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Effects of Oral Anti-diabetic Agent Sitagliptin on Total Antioxidant and Oxidant Status in Rats with Type 2 Diabetes Mellitus

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Abstract
With the presented study, it is aimed to examine the effect of dipeptidyl peptidase (DPP) 4 inhibitor sitagliptin, which is an oral anti-diabetic medicine, on total antioxidant and oxidant status in the rats which were induced with the experimental type 2 diabetes (T2DM) model. The study was planned to cover 24 weeks. In rats that are left out of the control group, after 8 weeks of High-Fat Diet (HFD) feeding, the experimental T2DM model was constructed by a low double dose streptozotocin (STZ) injection per week. Sitagliptin was applied to treatment groups at a dose of 10mg/kg-day. At the end of the study, Fasting Plasma Glucose (FPG), ALT, AST, LDH, HbA1c, insulin, Total Antioxidant Status (TAS), Total Oxidant Status (TOS) levels and Oxidative Stress Index (OSI), HOMA-IR and HOMA-β indices were measured. It was determined that sitagliptin decreased FPG and HbA1c levels, increased insulin levels, and did not affect ALT, AST, LDH, TOS levels and OSI in the diabetic treatment group compared to the diabetic group. However, it was identified that sitagliptin decreased TAS levels in pancreatic tissue in diabetic rats, and decreased TOS levels and OSI in the HFD group. It was detected that in diabetic rats sitagliptin balances glucose homeostasis and protects beta cells; whereas it increases oxidative stress in pancreatic tissue and conversely it decreases oxidative stress in pre-diabetic obese rats (HFD groups).

Key words: Type 2 diabetes, Sitagliptin, Oxidative stress, TAS, TOS, OSI

INTRODUCTION

Type 2 Diabetes Mellitus (T2DM) is a social problem, which has serious results regarding the patient and the society, and its incidence increases day by day in the world. Genetic factors, inactive life style, and obesity play an important role in the development of T2DM. Because the anti-diabetics used in the treatment of T2DM have side effects such as weight gain, hyperglycemia, and gastrointestinal research related to new treatment options are still in the center of attention [1, 2].

In this context, recently a new oral anti-diabetic agent, sitagliptin, which is a dipeptidyl peptidase 4 (DPP 4) enzyme inhibitor started to be used in the clinics in 2006 [3]. Sitagliptin is reported to ensure and tolerate glucose control safely even in high doses in T2DM treatment while minimizing side effects such as dose problems, weight gain, and hypoglycemia [4]. Sitagliptin is suggested to be used as mono-therapy [5, 6] or combined with other anti-diabetics [7, 8]. DPP 4 activity is known to increase in T2DM patients [9]. DPP 4 inactivates the incretin hormones (GLP-1 and GIP) which are produced in the bowels as glucose is taken to the body, and which alert the insulin synthesis or secretion from the pancreas. Consequently, enough insulin cannot be secreted from the pancreas and hyperglycemia develops [10, 11].

Sitagliptin prevents incretins from being inactivated by inhibiting DPP 4. Therefore, by contributing to pancreatic insulin synthesis and secretion, it both protects beta cells and balances glucose homeostasis [3, 12].

One of the main purposes of diabetes treatment is to protect the patient from the complications of diabetes. Oxidative stress is one of the main factors playing a role in the development of diabetes complications such as retinopathy and nephropathy. Because of this, the agent used in diabetes treatments to reduce oxidative stress is important in terms of preventing diabetes complications. Therefore, it is suggested that oral anti-diabetics such as metformin and gliclazide can reduce the development of diabetes complications by decreasing oxidative stress [13, 14]. Within this context, there are no studies in the literature regarding how sitagliptin used in T2DM treatment affects oxidant-antioxidant balance. With this study, it is aimed to present how sitagliptin influences oxidative stress in serum and pancreatic tissue. For this purpose, we experimentally investigate the effect of sitagliptin on beta cell functions and insulin resistance in T2DM induced rats.

MATERIALS and METHODS

Sixty 6 to 8 weeks old male (140-200g) wistar rats obtained from the Isparta Suleyman Demirel University Research Center of Experimental Animals we used in the research. During the study, the care and feeding of rats was performed at 21±2ºC ambient temperature, 55–60% humidity, and 12:12 hours of light-dark cycle conditions. The rats were fed with high energy and normal energy feed depending on the experiment groups (test and control) and provided with fresh water on daily basis. During the study, all interventions made to animals took place in Afyon Kocatepe Universty (AKU) Experimental Animal Research and Application Center, in accordance with the approval (reference number: AKUHADYEK-33-09; date: 05.04.2009) of AKU Experiment Animals Local Ethics Committee. After ensuring that the rats brought to the research center accommodated to the environment, 10 rats were allocated to the control group. A standard diet, which includes 4% fat, 76% carbohydrate, and 17% protein with a metabolic energy level of 2600 cal/kg, was administered to the control group rats. A high fat diet (HFD) with 57% fat, 30% carbohydrate and 14% protein was administered to other rats. The HFD was prepared manually using 40% standard food (powder), 50% tallow, 5% poultry meat and 5% fat soybean, and was transformed into pellet form prior to administration. The metabolic energy value of the prepared HFD was measured as 4930 cal/kg in Afyon Food Control Laboratories.

Experimental T2DM model

In the 8th week of the study, after fasting the rats for 12 hours, their fasting blood glucose levels were measured (Accu-Check Go, Bayer). In order to induce T2DM, streptozocin (STZ) was administered to rats where insulin resistance was formed via HFD. STZ (sigma), which is dissolved in citrate buffer (pH: 4.5), was administered to rats via intra-peritoneal injection twice a week at a dose of 30mg/kg. One week after the final injection, by checking the plasma glucose levels of the rats; it was concluded that T2DM was induced in rats with plasma glucose levels of 300 mg/dl and over [15].

For the next phase of the study, five groups were formed from the T2DM induced rats. A control group (CONT) was created with healthy rats that were fed standard feed throughout the study. An HFD group was formed of rats that were fed with a high fat diet only during the study. The diabetes control group (DYB-CONT) was formed with T2DM-induced rats that were fed a high fat diet throughout the study. In the diabetes treatment group (DYB-SIT); sitagliptin treatment was executed for 12 weeks to T2DM-induced rats, which were fed a high fat diet during the study. In the treatment group that was given a High Fat Diet (HFD-SIT), sitagliptin was given for 12 weeks to rats that were fed a high fat diet during the study. The weight changes of the rats were measured each week, while fasting glucose levels were measured every four weeks with strips (Accu-Chek Go, Bayer). Sitagliptin (Merck) was administered to rats having treatment every day, by dissolving it in pure water at a dose of 10mg/kg [16] via gavage.

Insulin tolerance test (ITT) application

Rats were fasted for two hours before the test, in order to minimize the differences in the plasma insulin and glucose levels that might result from food remaining in the stomachs of the rats, during insulin tolerance test application. Afterwards, insulin (humolog/novorapid) was injectted at a dose of 0.5U/kg under the skin [20]. Plasma glucose levels were measured on the 0th, 15th, 30th and 60th minutes. Assuming the starting values of the glucose levels identified as a result of ITT administered in the final week of the study as 100 before the insulin injection, transformation of the variations in the glucose levels were carried out [15]; afterwards, differences between the groups were tested statistically. In many studies where ITT was applied, it was reported that the glucose-lowering effect of insulin continues until the 30th minute, however, after the 30th minute glucose level returns to the starting level in the plasma owing to the effect of gluconeogenesis and hepatic glycogenolysis. Because of this, the peak point in the anti-hyperglycemic activity of insulin is considered to take place at the 30th minute, and the data recorded at the 30th minute is used for ITT [20]. In this context, when assessing the results of ITT studies, the 30th minute data were used.

Biochemical Analyses

Following the experimental procedures, the rats were sacrificed after an overnight fasting period (12 hours) under anesthesia with an intra-peritoneal injection of 65mg/kg ketamine and 7mg/kg xylazine. Blood samples from the rats were collected in two tubes anti-coagulated with heparin. Blood was centrifuged promptly at 3500 x *g* for ten minutes at + 4ºC for plasma. Pancreas tissues were rapidly excised, washed in ice-cold PBS and all samples were stored at −80°C until use.

All biochemical tests were performed in the biochemistry laboratories of Afyon Kocatepe University. Glycated hemoglobin (HbA1c) levels were measured in blood. Insulin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and lactate dehydrogenase (LDH) levels were determined in the plasma. AST, ALT, and LDH levels were measured using an autoanalyser (Roche Cobas C501). Plasma insulin concentrations were analyzed with a specific insulin kit for rats (Millipore, lot number: 1729070) at ELISA. HbA1c levels were measured immediately with the high-performance liquid chromatography assay (Variant II, Bio-Rad Laboratories, Hercules, CA, USA).

Measurement of TAS and TOS levels

Total antioxidant status (TAS) levels were measured according to the method of Erel (2004), which represents the quantity of the pancreas tissue and plasma antioxidants [17], and were determined using a kit from Rell Assay Laboratories in Turkey. The results were expressed as millimol Trolox equivalents per liter (mmol Trolox Eq/L). Also, the total oxidant status (TOS) levels were determined using a novel automated measurement method, which was developed by Erel (2005), in the pancreas tissue and plasma [18]. The results were calibrated with hydrogen peroxide and expressed in terms of micromolar hydrogen peroxide equivalent per liter (μmolH2O2 equiv./L)

Calculation of OSI, HOMA-β and HOMA-IR

The Oxidative Stress Index (OSI), which is an indicator of the degree of oxidative stress, was calculated according to the formula below, from the TAS and TOS kit protocol of Rell Assay.

OSI = [(TOS, μmol/L)/(TAS, (mmolTroloxEquiv/L) x 100]

β cell function (HOMA-β) and insulin resistance (HOMA-IR), respectively, were assessed using the homeostasis model assessments [19] according to the formula below, where fasting insulin and glucose levels were determined after twelve hours of fasting. At the same time, insulin resistance

was determined with an insulin tolerance test [15, 20] and the results of ITT were compared with the data of HOMA-IR HOMA-β=[20 x fasting insulin levels (mU/I)]/[fasting glucose levels (mmol/L)-3.5]
HOMA-IR=[fasting insulin levels (mU/l) x fasting glucose levels (mmol/L)/22.5

Statistical analysis

All numerical results are expressed as mean ± standard deviation of the mean for the indicated number of experiments. Statistical significance was calculated with the ANOVA test with the Duncan post-test and was considered significant at the $p<0.05$ level. Data was analyzed with the Statistical Package for the Social Sciences (SPSS) version 15.0 for Windows.

RESULTS

Feeding with HFD and Weight Increase

In order to experimentally induce T2DM in rats, which is observed in humans in a way characterized with insulin resistance and beta cell dysfunction, a low dose of STZ injection (two injections per week) was carried out after feeding the rats with HFD. It is shown in Figure 1 that HFD effectively increased body weights of the rats.

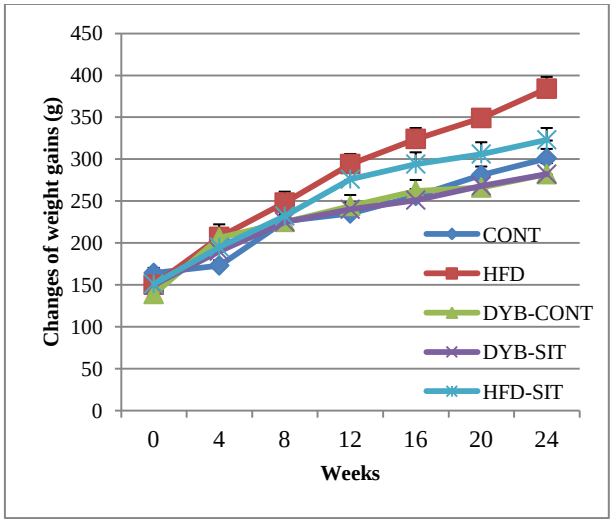

Figure 1. Observed weight increases in study groups during the study

While the average rat weight was (380 ± 14 g) in the HFD group after the 24th week, it is observed that the average weight was (300 ± 11 g) in the CONT group, and (320 ± 16 g) in the HFD-SIT group (Fig. 1). That is, sitagliptin reduced weight gain in the HFD group, which became mildly obese. In the groups where HFD was administered and diabetes was induced (DYB-CONT and DYB-SIT) instead, it is observed that sitagliptin did not have an effect on weight gain.

Effect of Sitagliptin on Biochemical Parameters

While the Fasting Plasma Glucose (FPG) levels of all groups were almost the same (90 ± 14 mg/dL); with the high fat diet administration, FPG levels started to increase. At the end of the study, while the FPG levels of the CONT group remained the same, it was determined that the FPG levels of the HFD and HFD-SIT groups increased statistically significantly ($p<0.05$) in comparison to the CONT group with an average of (130 ± 4 mg/dL). At the same time, it was observed that 12 weeks of sitagliptin treatment did not affect the FPG levels in the HFD groups (Fig 2, Table 1).

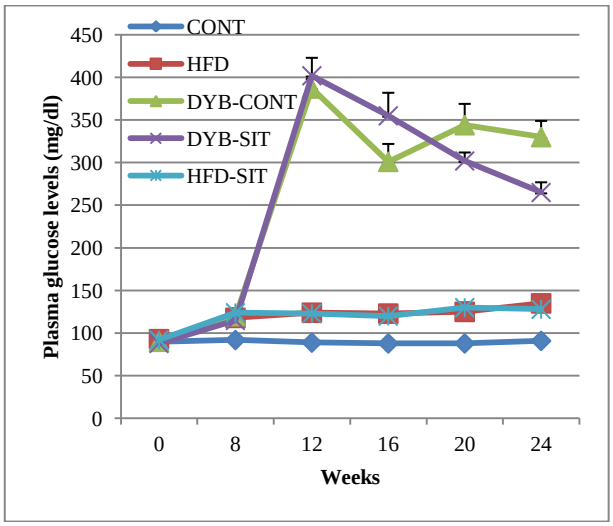

Figure 2. Fasting glucose levels of experiment groups during the study

It was identified that a low dose STZ injection performed in the 8th and 9th weeks of the study increased the FPG levels statistically significantly ($p<0.05$) in diabetic groups compared to other groups. It was observed that in the DYB-CONT group, the FPG levels started to balance in the 20th week, after eight weeks of STZ injection. It was determined that sitagliptin treatment decreased glucose levels in a significant way ($p<0.05$) in diabetic rats (Table 1).

When the HbA1c levels of study groups were analyzed, it was determined that HbA1c levels were (7.36 ± 0,66 %) in the CONT group, (8.7 ± 0,29 %) in the HFD group, and (9.16 ± 0,18 %) in the DYB-CONT group. It was observed that the HbA1c levels increased in the HFD and DYB-CONT groups in a statistically significant way compared to the CONT group. However, it was found that this increase decreased in the DYB-CONT and HFD-SIT groups because of the sitagliptin treatment; although when a comparison was made with the CONT group, a statistical difference was not found (Table 1).

At the end of the study, it was understood that insulin levels increased nearly four times in rats fed with HFD compared to CONT group rats, and hyper-insulinemia was induced. It was determined that the STZ injection reduced insulin levels in the DYB-CONT group compared to the HFD group, although this reduction was not statistically significant. It was observed that sitagliptin treatment did not change insulin levels statistically in either the diabetes or HFD groups (Table 1).

It was found that no significant difference occurred between ALT and LDH levels, which are among the liver enzymes analyzed in study groups. However, when AST levels are analyzed, it was detected that AST levels decreased significantly in the HFD, DYB-CONT and DYB-SIT groups in comparison to the CONT group. At the same time, when the DYB-CONT group and DYB-SIT group are compared, it was determined that sitagliptin treatment did not affect AST levels.

Table1. Analysis results of biochemical parameters of experiment groups at the end of the study

	Study groups				
	CONT	**HFD**	**DYB-CONT**	**DYB-SIT**	**HFD-SIT**
FPG (mg/dL)	91 ± 2 [a]	135 ± 4 [b]	330 ±19 [d]	265 ± 12 [c]	128 ± 3 [b]
İnsulin (ng/mL)	0.09 ± 0.3 [a]	0.39 ± 0.7 [b]	0.16 ± 0.4 [a, b]	0.32 ± 0.1 [b]	0.39 ± 0..07 [b]
HbA1c (%)	7.36 ± 0.66 [a]	8.70 ± 0.29 [b]	9.16 ± 0.18 [b]	8.32 ± 0.29 [a, b]	8.11 ± 0..33 [a, b]
ALT (U/L)	54.8 ± 7.8	51.4 ± 7.2	55.9 ± 4.3	56.1 ± 10	55.1 ± 7.2
AST (U/L)	129 ± 23 [b]	79 ± 10 [a]	78 ± 9.6 [a]	63 ± 8.7 [a]	98 ± 3.1 [a, b]
LDH (U/L)	449 ± 59.4 [a, b]	407 ± 80.1 [a, b]	522 ± 133 [b]	433 ± 47.2 [a, b]	237 ± 11.3 [a]
HOMA-β	0.021 ± 0.009 [a]	0.058 ± 0.011 [b]	0.010 ± 0.002 [a]	0.026 ± 0.008 [a]	0.065 ± 0..013 [b]
HOMA-IR	0.355 ± 0.14 [a]	2.391 ± 0.42 [b]	2,250 ± 0.56 [b]	1,751 ± 0.65 [b]	2.130 ± 0.37 [b]

Values are mean ± standard deviation; *n*=10.

[a, b, c, d]: Different letters in the same line represent statistically significant differences ($P<0.05$). FPG: fasting plasma glucose level, AST: aspartate aminotransferase, ALT: alanine aminotransferase, LDH: lactate dehydrogenase, HOMA-β: homeostasis model assessments for β cell, HOMA-IR: homeostasis model assessments for insulin resistant, CONT: control group, HFD: The group that was given only high fat diet, DYB-CONT: The group that were fed a high fat diet throughout the study and in which T2DM was generated, DYB-SIT: Diabetic group treated with sitagliptin, HFD-SIT: Sitagliptin was given for 12 weeks to rats that were given a high fat diet during the study.

When the HbA1c levels of study groups were analyzed, it was determined that HbA1c levels were (7.36 ± 0,66 %) in the CONT group, (8.7 ± 0,29 %) in the HFD group, and (9.16 ± 0,18 %) in the DYB-CONT group. It was observed that the HbA1c levels increased in the HFD and DYB-CONT groups in a statistically significant way compared to the CONT group. However, it was found that this increase decreased in the DYB-CONT and HFD-SIT groups because of the sitagliptin treatment; although when a comparison was made with the CONT group, a statistical difference was not found (Table 1).

At the end of the study, it was understood that insulin levels increased nearly four times in rats fed with HFD compared to CONT group rats, and hyper-insulinemia was induced. It was determined that the STZ injection reduced insulin levels in the DYB-CONT group compared to the HFD group, although this reduction was not statistically significant. It was observed that sitagliptin treatment did not change insulin levels statistically in either the diabetes or HFD groups (Table 1).

It was found that no significant difference occurred between ALT and LDH levels, which are among the liver enzymes analyzed in study groups. However, when AST levels are analyzed, it was detected that AST levels decreased significantly in the HFD, DYB-CONT and DYB-SIT groups in comparison to the CONT group. At the same time, when the DYB-CONT group and DYB-SIT group are compared, it was determined that sitagliptin treatment did not affect AST levels.

When HOMA- β index data, which is considered an indicator of beta cell function, was analyzed, it was observed that the HOMA- β index increased nearly three times in the HFD groups (HFD and HFD-SIT) compared to the CONT group. Conversely, in the group (DYB-CONT) in which rats fed a HFD were given an STZ injection, it was identified that the HOMA- β index decreased nearly six times ($p<0.05$) and beta cell dysfunction formed, compared to the HFD group. It was determined that sitagliptin treatment in diabetes groups increased the HOMA-β index, however this increase was not statistically significant.

When HOMA-IR data, which are found by using fasting glucose and insulin levels at the end of the study and which are considered an indicator of insulin resistance, were analyzed; a statistically significant increase was observed in all other groups compared to the CONT group. However, it was understood that sitagliptin treatment did not affect the HOMA-IR index neither in the HFD group nor in diabetic rats (Table 1). At the same time, an Insulin Tolerance Test (ITT) was also performed on rats in order to identify the effect of stagliptin to IR. When data was compared to the data recorded 30 minutes after the insulin injection, it was determined that the lowering rate of glucose levels in the DYB-CONT group were statistically significantly lower ($p<0.05$) than the lowering rate of glucose levels of the CONT, HFD and DYB-CONT groups. In a similar way, when the HFD group and the HFD-SIT group were compared, it was determined that sitagliptin reduced IR in the HFD group (Fig. 3).

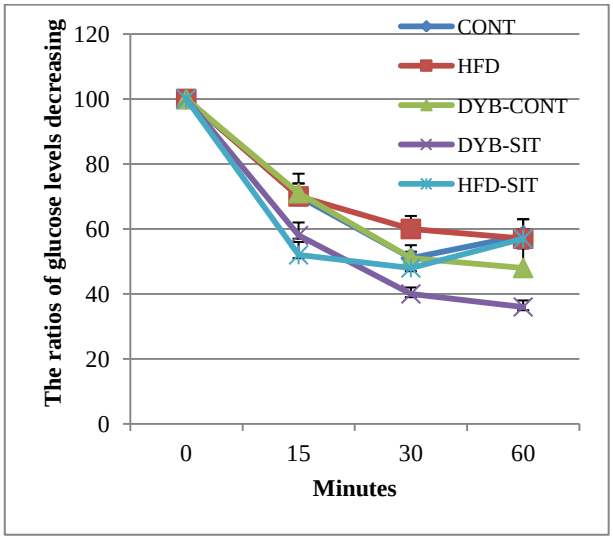

Figure 3. ITT results belonging to study groups

Effect of Sitagliptin on the Oxidative Stress Paramete Plasma and Pancreatic Tissues

Oxidative stress parameters TAS, TOS, and OSI levels were determined in both plasma and pancreatic tissue. No statistically significant difference was detected between groups in the analyses done in plasma. When pancreatic tissue TAS levels were analyzed, no difference was found between the control group and other groups. However, when the TAS data of the DYB-CONT group and DYB-SIT group were compared, it was identified that the TAS levels (0.55 ± 0,04 µmol Trolox equivalent/ L mg protein) of the DYB-SIT group were statistically significantly ($p<0.05$) lower than the TAS

levels (1.39 ± 0,27 µmol Trolox equivalent/ L mg protein) of the DYB-CONT group. Conversely, when pancreatic tissue TOS levels were analyzed, it was observed that the TOS levels of the HFD, DYB-CONT and DYB-SIT groups came out higher than those of the CONT group. When treatment groups were analyzed, no difference was found between the TOS and OSI levels of the DYB-CONT and DYB-SIT groups. At the same time, it was found that the HFD-SIT group had lower TOS levels and OSI ($p<0.05$) than the HFD group. In short, it was determined that sitagliptin treatment reduced TAS levels in diabetic rats, and TOS levels and OSI in HFD group rats (Table 2).

DISCUSSION

It is known that many mechanisms play a role in the development of diabetes and its complications. Among these, one of the most important is oxidative stress, which occurs due to obesity and hyperglycemia as well as lipotoxicity and glucotixicity. Just as oxidative stress in T2DM may cause β cell damage and IR development, it also paves the way for diabetic complications to emerge [13, 21]. Because of this, it is extremely important that the anti-diabetic medicine used in T2DM treatment supports the oxidant-antioxidant balance [14, 22], because oxidant metabolites like the end products of lipid peroxidation formed as a result of an increase in oxidative stress cause antioxidant levels like SOD, CAT, and GSH to reduce [23-25]. Conversely, this situation causes TAS levels to decrease in plasma and tissues, and TOS levels to increase [26, 27]. In this context, one of the reasons that anti-diabetic medicines like metformin and gliclazide are used widely in T2DM treatment today originates from the fact that these medicines support the antioxidant system [13, 14, 22]. That is why, the presented study attempted to identify the extent that sitagliptin affects the oxidant-antioxidant system in plasma and pancreatic tissue.

In the analyses carried out in plasma, it was determined that sitagliptin did not have any effect on the analyzed oxidative stress parameters. In the pancreatic tissue however, it was observed that while sitagliptin did not affect TOS levels and the OSI in the DYB-SIT group compared to the DYB-CONT group; it did not support the antioxidant system by reducing TAS levels. In the HFD and HFD-SIT groups, which became pre-diabetic as a result of 24 weeks of a high fat diet administration, it was determined that sitagliptin did not affect TAS levels, whereas it reduced oxidative stress by decreasing TOS levels and the OSI in the HFD-SIT group compared to the HFD group. The reason why sitagliptin increased oxidative stress in the diabetes treatment group while decreasing it in the HFD and HFD-SIT groups might be excessive glucose toxicity, which cannot be observed in the HFD groups and arises due to diabetes. In a study carried out by Ferreira et al. (2010), which is the first study intended for determining the effect of sitagliptin on the metabolism, inflammation, and oxidative stress in diabetic rats, it was reported that MDA levels, which is one of the oxidant parameters, decrease in diabetic pancreatic tissue [28]. That study and the present study seem to contradict with each other. This contradiction can be explained as follows. Although the lipid peroxidation end product MDA is an important oxidant molecule, it may not be enough alone to draw conclusions on oxidative stress, because other than MDA, there might be Nitric Oxide (NO) and oxidant metabolites such as Advanced Glycation End (AGEs) products that might influence oxidative stress [29]. TOS analyses on the other hand, rely on all oxidants found in the sample to transform ferrous (Fe^{+2}) ion complexes in the reagent used in the analysis by ferric (Fe^{+3}) oxidation [18]. TAS analyses similarly rely on all antioxidants that occur in the sample in order to transform dark green $ABTS^{+}$ (2,2'-azinobis-3-etilbenzotiazolin-6-sulfonic acid) radical cation in the reagents used in the ABTS molecule by degrading [17]. Because of this, it can be stated that the results obtained from the presented study are supportive in identifying the effect of sitagliptin on oxidative stress.

With the presented study, the effect of sitagliptin on the FPG, HbA1c, insulin, ALT, AST, and LDH levels in the rats was also examined. It is understood from the results obtained that as is the case in the literature [3, 5, 6, 7, 8, 10], sitagliptin reduced FPG and HbA1c levels, increased insulin levels and did not affect ALT, AST, and LDH levels in the treatment group (DYB-SIT) compared to the diabetic group (DYB-CONT). Furthermore, according to the ITT results, it was observed that sitagliptin reduced IR in diabetic rats and this result is in accordance with the previous studies in the literature [28].

The insulin and FPG levels obtained at the end of the study show that insulin resistance developed in rats administered with the HFD because it was determined that insulin levels (0.39ng/mL) increased four times on average in HFD rats compared to the control group (0.09ng/mL) rats. The fact that hyper-insulinemia and IR develops (according to ITT data) together with weight gain in rats fed a HFD indicates that obesity is formed. However, when the HFD group and HFD-SIT group are compared, it is observed that sitagliptin did not have any effect on FPG, HbA1c, and insulin levels. These results show that sitagliptin is not very effective on glucose homeostasis in pre-diabetic obese rats.

Table 2. TAS, TOS levels and OSI index data in plasma and pancreatic tissue of experiment groups

	Plasma			**Pancreatic tissue**		
	TAS (µmol Trolox equivalent/ L)	**TOS** (µmol H_2O_2 equivalent/L)	**OSI**	**TAS** (µmol Trolox equivalent / L mg protein)	**TOS** (µmol H_2O_2 equivalent / L mg protein)	**OSI**
CONT	1.38 ± 0.17	3.18 ± 0.74	251 ± 52	1.04 ± 0.1 $^{a, b}$	0.51 ± 0.05 ^{a}	50 ± 7 ^{a}
HFD	1.53 ± 0..14	5.08 ± 0.52	355 ± 71	1.36 ± 0.20 ^{b}	2.71 ± 0.31 ^{b}	233 ± 70 $^{a, b}$
DYB-CONT	1.74 ± 0..12	4.85 ± 1,12	288 ± 71	1.39 ± 0.27 ^{b}	2.53 ± 0.34 ^{b}	240 ± 88 $^{b, c}$
DYB-SIT	1.51 ± 0.07	4.50 ± 0.84	298 ± 58	0.55 ± 0.04 ^{a}	2.15 ± 0.19 ^{b}	404 ± 49 $^{b, c}$
HFD-SIT	1.58 ± 0.30	5.20 ± 0.13	384 ± 79	0.91 ± 0.8 $^{a, b}$	1.18 ± 0.26 ^{a}	125 ± 63 ^{c}

Values are mean ± standard deviation; n=10.

$^{a, b, c}$: Different letters in the same column represent statistically significant differences ($P<0.05$). TAS: total antioxidant status, TOS: total oxidant status, OSI: oxidative stress index, CONT: control group, HFD: The group that was given only high fat diet, DYB-CONT: The group that were fed a high fat diet throughout the study and in which T2DM was generated, DYB-SIT: Diabetic group treated with sitagliptin, HFD-SIT: Sitagliptin was given for 12 weeks to rats that were given a high fat diet during the study.

Studies carried out in the literature are directed towards the fact that low double dose STZ injection creates beta cell damage [15]. Also in the presented study, the fact that HOMA-β index data came out lower in the DYB-CONT group compared to the CONT group shows that β cell dysfunction was formed in rats by a low double dose STZ injection with a one week interval. This data is an indicator that after IR development with HFD feeding, an experimental T2DM model was created via STZ injection that is suitable to T2DM physiopathology which is seen in humans. When the effects of sitagliptin on the HOMA-β index in this model were examined, it was observed that the HOMA-β index levels of the DYB-SIT group were nearly 2.5 times more than those of the DYB-CONT group. This difference was not reflected in the statistics, as FPG and insulin levels used in HOMA-β index calculations were not close to each other but had extreme values. However, protective effect of sitagliptin against beta cells also identified in the present study is also supported by the extant literature [14, 30].

If all studies carried out are generalized, it can be stated that in diabetic rats sitagliptin balances glucose homeostasis, protects beta cells, although it increases oxidative stress in pancreatic tissue. Because of this, it is understood that sitagliptin puts forward an anti-diabetic medicine profile that does not support the antioxidant system. Sitagliptin is considered to be much more advantageous compared to other anti-diabetics because it can be used without causing dose problems and hypoglycemia risk since it induces insulin secretion and synthesis depending on glucose [1, 3, 4].. When its negative effect on the oxidant-antioxidant balance, proliferation of β cells [14, 30], and diabetic nepropathy [31] are considered, it can be recommended to be used in combination with various antioxidants in order to increase its effectiveness. This is because it is thought that sitagliptin will be able to enable glucose control in a more effective way, since the oxidative stress level also effecting insulin resistance will decrease with antioxidant support.

Ethical Issues

During the study, all interventions were made to animals in the AKU Experimental Animal Research and application Center, in line with the approval (reference number: AKUHADYEK-33-09, date: 05.04.2009) of AKU Experiment Animals Local Ethics Committee.

Knowledge and Acknowledgement

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Conflict of Interest

There are no conflicts of interest between the authors who contributed to the present study.

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