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Article

Oleic acid acutely impairs glucose homeostasis in standard chow diet but not high-fructose, high-fat diet-fed mice by acting on free fatty acid receptor 1

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This study aimed to investigate the acute effects of oleic acid (OA) on glucose homeostasis in mice fed a standard chow diet (SCD) and a high-fructose, high-fat diet (HFrHFD); moreover, the role of free fatty acid receptor 1 (FFAR1) was evaluated. The mice in the two groups were further divided into three subgroups as follows: control, OA (40 mg/kg), and OA + GW1100 (0.4 mg/kg, selective FFAR1 blocker). After a 16-week feeding period, the mice received the drugs via intraperitoneal (i.p.) injection followed by an i.p. glucose tolerance test (IPGTT) 30 min later. After 3 days, the mice received the same drugs to examine the effects of the drugs on the hepatic levels of phosphatidylinositol-4,5-bisphosphate (PIP2) and diacylglycerol (DAG). OA in the SCD-fed mice significantly increased the blood glucose level (48%, $P < 0.001$) after 30 min of glucose load compared to that in the control group, but did not affect the levels of PIP2 and DAG. Pre-injection with GW1100 significantly decreased the area under the curve of the IPGTT (28%, $P < 0.05$) in the SCD group compared to that in the SCD + OA group. OA reduced the blood glucose level (35%, $P < 0.001$) after 120 min of glucose load in the HFrHFD-fed mice; in addition, it increased hepatic PIP2 (160%, $P < 0.01$) and decreased hepatic DAG (60%, $P < 0.001$) levels. Pre-injection with GW1100 blocked the effects of OA on hepatic PIP2 and DAG without affecting the glucose tolerance. In conclusion, OA acutely impaired the glucose tolerance in the SCD-fed mice by acting on FFAR1 but did not improve it in the HFrHFD-fed mice.

Keywords: OA. GW1100. FFAR1. Glucose tolerance. High-fructose. High-fat diet. Insulin resistance.

INTRODUCTION

Oleic acid (OA) is an omega-9 long-chain fatty acid abundantly present in vegetable oils, such as olive oil (Orsavova *et al*., 2015), and acts as a ligand for free fatty acid receptors 1 and 4 (FFAR1 and FFAR4, respectively) (Schnell, Schaefer, Schöfl, 2007; Kim *et al*., 2016). However, the interactions between OA and these receptors are not well characterized, both in vitro and in vivo.

Notably, FFARs are G-protein-coupled receptors (GPCRs) and the following six types have been identified so far: FFAR1, previously known as GPR40, is stimulated by medium and long-chain fatty acids and is highly expressed in pancreatic ***β***-cells (Salehi *et al*., 2005); FFAR2, previously known as GPR43, is activated by short-chain fatty acids and is highly expressed in entero-endocrine L-cells (Kaji *et al*., 2011); FFAR3, previously known as GPR41, is a short-chain fatty acid receptor and is highly expressed in the adipose tissue and intestine (L**ó**pez Soto *et al*., 2014); FFAR4, previously known as GPR120, is a medium and long-chain fatty acid receptor highly expressed in adipose tissues (Moniri, 2016); GPR84 is a medium-chain fatty acid receptor, which is highly expressed in immune cells (Wang *et al*., 2006); and GPR119 is a long-chain fatty acid receptor highly expressed in pancreatic ***β***-cells (Hansen *et al*., 2012).

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Both FFAR1 and FFAR4 are G***α***qPCRs, and the activation of these receptors by saturated fatty acids, such as palmitic acid and stearic acid, induces the breakdown of phosphatidylinositol 4,5 bisphosphate (PIP2) by phospholipase C (PLC) into inositol triphosphate (IP3) and diacylglycerol (DAG) (Yamada *et al*., 2016). IP3 elevates the levels of intracellular Ca^{+2} and mediate insulin secretion from the pancreas (Ferdaoussi *et al*., 2012; Yamada *et al*., 2016). However, elevated levels of DAG activate protein kinase C (PKC), which phosphorylates and degrades insulin receptor substrate 1 (IRS1) leading to insulin resistance (IR) (Jornayvaz, Shulman, 2012). Therefore, the metabolic effects of high-fat diets are mainly attributed to the saturated fatty acid content. Moreover, it has been found that a high-fructose diet in combination with a high-fat diet worsens metabolic impairment and increases the complications associated with IR (Panchal *et al*., 2011).

In the same context, the activation of FFARs stimulates the translocation and binding of ***β***-arrestins to the membrane receptors, thereby promoting their desensitization and internalization (Nuber *et al*., 2016). ***β***-arrestins have been found to play a potential role in glucose homeostasis and insulin signaling. They form complexes with IRS1, Src, and Akt proteins and promote Akt phosphorylation, which in turn mediates glucose uptake by activating the glucose transporters (GLUTs) (Luan *et al*., 2009). Moreover, ***β***-arrestins can activate several downstream signals, including the formation of PIP2 through stimulation of phosphatidylinositol 4-phosphate 5-kinase (PIP5K) Iα (Nelson *et al*., 2008) and conversion of DAG into phosphatidic acid by activating DAG kinase (Cai *et al*., 2009).

Therefore, FFAR1/4 ligands that activate the G-protein pathway to a greater extent than the ***β***-arrestin pathway should induce IR. On the contrary, FFAR1/4 ligands that activate the ***β***-arrestin pathway more than the G-protein pathway should enhance insulin-mediated glucose uptake.

In this context, the acute effects of OA on glucose homeostasis are highly controversial. Tsuchiya *et al*. reported that OA (1 μM) stimulates insulin (0.1 nM)-induced phosphorylation of the insulin receptor at Tyr1185 and increases insulin (0.1 nM)-induced phosphorylation of Akt at Thr308 and Ser473 in differentiated 3T3-L1-GLUT4 myc adipocytes (Tsuchiya *et al*., 2014). In addition, they reported that OA (1 μM) can activate the insulin receptor/PI3K/PDK1/Akt/Rac1 axis, which facilitates insulin-induced glucose uptake into adipocytes; however, OA, by itself, did not affect the glucose uptake (Tsuchiya *et al*., 2014). (Al Jamal, 2011 #24)On the contrary, the addition of 2.0 mM OA into the culture media of HepG2 cells for 24 h resulted in the accumulation of lipid droplets and increased the production of triacylglycerol, lipid peroxidation, and inflammatory cytokines; in addition, the number of antioxidant molecules, glucose uptake, and cell proliferation were decreased (Vidyashankar, Varma, Patki, 2013).

The time frame for the downstream signaling of GPCRs extends from 1 to 30 min. G-protein signaling takes 1 to 5 min after ligand binding, but ***β***-arrestin signaling starts 10 min later and extends until 30 min (Ibrahim, Nakaya, Kurose, 2013). Therefore, significant changes in glucose homeostasis can be observed within and after 30 min of FFAR stimulation in vivo.

Glucose tolerance tests reveal information about the secretion and action of insulin, and the liver is one of the main sites of glucose disposal; moreover, OA has been shown to reduce the uptake of hepatic glucose (Vidyashankar, Varma, Patki, 2013). Therefore, this study aimed to investigate the acute effects of OA on glucose tolerance and hepatic lipid signaling (PIP2 and DAG) in mice fed a standard chow diet (SCD) and high-fructose, high-fat diet (HFrHFD). Additionally, because of the high expression level of FFAR1 in the pancreas and its effect on insulin secretion during the glucose tolerance test, this study investigated its role in mediating the effects of OA using a selective FFAR1 blocker (GW1100) in combination with OA.

MATERIAL AND METHODS

Animals

Experiments were performed on adult male Swiss albino mice (20 ± 5 g, 8 weeks old) purchased from the Faculty of Veterinary Medicine, Zagazig University, Egypt, and housed in the animal care unit of the Faculty

of Pharmacy, Zagazig University. The housing cages were well ventilated, and the room temperature was adjusted at 28ºC to 30ºC to minimize the activity of the brown adipose tissues. The light and dark cycles were adjusted to approximately 12 h each. During the acclimatization period, which extended for two weeks, the animals were fed a standard pellet chow diet and allowed free access to tap water. All procedures were conducted in accordance with the accepted principles for the care and use of laboratory animals and were approved by the animal ethics committee of the Faculty of Pharmacy, Zagazig University (Protocol # P1/6/2017).

Drugs and chemicals

OA was purchased from Carbosynth Co., USA. Dimethyl sulfoxide (DMSO) was purchased from Sigma-Aldrich, USA. GW1100 was purchased from MedChem Express, USA. All chemicals used in this study were of analytical grade.

Experimental design and induction of IR

The mice were divided into two main groups as follows: SCD-fed and HFrHFD-fed. The feeding period for both groups was 16 weeks (Panchal *et al.*, 2011; Ibrahim *et al.*, 2020). The HFrHFD was composed of 155 g of chow diet, 200 g of beef tallow, 170 g of fructose, 320 g sweetened condensed milk, 100 g corn gluten (60% protein), 25 g of salt mixture, and 30 g of water per kg of diet; in addition, the animals in these groups received fructose (20% w/v) in drinking water for 16 weeks (Panchal *et al.*, 2011; Ibrahim *et al.*, 2020). All nutritional parameters of this diet met or exceeded the guidelines of the National Research Council, Canada, for rats and mice (Tables I and II).

Subsequently, the mice in the two main groups were randomly distributed into three subgroups as follows (n = 6 each):

- control group (SCD or HFrHFD), which received a vehicle (25% DMSO, 75% distilled water; 100 µl/40 g body weight);
- OA group (SCD + OA or HFrHFD + OA), which received two doses (40 mg/kg) (Gonçalves de Albuquerque *et al*., 2012) every 3 days (El-Fayoumi *et al*., 2020): the first dose for the glucose tolerance test and the second dose to examine the effects on the levels of both hepatic PIP2 and DAG;
- OA + GW1100 group (i.e., SCD + OA + GW or HFrHFD + OA + GW), which received 0.4 mg/kg of the blocker first (Nakamoto *et al*., 2013) followed by OA, after 30 min. Both drugs were given in the same sequence as that in the OA group.

All drugs were dissolved in the previously mentioned vehicle and injected intraperitoneally. All mice were made to fast overnight before the injection of the drugs in both experiments (Jensen *et al*., 2013).

TABLE I - Composition of standard chow diet (SCD) and high-fructose, high-fat diet (HFrHFD)

	SCD	HFrHFD
Carbohydrate	60%	46%
Protein	22%	18%
Fat	2.5%	24%
Others (fibers, minerals, etc.)	15.5%	12%

TABLE II - Fatty acid composition of standard chow diet (SCD) and high-fructose, high-fat diet (HFrHFD)

	Fatty acids	Relative concentration (%)	
		HFrHFD	SCD
1	Capric acid (C10:0)	0.12	---
2	Lauric acid (C12:0)	0.13	---
3	Tridecanoic acid (C13:0)	0.15	---
4	Myristic acid (C14:0)	2.65	---
5	Myrist-oleic acid (14.1)	0.27	---
6	Pentadecanoic acid (C15:0)	0.38	---
7	Cis-10-Pentadecenoic (C15:1)	0.45	---
8	Palmitic acid (C16:0)	21.91	10.71
9	Palmitoleic acid (C16:1n 7)	1.05	1.53
10	Palmitoleic acid (C16:1n 9)	0.59	----
11	Heptadecanoic acid (C17:0)	0.95	0.29
12	Stearic acid (C18:0)	29.61	2.17
13	Oleic acid (C18:1n9c)	34.23	26.15
14	Elaidic acid (C18:1n9t)	3.01	0.87
15	Linoleic acid (C18:2n6c)	2.15	45.29
16	Linolelaidic acid (C18:2n6t)	0.39	3.75
17	Arachidic acid (C20:0)	0.30	6.67
18	γ- Linolenic acid (C18:3n6)	0.13	1.84
19	Cis-11- Eicosenoic acid (C20.1)	0.34	---
20	Linolenic acid (C18:3n3)	0.39	---
21	Cis -11,14- Eicosadienoic acid (C 20.2)	0.20	0.26
22	Behenic Linolenic acid (C22:0)	0.37	0.48

Measurement of blood glucose level

Blood glucose level was measured in a drop of blood obtained from the tip of the tail of a mouse, using an automated glucometer (GM100, Bionime GmbH, Berneck, Switzerland).

IPGTT

Thirty minutes after injecting the OA (or vehicle in the control groups), drops of blood obtained from the tails of the mice were used to measure the glucose levels (0 min) with the aid of a glucometer (GM100, Bionime GmbH). Subsequently, glucose (1 g/kg, 10% solution) was injected intraperitoneally, and the blood glucose levels were measured after 30, 60, and 120 min of glucose load (Deisl *et al*., 2016).

Preparation of liver samples

After 30 min of OA or vehicle injection, the mice were euthanized by decapitation. Liver samples were

collected, immediately frozen in liquid nitrogen, and stored at −80ºC until further analysis.

Enzyme-linked immunosorbent assay

Liver tissue (100 mg) was weighed and homogenized in 500 µl phosphate-buffered saline using a Con-Torque Eberbach Tissue Homogenizer (Michigan, USA). Each homogenate was centrifuged at 10,000 rpm and 5ºC for 10 min using a cooling centrifuge. The supernatant was collected into a new microcentrifuge tube (1.5 ml) and used for the biochemical tests. Units are expressed as per mg protein. The hepatic levels of PIP2 and DAG were measured using kits supplied by Nova Lifetech Limited (BlueGene Biotech, Shanghai, China [Cat. No. E03D0010] and LifeSpan BioSciences, WA, USA [Cat. No. LS-F18999], respectively). Serum insulin levels were measured using kits supplied by CUSABIO (Cat. No. CSB-E05071 m; Huston, USA).

Calculation of IR

The IR index was calculated using a homeostatic model assessment of IR (HOMA-IR) (Matthews *et al.*, 1985), according to the following equation: HOMA-IR = fasting glucose level (mg/dl) × fasting insulin level (IU/ml)/405.

Statistical analysis

Data are expressed as mean ± standard error of the mean (S.E.M). The statistical analysis was performed using unpaired Student's t-test, one-way analysis of variance (ANOVA) followed by Tukey's post hoc test, and two-way ANOVA followed by Bonferroni's post hoc test. P-values <0.05 were considered significant. All tests were performed using the GraphPad Prism software version 5 (GraphPad Software, Inc., CA, USA).

RESULTS

Feeding the HFrHFD mice for 16 weeks impaired glucose tolerance, induced IR, decreased hepatic PIP2, and increased hepatic DAG

As shown in Figure 1A, the body weights of the HFrHFD-fed mice were slightly increased during the first 15 weeks of the study and decreased during week 16 when compared to those of the SCD-fed mice. Furthermore, slight increases in the blood glucose levels of the IPGTT were observed in the HFrHFD-fed mice compared to those of the SCD-fed mice (Figure 1B). A slight increase in the area under the curve (AUC) of the IPGTT was noted in the HFrHFD-fed mice compared to that in the SCD-fed mice (P = 0.067; Figure 1C). Likewise, significant increases in serum insulin levels (98%, Figure 1D) and the HOMA-IR index (500%. Figure 1E), a significant decrease in hepatic PIP2 (63%; Figure 1F), and a significant increase in hepatic DAG (377%, Figure 1G) were observed in the HFrHFD-fed mice when compared to the corresponding values in the SCD-fed mice.

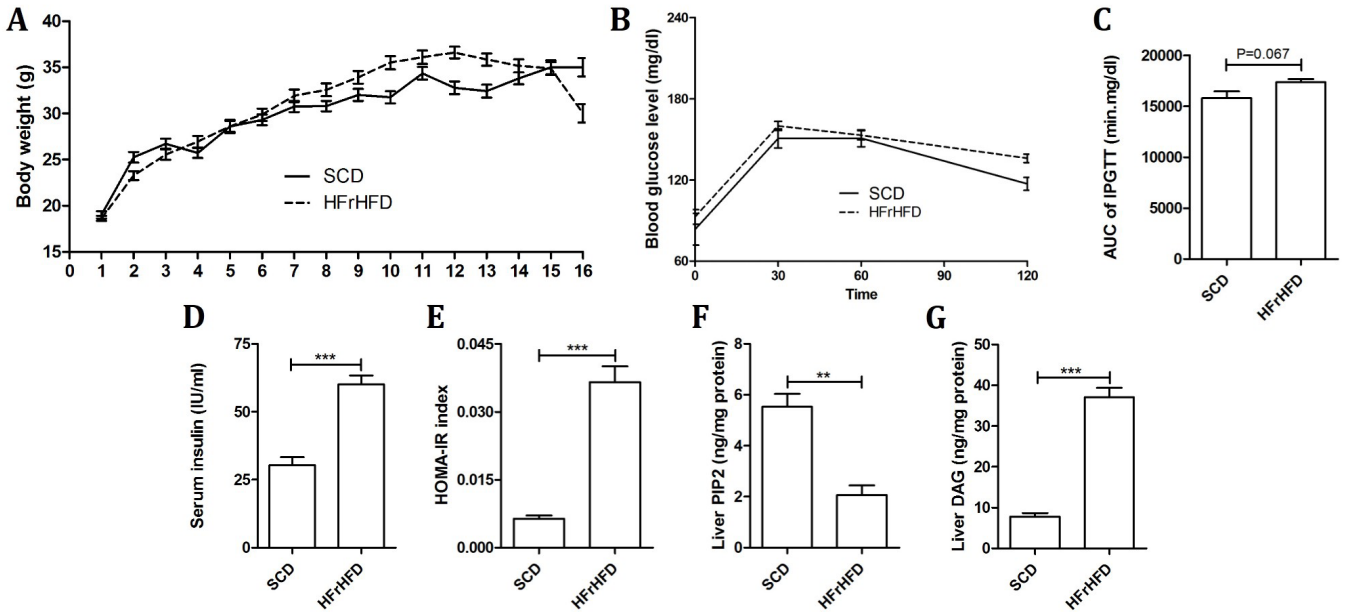

FIGURE 1 - Effect of feeding a high-fructose, high-fat diet (HFrHFD) for 16 weeks on the body weight, intraperitoneal glucose tolerance test (IPGTT), insulin resistance, and levels of hepatic phosphatidylinositol 4,5 bisphosphate (PIP2) and hepatic diacylglycerol (DAG). Graphical presentation of **A**: body weights; **B:** IPGTT; **C:** area under the curve (AUC) of the IPGTT; **D**: serum insulin level; **E:** homeostasis model of insulin resistance (HOMA-IR) index; **F:** hepatic levels of PIP2; and **G**: hepatic levels of DAG. Statistical analysis was performed using an unpaired Student's t-test; values are represented as mean ± standard error of the mean (S.E.M.). n = 6 in the IPGTT; 5 for the serum insulin and HOMA-IR; 3 for the PIP2 and DAG. **, *** P < 0.01, 0.001.

Effect of OA on glucose tolerance and PIP2 and DAG levels in the SCD-fed mice, and the role of FFAR1

Intraperitoneal injection of OA for 30 min in the SCD-fed mice (SCD + OA group) significantly increased the blood glucose levels at 30 min (222.5 ± 15.2 vs. 150.7 ± 7 mg/dl) of glucose load compared to that in the SCD group (Figure 2A). In contrast, the SCD + OA + GW1100 group presented with significantly decreased blood glucose levels at 30 (32%) and 60 (30.4%) min of glucose load compared to that in the SCD + OA group (Figure 2A). In addition, OA presented with a slightly increased AUC of IPGTT (P = 0.07, Figure 2B), whereas pre-injection with GW1100 significantly decreased the AUC of the IPGTT (28%) compared to that in the SCD + OA group (Figure 2B). However, both OA and OA + GW1100 did not induce significant changes in both hepatic PIP2 and DAG levels compared to those in the SCD group (Figures 2C and 2D).

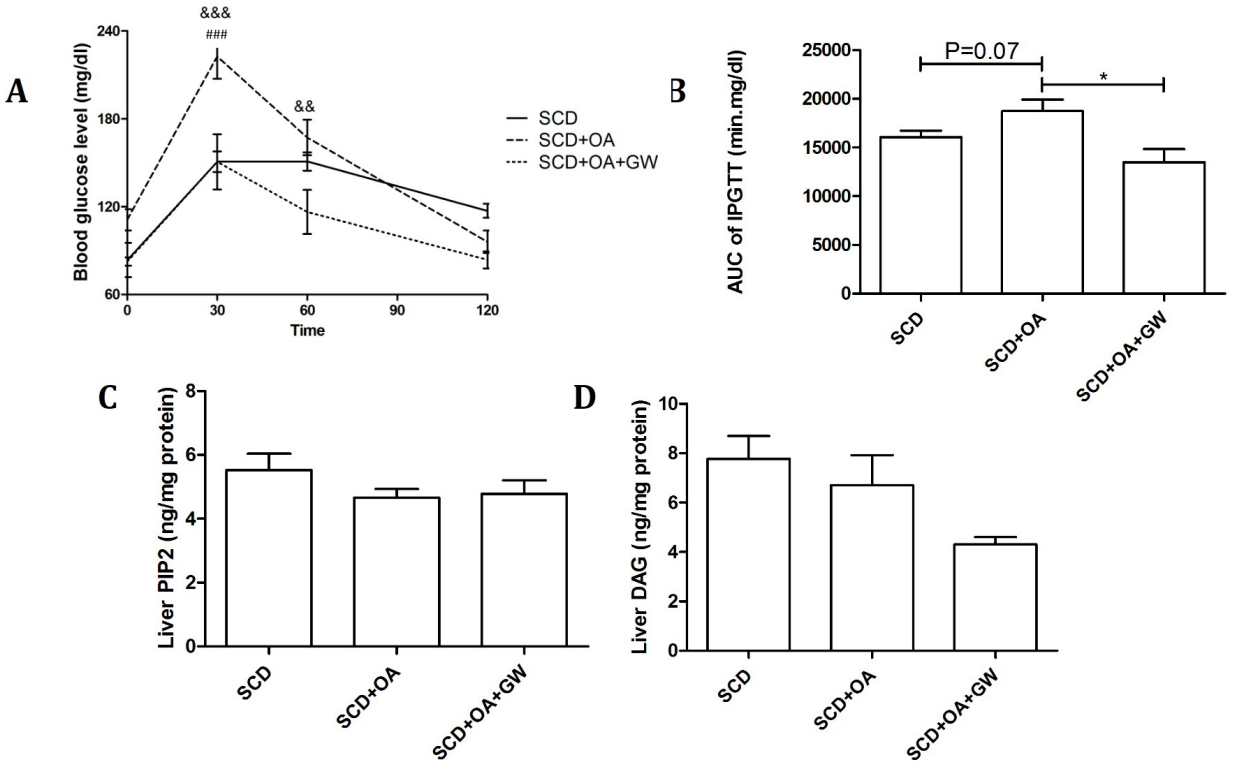

FIGURE 2 - Acute effect of OA on intraperitoneal glucose tolerance test (IPGTT), and levels of hepatic phosphatidylinositol 4,5 bisphosphate (PIP2), and hepatic diacylglycerol (DAG) in the standard chow diet (SCD)-fed mice. Graphical presentation of **A**: IPGTT; **B:** AUC of the IPGTT; **C**: hepatic levels of PIP2; and **D**: hepatic levels of DAG. SCD: Mice were fed a standard show diet for 16 weeks. SCD + OA: SCD-fed mice received OA (40 mg/kg/i.p.). SCD + OA + GW: SCD-fed mice received GW1100 (0.4 mg/kg/i.p), 30 min before OA administration. OA and GW1100 were dissolved in the same vehicle (25% DMSO, 75% distilled water; 100 µl/40 g body weight). Statistical analysis was performed using two-way ANOVA followed by Bonferroni's post hoc test for the IPGTT and one-way ANOVA followed by Tukey's post hoc test for other parameters; values are represented as mean ± S.E.M. n = 6 in the IPGTT; 3 for PIP2 and DAG. In IPGTT, ### $P < 0.001$ vs. SCD, &&, &&& $P < 0.01$ and 0.001, respectively, vs. SCD + OA + GW group. * $P < 0.05$.

Effect of OA on glucose tolerance and PIP2 and DAG levels in the HFrHFD-fed mice, and the role of FFAR1

Injection of OA for 30 min in the HFrHFD-fed mice significantly decreased the blood glucose levels (35% decrease) after 120 min of glucose load compared to that in the HFrHFD group (Figure 3A). In contrast, pretreatment with GW1100 significantly decreased the 0-min blood glucose level (35% decrease) compared to that in the HFrHFD + OA group (Figure 3A). No significant changes in the AUC of IPGTT were observed in all the HFrHFD subgroups (Figure 3B). Notably, injection of OA for 30 min significantly increased hepatic PIP2 (160%; Figure 3C) and decreased DAG (60%; Figure 3D) levels when compared to those in the HFrHFD group. In addition, pretreatment with GW1100 significantly altered the effect of OA on the hepatic levels of both PIP2 (73% decrease) and DAG (267% increase) compared to those in the HFrHFD + OA group (Figures 3C and 3D, respectively).

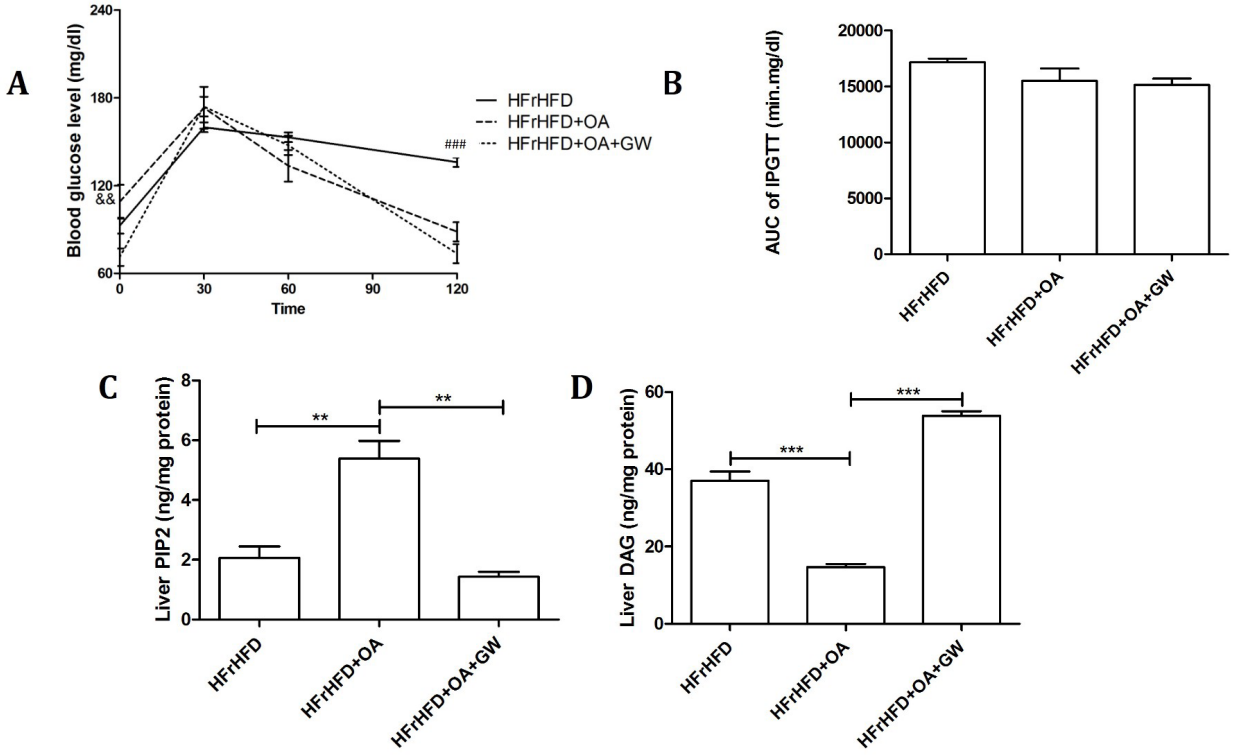

FIGURE 3 - Acute effect of OA on IPGTT, hepatic PIP2, and hepatic DAG in the HFrHFD-fed mice. Graphical presentation of **A**: IPGTT; **B:** AUC of the IPGTT; **C**: hepatic levels of PIP2; and **D**: hepatic levels of DAG. HFrHFD: Mice were fed a high-fructose, high-fat diet for 16 weeks. HFrHFD + OA: HFrHFD-fed mice received OA (40 mg/kg/i.p.). HFrHFD + OA + GW: HFrHFD-fed mice received GW1100 (0.4 mg/kg/i.p), 30 min before OA administration. OA and GW1100 were dissolved in the same vehicle (25% DMSO, 75% distilled water; 100 µl/40 g body weight). Statistical analysis was performed using two-way ANOVA followed by Bonferroni's post hoc test for the IPGTT and one-way ANOVA followed by Tukey's post hoc test for other parameters; values are represented as mean ± S.E.M. n = 6 in the IPGTT; 3 for PIP2 and DAG. In IPGTT, && $P < 0.01$ vs. HFrHFD + OA + GW group, ### $P < 0.001$ vs. HFrHFD group. **, *** $P < 0.01$ and 0.001, respectively.

DISCUSSION

FFARs are important players in the regulation of both insulin secretion and sensitivity (Miyamoto *et al*., 2016). Therefore, the ligands of these receptors are promising modulators for IR and type 2 diabetes and can be broadly classified as follows: full agonists that activate both G-proteins and β-arrestin pathways, such as palmitic acid; G-protein-biased agonists; β-arrestin biased agonists; inverse agonists that downregulate the basal activity of both pathways to a lower level; and antagonists that have no intrinsic activity (Wootten *et al*., 2018).

OA is classified as a ligand for both FFAR1 and FFAR4 (Schnell, Schaefer, Schöfl, 2007; Kim *et al*., 2016). However, no information is available about the exact effect of OA on the downstream signaling of these FFARs. Moreover, the effect of OA on IR is highly controversial (Vassiliou *et al*., 2009). FFAR1 is highly expressed in the pancreas and, to a lesser extent, in the liver, whereas FFAR4 is mainly expressed in the adipose tissues and skeletal muscles. Thus, we assumed that FFAR1 has a potential role in regulating glucose homeostasis, which can be estimated using the glucose tolerance test. The liver is one of the main classical insulin target sites in the body and is associated with fatty changes in HFrHFD-fed mice. Therefore, this study aimed to investigate the acute effects of OA on glucose homeostasis and hepatic lipid signaling (PIP2 and DAG) in SCD- and HFrHFD-fed mice, focusing on the role of FFAR1.

The current study showed a slightly impaired glucose tolerance and AUC of the IPGTT and significant increases in the serum insulin levels and HOMA-IR index in the

HFrHFD-fed mice when compared to those in the SCD-fed mice. Furthermore, HFrHFD significantly decreased the hepatic level of PIP2, an important intracellular signal that enhances insulin signaling by elevating the Akt activity (Yamada *et al*., 2016). Moreover, HFrHFD significantly increased the hepatic DAG level, which contributes to IR by activating PKC and, subsequently, degrading IRS-1 (Jornayvaz, Shulman, 2012). These hepatic changes are consistent with the conditions of hepatic IR and fatty liver disease associated with this dietary model (Cornall *et al*., 2011).

By contrast, OA demonstrated time-dependent dual effects on the glucose tolerance in the SCD-fed mice. OA injection for 30 min significantly increased the blood glucose levels at 30 min of the IPGTT; however, a decrease was noted after 120 min when compared to the corresponding levels in the SCD group. A possible interpretation for this finding is that glucose tolerance is affected by both insulin secretion and insulin sensitivity. During the first 30 min of the IPGTT, the blood glucose level is mainly regulated by the rate and extent of insulin secretion; however, after 120 min of glucose load, the blood glucose level is mainly regulated by insulin sensitivity (Abdul-Ghani *et al*., 2007). Therefore, OA might have reduced insulin secretion and improved insulin sensitivity to a lesser extent in the present study.

Chronic use of OA significantly reduced serum insulin levels and slightly improved IR in HFrHFD-fed mice (Mansour *et al*., 2019). The suppression in the serum insulin level was thought to be mediated by the direct suppression of insulin secretion, in addition to the indirect compensatory effect of improved insulin sensitivity (Mansour *et al*., 2019).

In contrast to that in the glucose tolerance test, OA did not show significant effects on the hepatic levels of both PIP2 and DAG in the SCD-fed mice. Taking into consideration that both PIP2 and DAG act as downstream signaling molecules for FFAR1 and FFAR4 (Yamada *et al*., 2016), this finding might be attributed to the following reasons: the possibility that OA does not act as a full agonist for FFAR1 or FFAR4 and the low expression levels of both FFAR1 and FFAR4 in the liver (Yamada *et al*., 2016).

Pre-injection with GW1100 (selective FFAR1 blocker) significantly decreased the effects of OA on the 30-min, but not the 120-min, blood glucose levels of the IPGTT. This confirms that FFAR1 mediates the effects of OA on the 30-min blood glucose levels of the IPGTT.

OA is a ligand for both FFAR1 and FFAR4 (Schnell, Schaefer, Schöfl, 2007; Kim *et al*., 2016); hence, blocking FFAR1 will increase the concentration of OA on FFAR4, mainly in the adipose tissues. As shown previously, liver FFARs have no role in these effects. It is well established that FFAR4 can mediate insulin-sensitizing effects in the adipose tissue (Im, 2018). Therefore, we presume that the effect of OA on the 120-min blood glucose level was mediated by the FFAR4 in the adipose tissue.

In contrast, OA injection for 30 min in the HFrHFD-fed mice significantly decreased the blood glucose levels after 120 min of glucose load when compared to that in the HFrHFD group, without affecting the 0- and 30-min levels. A possible interpretation for this finding is that, the expression level of FFAR1 decreases in HFrHFD-fed mice (Shen *et al*., 2014). Alternatively, because of the increase in adipose tissue volume in HFrHFD-fed mice along with increases in fatty deposits in the other organs, the level of FFAR4 is greatly increased in HFrHFD-fed mice (Cornall *et al*., 2011). This explains the sharp decrease in the 120-min blood glucose level of the IPGTT in the OA-treated mice compared to the vehicle-administered HFrHFD-fed mice.

OA significantly increased the hepatic levels of PIP2 and significantly decreased the hepatic levels of DAG compared to those in the vehicle-administered HFrHFD-fed mice. The liver in the HFrHFD-fed mice is fatty with a high number of infiltrating fatty cells. Therefore, the level of FFAR4 in this fatty liver is higher than that in the SCD-fed mice (Cornall *et al*., 2011). OA competes with other circulating FFAs on the hepatic FFAR4 leading to an increase in PIP2 and reduction in DAG levels. However, it is not clear as to whether this effect is attributed to the blocking action of the circulating FFAs on these receptors or whether it involves the direct activation of the β-arrestin/PIP2 pathway by OA. Additional studies are required to explore this phenomenon.

Pre-injection with GW1100 significantly decreased the 0-min blood glucose level of the IPGTT compared to

that in the OA-treated mice. This effect may be attributed to both the blocking of FFAR1 and the increased OA concentration on FFAR4 as explained previously. However, the reason for the significant decrease in the hepatic level of PIP2 and significant increase in the hepatic level of DAG following pre-injection with GW1100 remains unclear and needs to be investigated in the future.

In conclusion, although OA slightly improved the insulin sensitivity in both SCD- and HFrHFD-fed mice, it impaired glucose homeostasis, possibly by affecting the insulin secretion through its effect on FFAR1. These findings indicate that OA might not act as a promising insulin sensitizer.

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