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Could Increased *Glp1r* Expression via Sitagliptin in the GLP-1/GLP-1 Receptor Axis in the Diet-Induced Obesity Rat Model be Important in Liver Metabolism?

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ABSTRACT

Objective: The aim of this study was to evaluate the contribution of sitagliptin, which is used in the treatment of type 2 diabetes mellitus due to its insulinotropic effects, to the levels of glucagon-like peptide-1 (GLP-1) expressed in many systemic tissues in obesity, in liver, skeletal muscle, and fat tissue.

Materials and Methods: Adult Wistar albino rats (n=32) were randomly divided into four groups for 16 weeks of intervention. These groups were control (C) (n=8), obese (Ob) (n=8), sitagliptin (C+Stg) (n=8), and obese (Ob+Stg) given sitagliptin (n=8). *Glp1r* expression in rat liver, muscle, and adipose tissue was confirmed by quantitative real-time PCR.

Results: No significant change was detected in *Glp1r* expression levels in muscle and fat tissue in 4 groups. A 10.64-fold increase in *Glp1r* gene expression was observed in Ob compared to C (p=0.008). Additionally, a 4.03-fold increase in expression level was found in Ob+Stg compared to Ob (p=0.02) and a 12.52-fold increase in expression level was found in Ob+Stg compared to C (p=0.01).

Conclusion: The increased *Glp1r* expression intensity in obese individuals using sitagliptin compared with controls and obese individuals not using sitagliptin may play a role in the reorganization of liver metabolism that is impaired due to obesity, such as the gluconeogenesis process.

Keywords: Sitagliptin, GLP-1, obesity, liver metabolism, Glp1r, GLP1R

INTRODUCTION

Obesity is a risk factor for metabolic and cardiovascular complications and is a health problem with an increasing prevalence worldwide. Type 2 diabetes mellitus (T2DM), a major component of obesity, is associated with impaired glucose tolerance due to insulin resistance. Pancreatic beta cell damage affects insulin secretion and disrupts carbohydrate catabolism in the liver, adipose tissue, muscle, and many other tissues. Glucagon-like peptide-1 (GLP-1) regulation can be a therapeutic target for the correction of these metabolic problems (1).

GLP-1 and glucose-related insulinotropic peptide (GIP) are the main hormones involved in glucose regulation (2). Both the GIP receptor (GIPR) and the GLP-1 receptor (GLP1R) bind to the G protein-coupled receptor family, activate adenylate cyclase, and cause the activation of cAMP and protein kinase A. K^+ ions pass out of the cell and Na^+ ions pass into the cell, causing membrane depolarization, increasing insulin secretion together with the increase in intracellular

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Ca^{+2} (3). GLP-1 receptors are expressed in many systems, especially the nervous, circulatory, and respiratory systems. GLP-1 secretion of glucagon results in indirect stimulation of insulin and/or somatostatin secretion. The cellular mechanisms and effects of GLP-1 in preventing glucagon secretion may be better characterized (4).

In healthy individuals, after food intake, the previously released storage insulin (phase 1 insulin) is normally released from the pancreatic beta cells. Then, the insulin produced in the β cells (phase 2 insulin) is released and used. The intestinal hormones GLP-1 and GIP play important roles in glucose homeostasis by stimulating insulin release from the pancreas in the physiological axis and inhibiting glucagon secretion after food intake (5). In patients with T2DM and obesity, the insulin response that should increase after carbohydrate intake is reduced or delayed, glucagon secretion increases, resulting in postprandial hyperglycemia. In other words, pancreatic insulin secretion cannot occur at an adequate level due to the effect of GLP-1, and the insulinotropic effect of incretin hormones in the peptide structure is reduced in obese and T2DM patients (6). Dipeptidyl peptidase-4 (DPP-4) inhibitors are in the group of oral antidiabetic drugs and are currently used in the treatment of T2DM. DPP-4 inhibitors used to treat T2DM prevent the degredation of GLP-1, causing incretin such as GLP-1 to secrete insulin in response to increased blood glucose levels and lower blood glucose levels. This provides lower HbA1c levels (7, 8, 9). GLP-1 improves glucose tolerance and pancreatic α- and β-cell function by suppressing glucose-dependent insulin stimulation and glucagon secretion. It also has extrapancreatic effects, such as slowing gastric emptying and suppressing appetite. Inhibition of DPP-4 activity prolongs the half-life of intact biologically active GLP-1 (10).

Clinical investigations in the literature have focused on the correlation of GLP-1 levels with serum levels, and the reflections of sitagliptin on tissue biochemistry are quite limited. We aimed to evaluate the role of sitagliptin in increasing Glp1r (also known as GLP-1; GLP-R1) expression in muscle, liver, and fat tissue of Wistar albino rats.

MATERIALS AND METHODS

Animals

In this study, 32 Wistar albino adult male rats were provided by Gaziantep University Experimental Animal Research Center. For the study, Animal ethics committee approvals were obtained from Gaziantep University Animal Experiments Local Ethics Committee (HADYEK) dated 10.02.2020, numbered 133 protocol 2020/5 and dated 07/06/2020, numbered 32 Protocol 2020/16.

Animal Feeding Protocol and Tissue Procurement

The animals were cared for and fed at the Gaziantep University Experimental Animal Research Center in a room maintained at 20-24°C and 45-65% humidity during a 12-hour day and night cycle. The control (C) and sitagliptin-control (C+Stg) groups were fed with normal rat chow for 16 weeks, while the obese (Ob) and sitagliptin-obese (Ob+Stg) groups were fed with a high-fat diet (60%). The high fat diet content was 200 g of casein, 3 g of L-cystine, 7.8 g of corn starch, 100 g of maltodextrin10, 172.8 g of sucrose, 50 g of cellulose, 25 g of soybean oil, 177.5 g of animal fat, mineral mixture S10026, 13 g of dicalcium phosphate, 13 g of potassium citrate, 10 g of vitamin mixture W10001 10 g, Choline bitartrate 2 g, Yellow food coloring 0.05 g) (Arden AS, Ankara, Turkiye).

In sitagliptin dose selection, the dose with the lowest liver toxicity and highest bioavailability was selected according to the results of a previous study (11). Sitagliptin (Merck Pharmaceuticals) containing groups were created by gavage at a dosage of 10 mg/kg/day once daily for the second 8 weeks. As a sitagliptin source, sitagliptin hydrochloride monohydrate was prepared by dissolving it in 750 µL of distilled water with the help of a vortex. At the end of the sixteenth week, general anesthesia (Xylazine-10 mg/kg + Ketamine- 90-100 mg/kg, intraperitonial) was applied to obtain liver, muscle, and fat tissue samples from all groups, and the tissues were collected. Collected tissues were stored at -80°C.

RNA Isolation and Quantitative Real-Time Polymerase Chain Reaction

A tissue homogenate was created using ceramic bead tubes (Lysing Matrix D 2 mL MP BIO) for 50 mg tissue according to the manufacturer's instructions. RNA was obtained after alcohol-based washing and elution via spin column filter according to the kit instructions (GeneAll Cat No./ID: 305101). Measurements were performed using a spectrophotometer (NanoDrop 8000, DE 19810, Thermo Fisher, USA). cDNA synthesis was performed by following the instructions of the kit containing the Reverse Transcription enzyme (A.B.T.™ GenEx SYBR Assay, South Korea). Expression of the detected *Glp1r* gene was performed using quantitative real-time PCR (qRT-PCR) with Sybr Green PCR Master mix (A.B.T.™) with qRT–PCR Rotor Gene Q (Qiagen). According to the assay protocol, the primer annealing temperature was set to 60°C, and 45 qRT–PCR cycles were performed. Ct values were evaluated according to the formula $2^{-\Delta\Delta Ct}$ to calculate the relative fold change. In this calculation, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as an internal control in all tissues.

Statistical Analyses

The Ct values of *Glp1r* and the Ct values of *GAPDH* as the reference gene were determined for 4 groups in triplicates. The data normalized with *GAPDH* were used to determine fold changes between groups using the $2^{-\Delta\Delta Ct}$ formula. When the distribution of data from the samples was examined, it was observed that they did not show a normal distribution. Accordingly, due to the small number of tissues, the evaluation was performed using the Mann–Whitney U test. p values less than 0.05 were accepted as the significance limit.

RESULTS

Liver *Glp1r* Expression

The changes in the C+Stg, Ob, and Ob+Stg groups and other changes compared with group C are shown in Table 1 and Figure 1. Although a 1.17-fold (0.85 fold change) decrease in *Glp1r* expression was observed in C compared with C+Stg (p=0.84), and a 42.18-fold increase in *Glp1r* expression was observed in Ob+Stg compared with C (p=0.08), no statistical significance was found. On the other hand, a 10.64-fold increase in *Glp1r* gene expression was observed in Ob compared with C (p=0.008). In addition, a 4.03-fold increase in expression level was found in Ob+Stg compared with Ob (p=0.02), and a 12.52-fold increase in expression level was found in Ob+Stg compared with C (p=0.01).

Glp1r Expression in Skeletal Muscle and Lipid Tissue

The distribution of other groups according to C and fold change are shown in Table 2 and Figure 2. Compared with C, there was a 12.35-fold increase in *Glp1r* gene expression in C+Stg (p=0.14), a 2.95-fold increase in Ob (p=0.22), and a 4.75-fold decrease in Ob+Stg (fold change 0.21, p=0.039). Compared with the C+Stg group, there was a 41.47-fold decrease in the Ob+Stg group (p=0.57), and a 5.22-fold decrease in the Ob group (p=0.37). In addition, although mild, there was a 0.08-fold decrease in Ob+Stg compared with Ob (p=0.9).

DISCUSSION

DPP-4 inhibitors are agents that regulate glucose in an insulinotropic manner via GLP-1 (12). In a sitagliptin-treated T2DM rat model study, showed no change in blood glucose levels but decreased body weight (13). In a study conducted on obese individuals with T2DM, an increase in CB-1R level was detected in diabetic liver after treatment with sitagliptin. It was also reported that weight decreased in both the control and diabetic groups (14). In a study conducted on obese insulin-resistant subjects, an increase in the expression of GIP was detected. It was determined that the receptor status and GLP-1 signaling in adipose tissue increased in obese and insulin-resistant patients (15). In contrast to this study, no significant results were found for *Glp1r* expression in adipose tissue compared to our study when compared with the control. In a study conducted by Prakash et al. in 2020, they found that sitagliptin regulated adiponectin and activating protein kinase (AMPK) levels in the liver of obese mice (16). They reported that it reduced the amount of adipose tissue that is caused by obesity and that the increase in GLP-1 levels in metabolic syndrome and fatty liver had an effect that is independent of insulin (16). In a study conducted by Nahon et al. in 2018 on patients with prediabetes, they reported that they detected an increase in the expression of the PPAR-γ coactivator-β (*PGC1β*) gene, which encodes the mitochondrial biogenesis inducer in skeletal muscle, after 12 weeks of sitagliptin administration in males with prediabetes (17). In another study, the increase in genes responsible for the oxidation of fatty acids was evaluated through insulin sensitivity, and it was found that this process was supported by the use of sitagliptin (18, 19). In another study that detected a critical decrease in plasma glucose levels due to 5 weeks of sitagliptin use, the *GIP* expression level increased during this period, but there was no major change in adipose tissue (20). Similarly, significant decreases in VLDL and triacylglycerol levels were detected in overweight men after 6 weeks of sitagliptin use (21, 22). In a study conducted by Li et al. in 2017, sitagliptin and metformin were compared in a study conducted for the treatment of T2DM with an observation period of 12-24 weeks. In this study, they showed that the use of sitagliptin alone reduced weight gain and hypoglycemia. In obese patients, insulin treatment reduced body mass index, hypoglycemia, and cholesterol levels. In this regard, it was reported to be more effective than metformin (23). Supporting this, it has been reported that sitagliptin plays a role in reducing the adiponectin/leptin ratio (24). Although this ratio, which shows the dysfunction of the adipose tissue, suggests that sitagliptin may be an important mediator in lipid biochemistry, no significant result was obtained from the Glp1r level in our study. This may be due to the longer-term effect of sitagliptin on the adipose tissue or, as in the previous study, the pathophysiological process in the adipose tissue may be due to adiponectin/leptin and may be secondary to other factors.

In recent years, the roles of sitagliptin in regulating some pathophysiological processes that play a role in obesity and obesity-related disease patterns have been investigated. Results have been obtained indicating that sitagliptin may be effective in suppressing the increased inflammatory response in obesity via cytokines and in eliminating the oxidative

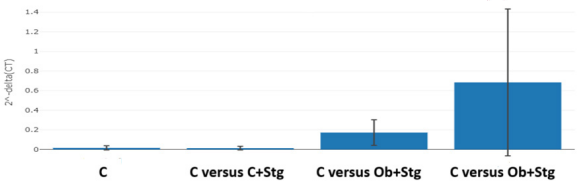

Figure 1. Distribution of *Glp1r* gene expression in liver according to groups.

Table 1. Comparison of *Glp1r* expression in the liver between groups and according to sitagliptin

Group	Fold Change	p value
C versus C+Stg	0.85	0.84
C versus Ob	10.64	0.008
C+Stg versus Ob+Stg	42.90	0.09
C versus Ob+Stg	12.52	0.01
Ob versus Ob+Stg	4.03	0.02
Ob versus C+Stg	42.18	0.08

Experimed 2024; 14(3): 210-214

Table 2. Comparison of *Glp1r* expression in lipid and muscle tissue between groups and according to sitagliptin

GROUPS	Fold Change	p value
Muscle Tissue		
C versus C+Stg	12.35	0.14
C versus Ob	2.95	0.22
C versus Ob+Stg	0.21	0.39
C+Stg versus Ob+Stg	-41.47	0.57
C+Stg versus Ob	-5.22	0.37
Ob versus Ob+Stg	0.08	0.91
Lipid Tissue		
C versus C+Stg	4.55	0.11
C versus Ob	395.03	0.33
C versus Ob+Stg	0.10	0.14
C+Stg versus Ob+Stg	-28.23	0.20
C+Stg versus Ob	86.77	0.33
Ob versus Ob+Stg	0.01	0.24

load brought about by inflammation via malondialdehyde (MDA) (25). As part of this process, because its atherogenic activity can be suppressed via lncRNAs targeting *GLP-1*, and thus endothelial nitric oxide synthase (eNOS) activity can be restored, results obtained can be interpreted as protective of the vascular system (26).

The limited sample size, lack of blood glucose and lipid levels, and single-dose sitagliptin administration are among the limitations of this study. Despite this, some important findings were identified in our study. Increasing the expression of *Glp1r*, which is known to reduce gluconeogenesis in the liver and reduce liver steatosis and thus have a blood sugar-regulating effect, is an important therapeutic target in obesity, which is a multifactorial condition. The increase in *Glp1r* expression between the control and obese groups in our study shows that we have created an experimentally accurate obesity model. This suggests that sitagliptin can be used to regulate liver metabolism to reverse obesity. In obese patients, muscle atrophy in muscle tissue, high fat retention in muscle, and impaired amino acid metabolism are also factors that increase progression. Increased muscle perfusion induced by GLP-1 receptor-mediated signals and the transport of oxygen and insulin to myocytes are among the factors that contribute to progression. In our study, *Glp1r* levels did not significantly increase in the sitagliptin-treated groups. This suggests that sitagliptin does not play an extensive role in the correction of muscle-based pathophysiological processes in obesity. Similarly, although a decrease in *Glp1r* was observed in Ob+Stg compared with C in fat tissue, no statistical relationship was found. This suggests that sitagliptin is limited in regulating processes such as fatty acid oxidation, intracellular cholesterol transport, and white adipocyte differentiation, which are associated with adipose tissue-based pathophysiology in obesity.

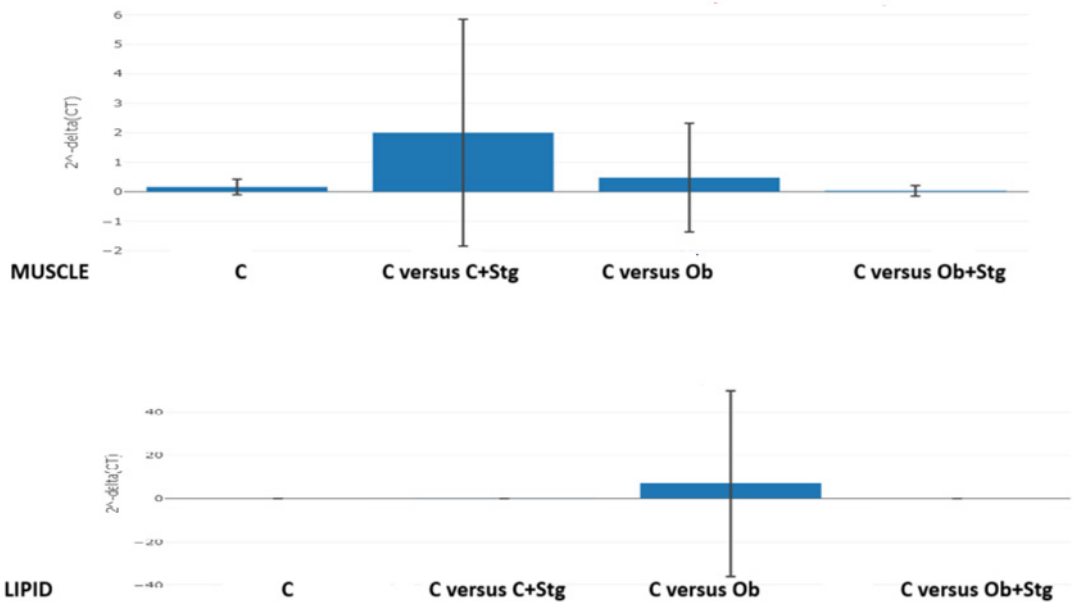

Figure 2. Distribution of *Glp1r* expression in lipid and muscle tissues according to groups.

Ethics Committee Approval: For the study, Animal ethics committee approvals were obtained from Gaziantep University Animal Experiments Local Ethics Committee (HADYEK) dated 10.02.2020, numbered 133 protocol 2020/5 and dated 07/06/2020, numbered 32 Protocol 2020/16.

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