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Hormonal and enzymatic analysis for pancreas of diabetic and obese mice in Iraq

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ABSTRACT

For our knowledge, this appears to be first Iraqi study aimed for detection the concentration of pancreatic exocrine enzymes and endocrine hormones as well as lipid profile in obese, diabetic as well as obese/diabetic mice. Totally, 80 adult male mice, *Mus musculus* were selected, prepared, divided to 4 groups, and submitted to experimentally period continued for 2 months, July and August 2021. The findings of pancreatic enzymes showed a significant decrease in values of amylase in diabetic and obese/diabetic mice. In the case of lipase, insignificant differences were observed between obese/diabetics, but not in obese/diabetic group. No significant differences were found between the concentrations of chymotrypsin throughout all study groups. Trypsin reduction was observed in diabetic and obese/diabetic groups. The findings of pancreatic hormones detected higher concentration of gastrin in diabetic mice, and lowered in obese/diabetic ones. Glucagon elevation was found in diabetic and obese/diabetic groups, while reduction in diabetic group. Though obese mice were revealed a high insulin concentration, diabetic and obese/diabetic groups showed lowering. There was significant reduction in levels of somatostatin in mice of diabetic and obese/diabetic groups. Significant decreases in values of vasoactive intestinal polypeptide were observed in mice of diabetic and obese/diabetic groups. In the case of lipid profile, there were significant increases in values of triglyceride among the groups of diabetic and obese/diabetic groups. Significant HDL reduction were recorded in diabetic and obese/diabetic groups, while higher values in obese group. In the case of LDL, total cholesterol and total cholesterol / HDL ratio exhibited significant increases among mice of obese/diabetic group, while decreases in obese/diabetics. In conclusion, pancreatic exocrine enzymes were positively impaired in diabetic as well as obese/diabetic groups but not in obese group; whereas, pancreatic endocrine hormones and lipid profiles were affected among all diseased groups when compared to control group. The role of pancreatic enzymes as well as hormones in the pathogenesis of metallic disorders warrants further investigations.

Keywords: Exocrine, Endocrine, Pancreatic Insufficiency, Diabetes mellitus, Streptozotocin.
Article type: Research Article.

INTRODUCTION

Pancreas is one of the important solid organs of the gastrointestinal system, which extends in human from the duodenum to the spleen and immersed in fatty tissue that fills the space behind the stomach in animal models as mice (Frantz *et al.* 2012; Yu *et al.* 2019). This organ is unique in that having both an endocrine and exocrine gland (Dybala *et al.* 2020). The exocrine portion involved >95% of pancreatic mass that responsible for the production and secretion of digestive amylase, chymotrypsin, lipase, and trypsin enzymes into duodenum. Whereas, the endocrine portions involved 1-2% of pancreatic mass that responsible for production and secretion of gastrin, glucagon, insulin, somatostatin, and vasoactive intestinal polypeptide (VIP) hormones into blood (Longnecker *et al.* 2018; Miyake *et al.* 2018; Freeman *et al.* 2019). Diabetes mellitus (DM) is a chronic illness resulted due to lacking the secretion of insulin because a progressive or marked inability of pancreas to yielding of insulin, or presence a defect in uptake of insulin by tissues, which alter the metabolism of carbohydrate, fat and protein

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(Ozougwu *et al.* 2013; Al Goblan *et al*. 2014). In types 2 DM (DMT2), the presence of several newly variables such as increasing the obesity's incidence throughout all ages and sexes, physical activities, poorly diets and urbanization meaning that the numbers of diagnosed DM patient being elevated (Ershow 2009). Obesity is a global problem, reaching epidemic proportions in many industrialized as well as numerous developed countries causing a change in a health and policy focusing from undernutrition to obesity and obesity-related diseases (Villela *et al.* 2009; Haththotuwa *et al.* 2020). In individuals with obesity, the development of diabetes becomes more inevitable since there is insufficiency in secretion of insulin accompanied by resistance to insulin (De Boer *et al.* 2012; Al Goblan *et al.* 2014). Even in non-diabetic patients, obesity has been related to development of insulin resistance in liver and kidney due to effect of adipocyte, as an endocrine entity, in secretion of several proteins that known as adipocytokines (Koopman *et al.* 2009). Additionally, obesity considers a risk factor for diseases of pancreas such as pancreatic cancer and pancreatitis (Kim & Han 2012). Given that exocrine and endocrine pancreas are derived from the same origin *in utero*, neogenesis and trans-differentiation from an exocrine to an endocrine compartment in postnatal period have been recorded in animal studies, suggesting that there is continues interplay between an exocrine and an endocrine pancreas during the life (Saisho 2016). Worldwide, rare studies were carried out to investigate the association of pancreatic insufficiency concerning to obesity (Saisho 2016), fatty pancreas (Miyake *et al.* 2018), pancreatic cancer (Eibl 2020), as well as acute (Tu *et al*. 2017) and chronic (Diéguez Castillo *et al.* 2020) pancreatitis. However, no available studies were found in Iraq to detect the association of pancreatic insufficiency to obese and/or diabetic patients/lab animals. Therefore, this appears to be the first Iraqi study aimed to detect the levels of pancreatic exocrine enzymes and endocrine hormones in obese and diabetic mice with additional investigation of lipid profiles.

MATERIALS AND METHODS

Ethical approval

This study was licensed by the Scientific Committee of the College of Education for Pure Sciences, University of Wasit, Wasit, Iraq.

Study animals and design

Totally, 80 adult laboratory male mice, *Mus musculus* of 22-30 gram in body weight were selected and adapted one week to preparation period, during which, all examined animals were fed pellets and drunk the tap water at the same cage. For experimentally study that continued for 2 months (July & August 2021), mice were divided equally to four groups including control, diabetic, obese, and diabetic and obese.

Induction of diabetes mellitus and obesity

For obese induction, mice of obese as well as diabetic and obese groups were fed on high fat diets (20% kcal protein + 20% kcal carbohydrate + 60% kcal fat) for additionally 1month post preparation period and prior to diabetic induction. As described by Furman (2015), diabetes mellitus type 2 (DMT2) was induced in mice of diabetic as well as diabetic and obese groups following the Basic Protocols 3 and 4, respectively and using Beta-Nicotinamide mononucleotide (1 mL kg^{-1}) and Streptozotocin (2 mL kg^{-1}; Fousi Chemical, China). On experimentally 10^{th} day, the blood glucose concentration was evaluated from a tail vein blood sample using a One Touch Basic blood glucose monitoring system. Mice having blood glucose of >150 mg dL^{-1} (8.3 mmol L^{-1}) were considered to be diabetic.

Blood sampling

At the final of experimentally period, 0.7- 1.8 mL of whole blood was collected directly from the heart of each examined mouse. Each blood sample was divided into an EDTA tube and a glass gel free-anticoagulant tube to obtain plasma and sera, respectively.

Enzymatic and hormonal evaluation

Following the manufacturers' instructions of each mouse ELISA kit, sandwich technique was applied to measure alpha amylase (AMY2A; Cusabio, USA), trypsin (Cusabio, USA), lipase (Sunlong Biotech, China), glucagon (Sunlong Biotech, China), insulin (Sunlong Biotech, China), somatostatin (Sunlong Biotech, China), vasoactive intestinal peptide (Sunlong Biotech, China), high density lipoprotein cholesterol (HDL) (Sunlong Biotech, China), low density lipoprotein cholesterol (LDL) (Sunlong Biotech, China), and triglyceride (Sunlong Biotech, China). In addition, competitive and colorimetric techniques were used to measure gastrin (Lifespan BioScience, USA)

and chymotrypsin (Novus Biologicals, USA), respectively. All ELISAs' kits were measured at an optical density (OD) of 450 nm. In addition, concentrations of targeted parameters in sera and plasma samples were obtained using the standard curve throughout plotting the standard ODs in Y-axis, and the respective concentrations in X-axis with interpolating the ODs of sera and plasma to evaluate their concentration. Additionally, values of total cholesterol were detected through summation the values of HDL and LDL of each sample; and then, the values of total cholesterol / HDL ratio were measured for each sample in accordance with the our obtained values previously.

Statistical analysis

The study results were analysed by the GraphPad Prism (Version 6.0.1). Two-Way ANOVA was applied to detect statistical differences between values of examined groups. Variation was considered significant at a $p<0.05$. Each value was represented as mean ± standard error (M ± SE) and range.

RESULTS

Pancreatic enzymes

Significant variation ($p < 0.05$) in values of pancreatic enzymes were showed between the experimentally diseased study groups (obese, diabetic, obese and diabetic) and control group (Fig. 1). For amylase (mU mL^{-1}), insignificant variation ($p > 0.05$) was detected in values of obese (99.95 ± 5.09) group when compared to control (103.02 ± 4.87) group. However, significant decreases ($p < 0.05$) were detected in groups of diabetic (91.73 ± 5.31) and obese/diabetic mice (83.97 ± 3.56). Although concentration of lipase (pg mL^{-1}) was differed insignificantly between the groups of obese (34.51 ± 1.57) and diabetic (32.29 ± 1.45), compared to control (35.17 ± 1.31), but not in obese/diabetic (27.43 ± 1.7) group which exhibited a significant reduction in values of their animals. In the case of levels of chymotrypsin (ng mL^{-1}), insignificant variation ($p > 0.05$) was found in values of obese (2.79 ± 0.26), diabetic (2.65 ± 0.25), and obese/diabetic (3.03 ± 0.26) groups, compared to values of control group (2.58 ± 0.23). In the case of trypsin (ng mL^{-1}), though the findings of obese group (24.62 ± 2.69) were differed insignificantly ($p > 0.05$) with the values of control group (21.67 ± 2.19), significant reduction ($p < 0.05$) was observed in values of diabetic (27.59 ± 2.44) and obese/diabetic (33.15 ± 2.4) groups.

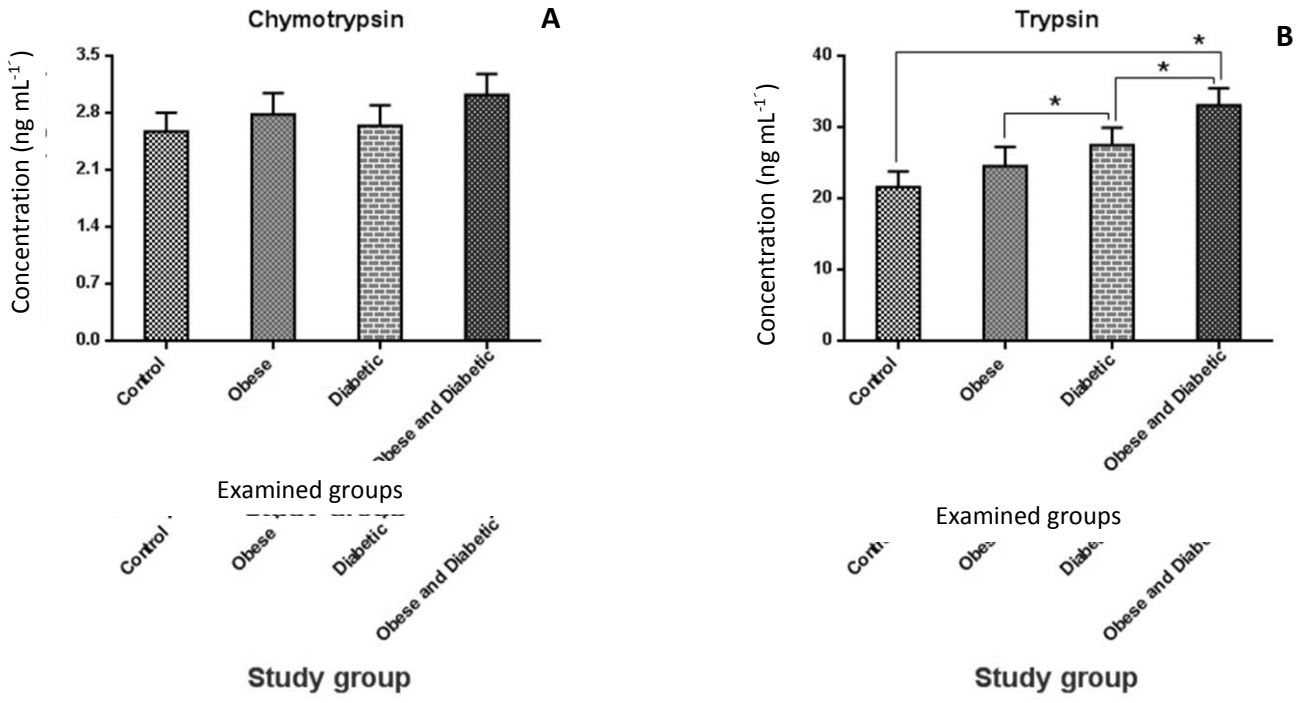


Fig. 1. (A-B). Concentration of pancreatic enzymes among examined groups.

The findings of pancreatic hormones revealed significant differences ($p < 0.05$) in values of obese, diabetic, in addition to obese/diabetics compared to control group (Fig. 2). Significantly, the highest concentration of gastrin (pg mL) was detected in diabetic mice (5.42 ± 0.2), whereas, the lowest was observed in obese/diabetic (3.25 ± 0.17) compared to obese (3.98 ± 0.24) and control (4.59 ± 0.31) groups. The highest levels of glucagon (pg mL^{-1}) were reported in diabetic (55.03 ± 4.08) as well as diabetic and obese (52.23 ± 3.58) groups, while the lowest level was recorded in diabetic (41.58 ± 2.88) in comparison with control group (49.83 ± 3.87). For insulin (mU L^{-1}), though mice of obese group revealed the higher concentration (3.06 ± 0.16), while the diabetic (1.84 ± 0.13) and obese/diabetic (1.67 ± 0.12) groups exhibited the lower concentration in comparison with control group (2.36 ± 0.15). Although, insignificant variation ($p > 0.05$) was observed between somatostatin levels (pg mL^{-1}) in obese (17.72 ± 1.39) and control (18.19 ± 1.33) groups, however, significant reduction ($P<0.05$) was found in those of diabetic (16.6 ± 1.28) as well as diabetic and obese (15.13 ± 1.2) groups. In the case of vasoactive intestinal polypeptide level (VIP; pg mL^{-1}), insignificant variation ($p > 0.05$) was recorded in diabetic (18.07 ± 1.03) and obese / diabetic (18.2 ± 1.04) groups; however, both groups exhibited the lowest significant levels ($P < 0.05$) compared to the obese (20.05 ± 1.06) and control (21.48 ± 1) groups.

Lipid profile

Significant variation ($p < 0.05$) was observed in the levels of lipid parameters among examined groups (Fig. 3). There were significant increases ($p < 0.05$) in triglyceride levels (ng mL^{-1}) of diabetic (74.96 ± 2.99) and obese/diabetic (77.51 ± 2.59) groups compared to obese (68.91 ± 3.56) and control (63.39 ± 1.77) ones. In the case of HDL (ng mL^{-1}), significant lowered levels ($p < 0.05$) were recorded in diabetic (11.71 ± 0.35) and obese/diabetic (11.27 ± 0.3) groups, while higher levels ($p < 0.05$) in obese (12.19 ± 0.38) and control (12.53 ± 0.29) ones. In addition, significant LDL (ng mL^{-1}) increases ($p < 0.05$) were observed in values of obese/diabetic group (633.59 ± 17), while significant reduction ($p < 0.05$) in obese (511.37 ± 18.6) and diabetic (537.3 ± 15.15) groups that both were higher than those in control group (425.37 ± 15.44). In the case of both total cholesterol level (ng mL^{-1}) and total cholesterol/HDL ratio, significant elevations ($p < 0.05$) were found in obese/diabetic group (642.26 ± 17.45 and 58.63 ± 2.04), while significant decreases ($p < 0.05$) in obese (524.33 ± 18.92 and 43.31 ± 1.89) and diabetic (548.61 ± 16.73 and 47.11 ± 1.72) groups respectively. However, all experimentally diseased groups were revealed high levels than control group (436.87 ± 16.85 and 35.02 ± 1.6 respectively).

DISCUSSION

Obesity has become a worldwide epidemic in the 21st century. In American alone, over 1/3 of adults are currently obese, with continuous rising of incidence rate in particular in younger ages resulting in significantly increasing the risk of numerous acute and chronic diseases like DM (Papachristou *et al.* 2006; Nöthlings *et al.* 2007; Flegal *et al.* 2010). Our findings revealed significant effects of obesity and DMT2 on the mice pancreatic exocrine enzymes and endocrine hormones as well as serum lipid profiles. We indicated that pancreatic enzymes were not impaired among obese group, compared to control one. However, significant decreases in amylase and significant elevation in trypsin were found clearly among the diabetic and obese/diabetic groups. In addition, lipase level was decreased significantly in the obese/diabetic group only, while chymotrypsin level was not affected significantly among all experimentally diseased and control groups. In the case of amylase, the findings of this study were in contrast to those found by Afsartala *et al.* (2016) who detected the over-expression of amylase in obese mouse hepatocytes. We suggest that there were minimum pathological changes due to obesity on the serum amylase level. Similarly, Yadav *et al.* (2013) reported significant reduction in the amylase of human individuals with DM in comparison with control group, and suggested that low serum amylase in those individuals might be related to insulin action impairment as a result of resistance to insulin or inadequately secretion of insulin. (Ko *et al.* 2020) concluded that low concentration of amylase could be related significantly to DMT1, DMT2, excess deposit, and metabolic syndrome. Significant decreasing of lipase in mice of obese/diabetic group might explain by the direct interaction between lipase and insulin. Shimada *et al.* (1995) investigated the role of lipase in mice with diabetes mellitus, and found that the lipase activity was decreased significantly in skeletal and cardiac muscles of diabetic mice suggesting that its activity in DM is regulated specifically by tissues. Fex *et al.* (2006) reporting that lipids are involved in β-cells stimulus-secretion coupling, and that lipase in β-cells is necessary for generating the coupling factors from intracellular lipids.

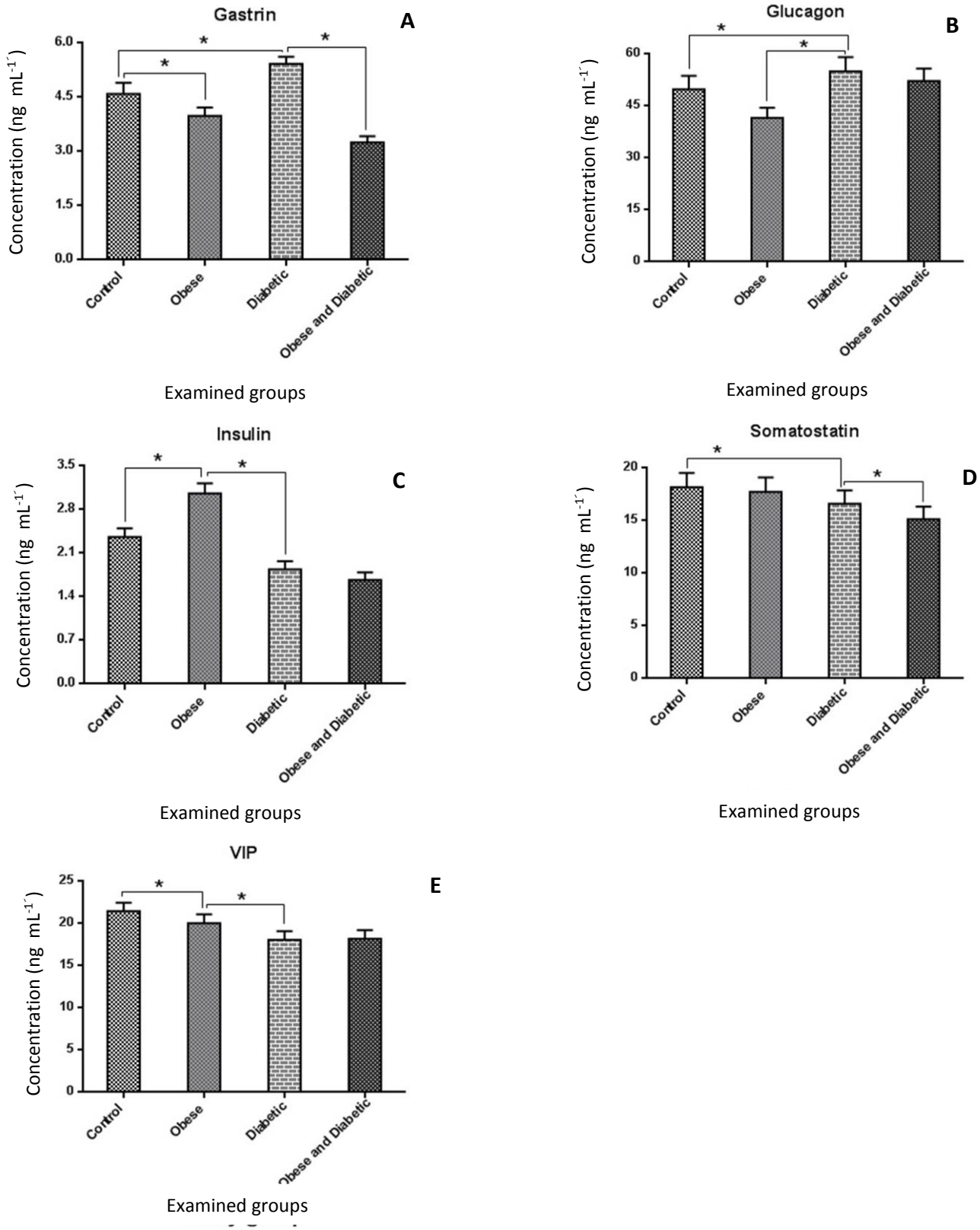
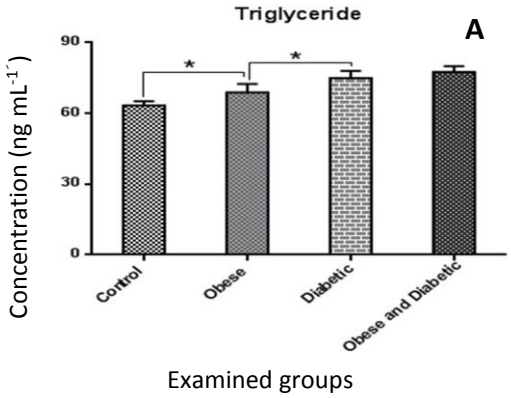
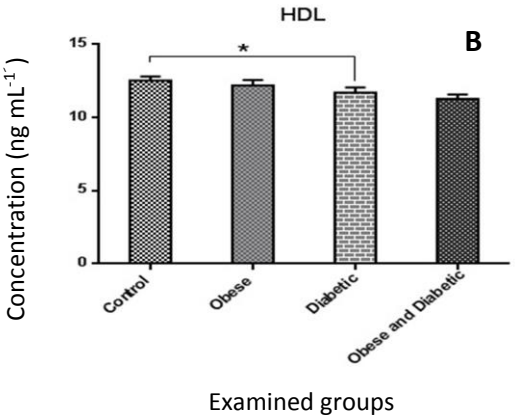
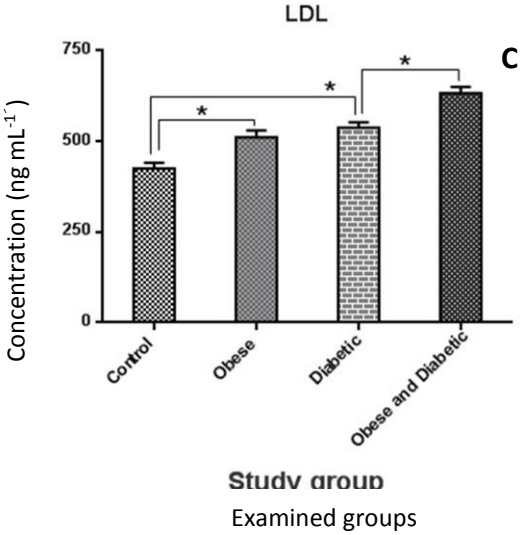
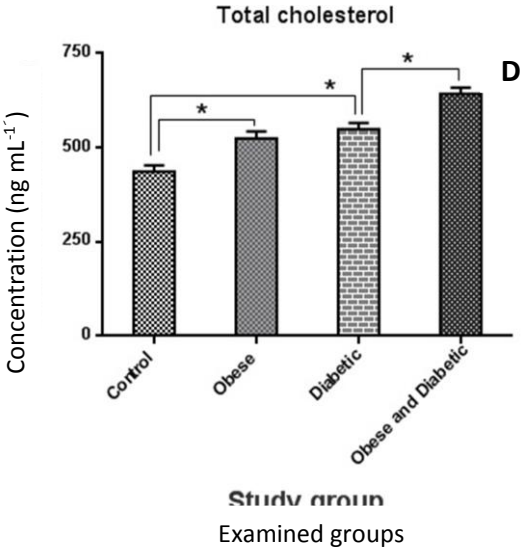


Fig. 2. (A-E). Pancreatic hormones concentrations among examined groups; A: Gastrin; B: Glucagon; C: Insulin; D: Somatostatin; E: VIP.









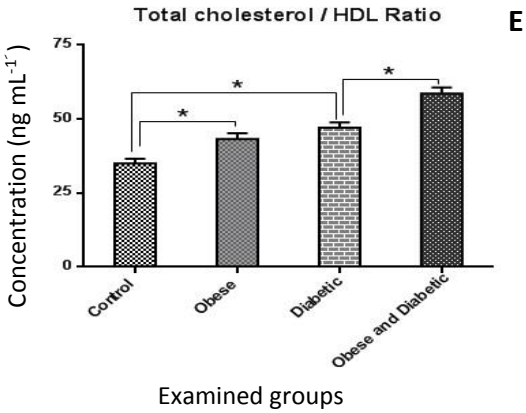


Fig. 3. (A-E). Lipid profile levels among examined groups; A: Triglyceide; B: HDL; C: LDL; D: Total cholesterol; E: Total cholesterol / HDL ratio.

Other studies demonstrated that resistance to insulin is related to rising lipid contents (Badin *et al.* 2011; Schweiger *et al.* 2017), and that deficiency of lipase influences the lipolysis and declines resistance to insulin induces by some diets (Taschler *et al.* 2011). In the case of trypsin, the mechanism that underlies the higher levels in obese/diabetic mice is not completely understood but may involve abnormal interaction between the endocrine and exocrine pancreas caused by β-cell dysfunction. In previously performed studies, it was found that trypsin had a marginal effect on glucose uptake (Barnett & Whitney 1965), and that its level represent a qualitative index for decreasing the function of pancreatic exocrine in DM, but without value in quantifying the degrees of insufficiency (Frier *et al.* 1980). Other authors summarized that the activity of pancreatic trypsin was increased significantly by replacement of insulin due to the role of trypsin in conversion of proinsulin to insulin (Duan *et al.* 1989) or degradation of insulin (Schilling & Mitra 1991).

In our study, there is evidence that the pancreatic hormones were influenced effectively due to the induction of obesity, diabetes mellitus or both. In comparison with mice of control group, the gastrin were elevated in diabetic group, and lowered in the obese and obese/diabetic groups, which is in agreement with results obtained by other authors (Kaba *et al.* 2015; Rehfeld 2016). Suarez-Pinzon *et al.* (2008) showed that treating non-obese diabetic mice using a combination of glucagon-like peptide-1 and gastrin stimulate the growth of islet cells and secretion of insulin to an extent that completely restored normal glycemia. In previous study (Starosel'tseva *et al.* 1988), it was shown that food intake correlates to elevating gastrin concentration in DMT2 individuals, but not with body mass and total level of insulin, suggesting that gastrin is related to an actively metabolic forms of insulin. In contrast to insulin, the glucagon level increased significantly in diabetic and obese/diabetic groups, but not in obese group that exhibited significant reduction in its level.

The opposite effects of insulin and glucagon in fuel homeostasis, the paracrine / endocrine inhibitory influences of insulin on glucagon secretion and the hyperglucagonemia in the pathogenesis of DMT2 have long been recognized (Godoy Matos 2014). Glucagon is an insulin counter-regulatory hormone produced from the pancreatic α-cells as a result of hypoglycemia. The rising of counter-regulatory hormones such as glucagon and epinephrine and suppressing the secretion of insulin represent the main protective mechanisms toward hypoglycemia (Haymond *et al.* 2019). However, hyperglucagonemia is a hallmark for obese and insulin resistance individuals promoting to hepatic glucose output, exacerbating hyperglycemia and predisposing to development of DMT2 (Stern *et al.* 2019). Therefore, decreased insulin secretion and upraised glucagon production in DM can influence the normal pancreatic milieu through reducing the total volume of pancreas as well as secreting amylase and bicarbonate (Yadav *et al.* 2013). Several studies have confirmed that levels of sera glucagon are increased in individuals with obesity (Madsbad 2014; Del Prato *et al.* 2021) and resistance to insulin (Okamoto *et al.* 2017; Adeva Andany *et al.* 2019). In addition, meal-induced suppression of glucagon could blunt in individuals having resistance to insulin (Fuglsang-Nielsen *et al.* 2020; Sharma *et al.* 2020). Accordingly, investigation concerning to the influence of feeding and fasting on insulin, glucagon, and glucagon-insulin ratio in obese individuals is of great importance to understand the hormonal regulation of hepatic metabolic response and metabolic alteration related to insulin resistance (Longuet *et al.* 2008; Foghsgaard *et al.* 2017; Hædersdal *et al.* 2018; Stern *et al.* 2019). We found that somatostatin reduced insignificantly in obese mice group, but more significantly in diabetic and obese/diabetic ones.

Throughout several peptide types participated in behaviour regulation of food-seeking, somatostatin displays a limitative activity on complicated process with maintenance releasing and secreting other types of peptide, integrity of neurons, as well as regulation of hormones (Kumar & Singh 2020). In addition, it serves as a link between peripheral and central tissues with an important effect on the behaviour of food intakes and expenditure of energy in obese patients (Nagulesparan *et al.* 1979; Boehm 2003; Surya *et al.* 2009; Gerich 2019; Kumar & Singh 2020). The existence of this hormone in D cells of pancreatic islets suggested that it might play a locally role in regulating the secretion of glucagon and insulin, and the altered functions of these cells in animal-model suggest that the peptides might include in diabetes pathogenesis (Somvanshi *et al.* 2018; Ni *et al.* 2021). Henquin *et al.* (2017) investigated the somatostatin stores in pancreas obtained at autopsy, and found that the peptide content was lower in DMT2. Hence, clinical studies confirmed the important application of this hormone in diabetic patients and its complications like obesity, nephropathy and retinopathy due to inhibition of insulin-like growth factor 1 (IGF-1) as well as the vascular endothelial growth factor (VEGF) together with insulin secretion and effects upon rennin-angiotensin-aldosterone system (Rai *et al* 2015; Gomes Porras & Cárdenas Salas 2020). The results of this study exhibited significant decreases in VIP levels among all experimentally diseased groups,

in particular, diabetic and obese/diabetic groups. These findings were similar to those reported by other authors (Baranowska 1991; Adeghate *et al.* 2001; Hogenboom *et al.* 2019; Atas *et al.* 2021). Many studies have reported the important role of VIP in the regulation of normal gut motility (Cao *et al.* 2005), intestinal secretion (Wu *et al.* 2015), and in water and ion transport in the gut (Jayawardena *et al.* 2017). In addition, VIP-containing neurons play a role in reception, accommodative relaxation and opening gastrointestinal sphincters (Lelievre *et al.* 2007; Iwasaki *et al.* 2019). Because of its broad spectrum of biological functions, VIP has emerged as a promising therapeutic agent for the treatment of many autoimmune diseases including diabetes (Sanlioglu *et al.* 2012; Vu *et al.* 2015; Iwasaki *et al.* 2019). Adeghate *et al.* (2001) reported that the number of VIP-immunoreactive neurons was significantly lower in the GIT of rats suffering from diabetes, suggesting this loss to macro- and micro-angiopathies, consistent complications of diabetes mellitus that resulting also in poor blood supply and noxious metabolites. Moreover, the ability of the local neurons to synthesize and / or store VIP might be impaired in diabetes (Ganea *et al.* 2015).

To determine the extent to which metabolic status influences insulin response of pancreatic islets to VIP, the insulin resistance has been investigated in obese mice and the findings revealed that VIP induces a strong insulin secretion from islets isolated from young and obese mice (Persson Sjögren *et al.* 2006). Thus, deregulated VIP signalling might be responsible for the reduced glucose-induced insulin secretion observed in patients with DMT2 and / or elderly individuals (Sanlioglu *et al.* 2012). Among findings of lipid profile, we found a significant increase in the triglyceride, LDL and total cholesterol levels as well as total cholesterol / HDL ratio with significant reduction in HDL level in the diabetic and obese/diabetic mice groups. Abnormalities in lipid metabolism are very commonly observed in patients who are obese, as approximately 60-70% of obese patients are dyslipidemic (Feingold 2020). Several lipid abnormalities have been reported in obese patients including particularly elevated triglyceride, LDL, VLDL, and apolipoprotein (Apo) B in addition to typically decreasing levels of HDL and Apo A (Lu *et al.* 2011; Klop *et al.* 2013; Firdous 2014). Although high triglyceride does not cause diabetes, instead, their levels indicate that your system for turning food to energy is not working properly. There is abundant evidence indicating the connection between triglyceride and DMT2, in addition to reports confirmed that high triglyceride may predict the incidence of DMT2 independently (Zhao *et al.* 2019). The potential mechanism linking triglyceride to DMT2 might refer the free fatty acids metabolic pathways, as a large amount of free fatty acids and other compounds released by oversized adipose tissue can produce insulin resistance (Boden 2008). However, insulin deficiency may cause a significant loss of adipose tissue by enhancing the lipolytic process (Blüher 2016).

Lipase is believed to play a role in the process of fat deposition through hydration of triglyceride on the capillary endothelium and mediates the uptake of free fatty acids by adipose tissue resulting in accumulation of triglycerides in adipose tissue (Jaworski et al., 2007). Goldberg *et al.* (2008) mentioned that recent guidelines consider the presence of diabetes as equivalent to the presence of known hyperlipidemia, and concluded that diabetic cholesterol-fed mice developed hyperlipidemia due to a non-LDL receptor defect in clearance of circulating ApoB-containing lipoproteins. Other studies suggested that anti-inflammatory function and anti-oxidant activity of HDL was altered or impaired in obese patients, compared to healthy ones (Sorrentino *et al.* 2010; Kim *et al.* 2015; Mousum *et al.* 2018). A number of authors investigated and concluded that the triglyceride/HDL ratio could be used as a potential cheap and available surrogate marker for insulin resistance and as a predictor of DMT2 in clinical practice (Casoinic *et al.* 2016; Femlak *et al.* 2020; Ganeva *et al.* 2021).

CONCLUSION

The results of this study indicated that pancreatic exocrine enzymes are positively impaired in diabetic as well as obese/diabetic groups but not in obese one, whereas pancreatic endocrine hormones and lipid profiles were affected among all experimentally-diseased groups compared to control. The worldwide rising incidence of obesity and DMT2 should lead to a surge in basic research to understand the specific mechanism by which pancreatic insufficiency has begun or emerge.

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