 6 litters/group). Data were analyzed using the two-way ANOVA followed by Tukey's post hoc test. $^{*}P<0.05$, $^{**}P<0.01$ and $^{***}P<0.001$ versus StD group; $^{\#}P<0.05$ and $^{\#\#}P<0.01$ versus StD-V group; $^{\wedge\wedge\wedge}P<0.001$ versus HFFrD; $^{+}P<0.05$, $^{++}P<0.01$ and $^{+++}P<0.001$ versus HFFrD-V group.

	StD	StD -V	StD -D	HFFrD	HFFrD-V	HFFrD -D
Glucose (mg/dl)	54 ± 3.6	68.5 ± 3.2	65.8 ± 2.2	85.8 ± 2.5***	90.3 ± 4.8***	69.7 ± 7.6^{+}
Insulin (mIU/L)	14.9 ± 1.2	12.1 ± 0.3	12.6 ± 1.02	15.3 ± 0.9	15.1 ± 1.2	14.6 ± 1.5
HOMA-IR	1.9 ± 0.1	2.04 ± 0.1	2.04 ± 0.1	3.2 ± 0.2*	3.3 ± 0.3**	2.4 ± 0.3
Triglyceride (mg/dl)	65.5 ± 4.6	74.4 ± 3.7	51.9 ± 2.2$^{\#\#}$	49.7 ± 5.5	50.4 ± 3.9	53.8 ± 2.8
Cholesterol (mg/dl)	70.3 ± 3.5	83.5 ± 3.8	74.7 ± 3.9	118.4 ± 5.2***	100.9 ± 9.2*	60.7 ± 8.7$^{\wedge\wedge\wedge++}$
LDL (mg/dl)	15.4 ± 0.6	20.7 ± 1.5	20.6 ± 0.2	39.8 ± 4***	38.9 ± 2.3***	47.3 ± 3.3***
HDL (mg/dl)	43.9 ± 2.08	46.8 ± 1.7	34.9 ± 5.6$^{\#}$	19.6 ± 0.4***	21.3 ± 0.5***	23.2 ± 1.8***
log Triglyceride/ HDL	0.04 ± 0.002	0.04 ± 0.001	0.05 ± 0.009	0.08± 0.002***	0.07± 0.001***	0.07± 0.005***
Cholesterol/ HDL	1.6 ± 0.08	1.7 ± 0.09	2.4 ± 0.4	6.06 ± 0.3***	4.7 ± 0.4***	2.6 ± 0.3$^{\wedge\wedge\wedge+++}$
LDL/ HDL	0.3 ± 0.01	0.44 ± 0.02	0.6 ± 0.1	2.03 ± 0.2***	1.8 ± 0.1***	2.1 ± 0.2***

StD: standard diet; StD-V: standard diet-Vehicle; StD-D: standard diet-Drug; HFFrD: high-fat-fructose diet; HFFrD-V: high-fat-fructose diet -Vehicle and HFFrD-D: high-fat-fructose diet -Drug; LDL: low-density lipoprotein; HDL: high-density lipoprotein; HOMA-IR: Homeostasis model assessment of insulin resistance

Protein assay and measurement of PANDER content in pancreas tissue

The total protein concentration of pancreas tissue was estimated using the Bradford method (Kruger 2009). The PANDER content was calculated as the ratio of pancreatic PANDER concentration using a rat PANDER ELISA kit (ZellBio, Germany; sensitivity: 6ng/ml) to its total protein level (4 rats/group, 4 litters/group).

Body and tissues weights

In each of the study groups, the body weights of animals were measured at the end of the protocol (digital scale; precision: 0.1g). In addition, the internal organs (including the thymus, heart, liver, spleen, pancreas and adrenals) were weighted (6 rats/group, 6 liters/group).

Statistical analysis

Results are expressed as mean±SEM. Graph Pad Prism software (version 8.3.0) was used for statistical analysis. Two-way analysis of variance (ANOVA) and Tukey's post-hoc test were performed, in which diet and treat considered independent factors. Differences were considered significant at $P< 0.05$.

Results

Plasma parameters analysis

According to table 2, two-way ANOVA with Tukey's post hoc test showed an increased glucose level in HFFrD and HFFrD-V relative to StD ($P<0.001$). While, it was declined in HFFrD-D relative to HFFrD-V ($P<0.05$) [diet: $F(1, 30)= 28.44$, $P< 0.0001$; drug: $F(2, 30)= 3.943$, $P= 0.0302$; diet× drug: $F(2, 30)= 5.154$, $P= 0.0119$]. The plasma insulin concentrations did not change in any of the experimental groups [diet: $F(1, 30)= 4.085$, $P= 0.0523$; drug: $F(2, 30)= 1.197$, $P= 0.3161$; diet× drug: $F(2, 30)= 0.7136$, $P= 0.4980$]. The HOMA-IR index was significantly increased in HFFrD ($P<0.05$) and HFFrD-V ($P<0.01$) relative to StD [diet: $F(1, 30)= 24.25$, $P< 0.0001$; drug: $F(2, 30)= 1.621$, $P= 0.2145$; diet× drug: $F(2, 30)= 1.894$, $P= 0.1680$]. The plasma triglyceride concentration was decreased in StD-D relative to StD-V ($P<0.01$) [diet: $F(1, 30)= 14.96$, $P= 0.0005$; drug: $F(2, 30)= 2.852$, $P= 0.0735$; diet× drug: $F(2, 30)= 5.492$, $P= 0.0093$]. The increment of plasma cholesterol concentration was seen in the HFFrD ($P<0.001$) and HFFrD-V ($P<0.05$) relative to StD. While the plasma cholesterol level was decreased in HFFrD-D ($P<0.001$ and $P<0.01$ relative to HFFrD and HFFrD-V, respectively) [diet: $F(1, 30)= 11.37$, $P= 0.0021$; drug: $F(2, 30)= 11.32$, $P= 0.0002$; diet× drug: $F(2, 30)= 12.38$, $P= 0.0001$]. The plasma LDL concentration was increased in HFFrD, HFFrD-V and HFFrD-D relative to StD ($P<0.001$) [diet: $F(1, 30)= 136.9$, $P< 0.0001$; drug: $F(2, 30)= 3.532$, $P= 0.0419$; diet× drug: $F(2, 30)= 1.623$, $P= 0.2142$]. The decrement plasma HDL concentration was seen in StD-D ($P<0.01$ relative to StD-V), HFFrD, HF-

TABLE 3: The weight of the tissues including the thymus, heart, liver, spleen, pancreas, adrenal, kidney and soleus muscle alteration due to the HFFrD consumption and/or 4-PBA administration. Results are presented as mean±SEM (6 rats/group, 6 litters/group). Data were analyzed using the two-way ANOVA followed by Tukey's post hoc test. $^{*}P<0.05$, $^{**}P<0.01$ and $^{***}P<0.001$ versus StD group; $^{\wedge}P<0.05$ and $^{\wedge\wedge\wedge}P<0.001$ versus HFFrD.

	StD	Std -V	StD –D	HFFrD	HFFrD-V	HFFrD -D
Body Weight	197.6 ± 0.8	179.7 ± 4.9	173.5 ± 2.6***	110.2 ± 0.4***	75.4 ± 3.3***^^^	63.8 ± 0.7***^^^
Thymus	0.24 ± 0.02	0.18 ± 0.006	0.17 ± 0.004*	0.26 ± 0.01	0.20 ± 0.02	0.22 ± 0.01
Thymus/BW (%)	0.12 ± 0.01	0.10 ± 0.005	0.09 ± 0.004	0.29 ± 0.01***	0.29 ± 0.04***	0.35 ± 0.03***
Heart	0.71 ± 0.03	0.72 ± 0.02	0.75 ± 0.02	0.60 ± 0.02*	0.58 ± 0.03**	0.54 ± 0.02
Heart/BW (%)	0.71 ± 0.03	0.41 ± 0.02	0.35 ± 0.01	0.66 ± 0.02***	0.82 ± 0.07***	0.85 ± 0.03***^
Liver	6.58 ± 0.1	5.62 ± 0.1	5.52 ± 0.3*	3.84 ± 0.2***	3.72 ± 0.2***	3.61 ± 0.1***
Liver/BW (%)	3.31 ± 0.06	3.19 ± 0.09	3.12 ± 0.18	4.27 ± 0.2	5.38 ± 0.71**	5.64 ± 0.18***
Spleen	0.78 ± 0.05	0.89 ± 0.1	0.70 ± 0.05	0.40 ± 0.003***	0.36 ± 0.01***	0.37 ± 0.009***
Spleen/BW (%)	0.39 ± 0.02	0.51 ± 0.06	0.40 ± 0.03	0.44 ± 0.003	0.51 ± 0.04	0.59 ± 0.01**
Pancreas	0.56 ± 0.02	0.58 ± 0.01	0.50 ± 0.03	0.38 ± 0.007***	0.33 ± 0.01***	0.32 ± 0.003***
Pancreas/BW (%)	0.28 ± 0.01	0.33 ± 0.01	0.28 ± 0.01	0.42 ± 0.008***	0.46 ± 0.04***	0.51 ± 0.01***
Adrenals	0.04 ± 0.005	0.06 ± 0.005	0.04 ± 0.004	0.07 ± 0.005	0.05 ± 0.005	0.05 ± 0.005
Adrenals/BW (%)	0.02 ± 0.003	0.03 ± 0.002	0.02 ± 0.002	0.08 ± 0.003***	0.07 ± 0.007***	0.08 ± 0.009***

StD: standard diet; StD-V: standard diet-Vehicle; StD-D: standard diet-Drug; HFFrD: high-fat-fructose diet; HFFrD-V: high-fat-fructose diet -Vehicle; HFFrD-Drug: high-fat-fructose diet -Drug; and BW: body weight.

FrD-V and HFFrD-D ($P<0.001$ relative to StD) [diet: $F(1, 30)= 86.46$, $P< 0.0001$; drug: $F(2, 30)= 1.741$, $P= 0.1925$; diet× drug: $F(2, 30)= 4.020$, $P= 0.0284$]. The ratio of plasma log triglyceride to HDL was increased in HFFrD, HFFrD-V and HFFrD-D relative to StD ($P<0.001$) [diet: $F(1, 30)= 83.43$, $P< 0.0001$; drug: $F(2, 30)= 0.9847$, $P= 0.3853$; diet× drug: $F(2, 30)= 3.636$, $P= 0.0386$]. The ratio of plasma cholesterol to HDL was elevated in HFFrD and HFFrD-V relative to StD ($P<0.001$). Whereas, it was decreased in HFFrD-D relative to HFFrD and HFFrD-V ($P<0.001$) [diet: $F(1, 30)= 92.88$, $P< 0.0001$; drug: $F(2, 30)= 8.267$, $P= 0.0014$; diet× drug: $F(2, 30)= 22.81$, $P< 0.0001$]. The increment of plasma LDL to HDL ratio was seen in HFFrD, HFFrD-V and HFFrD-D relative to StD ($P<0.001$) [diet: $F(1, 30)= 199.3$, $P< 0.0001$; drug: $F(2, 30)= 2.034$, $P= 0.1485$; diet× drug: $F(2, 30)= 0.7342$, $P= 0.4883$].

Plasma concentration and pancreatic content of PANDER

The plasma PANDER concentration was significantly elevated in HFFrD and HFFrD-V relative to StD ($P<0.001$). Whereas, it was decreased in HFFrD-D ($P<0.01$ and $P<0.05$ relative to HFFrD and HFFrD-V, respectively) [diet: $F(1, 30)= 42.80$, $P< 0.0001$; drug: $F(2, 30)= 3.519$, $P= 0.0424$; diet× drug: $F(2, 30)= 7.022$, $P= 0.0032$] (Figure 2A). The pancreatic PANDER content increment was seen in HFFrD ($P<0.05$), HFFrD-V ($P<0.001$) and HFFrD-D ($P<0.01$) relative to StD. While, it declined in StD-D relative to StD-V and HFFrD-D relative to HFFrD and HFFrD-V ($P<0.01$) (Figure 2B) [diet: $F(1, 18)= 181.4$, $P< 0.0001$; drug: $F(2, 18)= 17.46$, $P= 0<0.001$; diet× drug: $F(2, 18)= 2.018$, $P= 0.1619$].

Body and tissues weights analysis

As shown in table 3, the body, liver, spleen and pancreas weights were decreased in StD-D, HFFrD, HFFrD-V and HFFrD-D relative to StD ($P<0.001$). In addition, body weight was significantly decreased in HFFrD-V and HFFrD-D relative to HFFrD ($P<0.001$). The thymus weight was significantly lower in StD-D than in StD ($P<0.05$). The increment of thymus, pancreas and adrenals weights to body weight ratio was seen in HFFrD, HFFrD-V and HFFrD-D relative to StD ($P<0.001$). The heart weight decreased in HFFrD ($P<0.05$) and HFFrD-V ($P<0.01$) relative to StD. The ratio of heart weight to body weight elevation was seen in HFFrD-V and HFFrD-D relative to StD ($P<0.001$). Whereas, it was decreased in HFFrD ($P<0.001$ relative to StD)

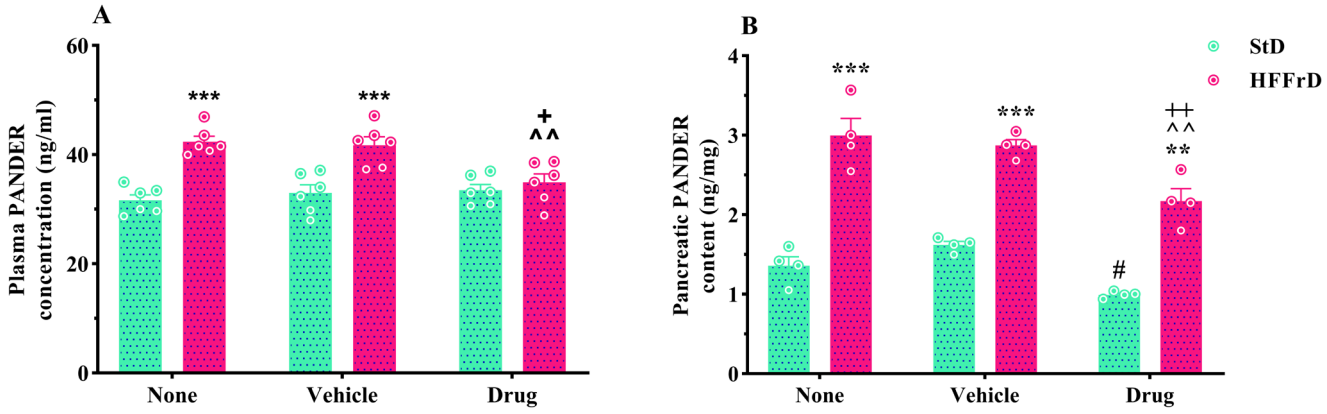

FIGURE 2. The plasma PANDER concentration (A) (6 rats/group, 6 litters/group), and pancreatic PANDER content (B) (4 rats/group, 4 litters/group) changes due to the HFFrD consumption and/or 4-PBA administration. Each column is represented as mean±SEM. Data were analyzed using the two-way ANOVA followed by Tukey's post hoc test. $^{*}P<0.05$ and $^{***}P<0.001$ versus StD group; $^{\wedge\wedge}P<0.01$ versus HFFrD group; $^{+}P<0.05$ versus HFFrD-V group.
StD: standard diet; StD-V: standard diet-Vehicle; StD-D: standard diet-Drug; HFFrD: high-fat-fructose diet; HFFrD-V: high-fat-fructose diet -Vehicle and HFFrD-D: high-fat-fructose diet -Drug.

TABLE 4: The F values of the two-way ANOVA for the body and tissues weight including thymus, heart, liver, spleen, pancreas and adrenal at the end of the protocol.

	Diet	Drug	Diet × Drug
Body Weight (BW)	F(1, 30) = 1391 P< 0.0001	F(2, 30) = 63.06 P< 0.0001	F(2, 30) = 6.116 P= 0.0059
Thymus	F(1, 30) = 116.6 P< 0.0001	F(2, 30) = 0.6414 P= 0.5336	F(2, 30) = 1.768 P= 0.1879
Thymus/BW (%)	F(1, 30) = 6.524 P= 0.0160	F(2, 30) = 9.475 P= 0.0006	F(2, 30) = 0.5417 P= 0.5874
Heart	F(1, 30) = 151.0 P< 0.0001	F(2, 30) = 6.640 P= 0.0041	F(2, 30) = 1.293 P= 0.2894
Heart/BW (%)	F(1, 30) = 46.64 P< 0.0001	F(2, 30) = 0.0082 P= 0.9918	F(2, 30) = 1.921 P= 0.1641
Liver	F(1, 30) = 48.68 P< 0.0001	F(2, 30) = 1.850 P= 0.1747	F(2, 30) = 3.040 P= 0.0628
Liver/BW (%)	F(1, 30) = 127.9 P< 0.0001	F(2, 30) = 4.315 P= 0.0225	F(2, 30) = 2.086 P= 0.1418
Spleen	F(1, 30) = 7.198 P= 0.0118	F(2, 30) = 3.589 P= 0.0400	F(2, 30) = 3.361 P= 0.0482
Spleen/BW (%)	F(1, 30) = 84.88 P< 0.0001	F(2, 30) = 1.349 P= 0.2748	F(2, 30) = 1.839 P= 0.1765
Pancreas	F(1, 30) = 98.22 P< 0.0001	F(2, 30) = 3.218 P= 0.0542	F(2, 30) = 3.261 P= 0.0523
Pancreas/BW (%)	F(1, 30) = 154.2 P< 0.0001	F(2, 30) = 4.377 P= 0.0215	F(2, 30) = 2.410 P= 0.1070
Adrenals	F(1, 30) = 109.3 P< 0.0001	F(2, 30) = 0.0401 P= 0.9607	F(2, 30) = 1.223 P= 0.3086
Adrenals/BW (%)	F(1, 30) = 0.2267 P= 0.6375	F(2, 30) = 5.138 P= 0.0121	F(2, 30) = 2.375 P= 0.1103

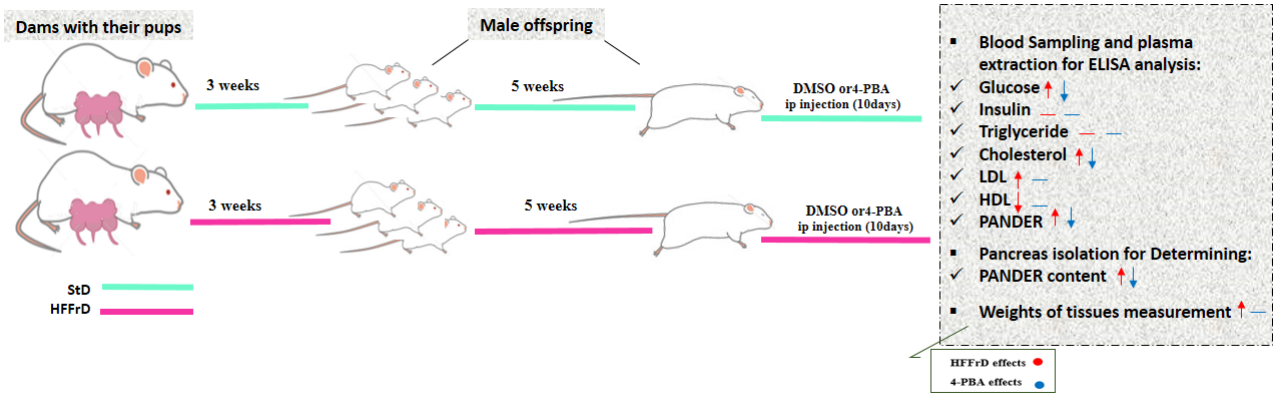

FIGURE 3. Summary of the results.

and increased in HFFrD-D (P<0.05 relative to HFFrD). The ratio of liver weight to body weight increased in HFFrD-V (P<0.01) and HFFrD-D (P<0.001) relative to StD. The ratio of spleen weight to body weight was increased in HFFrD-D relative to StD (P<0.01). The adrenals weights were not shown any significant changes in any of the experimental groups. The F values of these data are shown in table 4.

Discussion

The present study showed that long-term consumption of HFFrD increased the fasting glycemic level and led to an increase in HOMA-IR. In addition, plasma atherogenic indices were increased through increased cholesterol and LDL and decreased HDL in HFFrD-fed rats. These abnormalities were associated with PANDER increment in plasma and pancreas tissue. It is revealed that fructose metabolism in the liver stimulates lipogenesis and causes triglyceride accumulation, which contributes to hepatic insulin resistance (Altaş et al., 2010). In addition, the consumption of HFFrD for eight weeks resulted in insulin resistance with high serum glucose and insulin levels (Huang et al., 2013). In addition, chronic hyperglycemia and insulin resistance activate PANDER expression in pancreatic tissue (Wang et al., 2012). It has been reported that increased PANDER appears to act as a pro-inflammatory cytokine in both plasma and pancreatic tissue (Cieślak et al., 2015). Moreover, it is revealed that PANDER increment in the liver of 57Bl/6J mice promotes lipogenesis (Li et al., 2011). It seems that the increase in atherogenic indices is related to the increase in the level of pander in plasma and pancreas. Consistent with the body weight loss in HFFrD-fed rats, it has been found that increased intestinal inflammatory metabolites through high-carbohydrate intake lead to digestive disorders and body weight loss (Tan et al., 2021). Accordingly, the weight of the thymus and pancreas to the body weight increased in the HFFrD group, which increases the possibility of inflammation in these tissues. In this respect, consuming a high-carbohydrate diet for sixteen weeks increases liver inflammation, steatosis, and cardiac hypertrophy (Alam et al., 2013). In addition, consuming a high-fat diet causes abnormal fat accumulation in the liver, pancreas, and skeletal muscle tissues (Duan et al., 2018). It is notable that, in the present study 4-PBA decreased plasma glucose concentration. In this context, previous studies showed that 4-PBA reduces insulin resistance and hyperglycemia, leading to improved glucose homeostasis (de Pablo et al., 2021; Guo et al., 2017). In addition, the administration of 4-PBA decreased the cholesterol level and cholesterol to HDL ratio in the rats who consumed HFFrD. 4-PBA treatment reduces lipid accumulation and lipotoxicity through the activation of autophagy in hepatocytes exposed to palmitate (Nissar et al., 2017). Furthermore, the plasma and pancreatic PANDER levels decreased after 4-PBA administration in the HFFrD group. In this context, 4-PBA respectively inhibits activation and phosphorylation of NF-κB and mitogen-activated protein kinase in a collagen-induced arthritis mouse model, which alleviates inflammatory symptoms (Choi et al., 2021). It is worth noting that 4-PBA reduces ER stress and pro-inflammatory cytokine levels in mice treated with lipopolysaccharide (Zeng et al., 2017). In addition, 4-PBA attenuates the increase in plasma interleukin-1β and inflammation through the possible inhibition of ER

stress in twenty-week high-fat diet-fed rats (Binayi et al., 2023). Therefore, according to the above-mentioned statements, 4-PBA may induce ameliorative effects through the inhibition of ER stress.

Conclusion

Overall, since 4-PBA administration in the HFFrD group improved glucose and lipid metabolism as well as PANDER levels in both plasma and pancreatic tissue, it seems that the increased level of PANDER after long-term HFFrD intake could be the cause of hyperglycemia and dyslipidemia.

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Conflict of interest

The authors declare that they have no conflict of interest.

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