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Serum testosterone levels and oxidative stress in type 1 diabetes, type 2 diabetes, and obesity

Hamit Yasar Ellidag[1], Rahime Aslankoç[2], Mehmet Kök[3], Güzin Aykal[1], Özgür Aydın[1], Özlem Özmen[4], Remzi Can Çakır[5], Uğur Doğan[5]

[1]*Department of Clinical Biochemistry, Antalya Education and Research Hospital of Ministry of Health, Antalya, Türkiye*
[2]*Department of Physiology, Faculty of Medicine, Suleyman Demirel University, Isparta, Türkiye*
[3]*Department of Internal Medicine, Antalya Education and Research Hospital of Ministry of Health, Antalya, Türkiye*
[4]*Department of Pathology, Faculty of Veterinary Medicine, Mehmet Akif Ersoy University, Burdur, Türkiye*
[5]*Department of General Surgery Antalya Education and Research Hospital of Ministry of Health, Antalya, Türkiye*

Abstract

Introduction: Obesity, type 1 diabetes mellitus (T1DM), and type 2 diabetes mellitus (T2DM) are metabolic diseases that continue to be a global problem. Testosterone levels in men are affected by several factors, including obesity and DM. Although the relationship between diabetes and testosterone is not fully understood, oxidative stress is thought to play a major role. The aim of this study was to compare serum testosterone levels and oxidative stress markers [total antioxidant status (TAS), total oxidant capacity (TOS), oxidative stress index (OSI), and ischaemic modified albumin (IMA)] among the control group and experimentally induced obese, T1DM, and T2DM rats.

Material and methods: The study included 28 male Sprague-Dawley rats divided into 4 groups: the obesity group were fed a high-fat diet (HFD), the T2DM group received a HFD plus a single dose of streptozocin (STZ), the T1DM group received only STZ, and there was a control group. Serum testosterone, TAS, TOS, OSI, and IMA were analysed.

Results: Serum testosterone levels were lower in the T1DM and T2DM groups compared to the control and obesity groups. The TOS levels were highest in the T2DM group, followed by the T1DM group, the obesity group, and finally the control group. No significant difference was found between the obesity group and the control group in terms of TOS levels. Regarding TAS levels, the order observed was control group > obesity group > T2DM > T1DM. Testosterone was positively correlated with TAS and negatively correlated with TOS and OSI.

Conclusions: Increased oxidative stress in diabetes may be an important factor that decreases serum testosterone levels. **(Endokrynol Pol 2024; 75 (2): 183–191)**

Keywords: *obesity; type 1 diabetes mellitus; type 2 diabetes mellitus; free radicals; oxidative stress; testosterone*

Introduction

Although obesity and obesity-related diseases were briefly out of the spotlight during the COVID-19 pandemic, they remain a significant risk worldwide. Since 1980, the prevalence of obesity and type 2 diabetes mellitus have increased significantly in many countries [1, 2]. Comprehensive studies have indicated a more than 2-fold increase in the prevalence of type 1 diabetes (T1DM) and type 2 diabetes (T2DM) in men (from 4.3% to 9.0%) and a 1.5-fold increase in women (from 5.0% to 7.9%). It has been understood that almost 85-95% of this rise was attributed to T2DM [3, 4].

Environmental factors such as poor diet, a sedentary lifestyle, and genetic predisposition are the main causes of obesity [5, 6]. In obesity, adipose tissue releases more free fatty acids into the circulation through lipolysis [7]. Fatty acids increase in the circulation, leading to increased insulin resistance in muscles and the liver, and impaired pancreatic insulin secretion. Although the molecular mechanism between lipids and insulin resistance is not fully understood, it is hypothesised that metabolites formed as a result of lipid metabolism in the cell disrupt the insulin signalling pathway. As a result of insulin resistance, glucose entry into skeletal muscle and fat cells decreases, while gluconeogenesis increases in the liver, resulting in hyperglycaemia [7–9]. As a result, increased insulin resistance leads to metabolic syndrome and T2DM.

Unlike obesity and T2DM, T1DM is caused by genetic or autoimmune factors and usually occurs in childhood independently of lifestyle. Although regression and even remission of T2DM symptoms can be achieved with lifestyle and dietary changes, this is not the case in T1DM. Insulin replacement is absolutely necessary for treating T1DM because it can lead to severe complications such as diabetic ketoacidosis and nonketotic hyperosmolar coma if left untreated [10–13].

Assoc. Prof. Dr. Hamit Yasar Ellidag, Antalya Education and Research Hospital, Department of Clinical Biochemistry, Antalya Egitim ve ArastirmaHastanesi Varlik Mahallesi, Kazim Karabekir Caddesi Soguksu 07100, Antalya, Türkiye; e-mail: hayael1980@hotmail.com

In men, testosterone plays a vital role in maintaining muscle mass and bone density, regulating mood, and supporting male reproductive health. Testosterone levels in men may be affected by various factors including age, obesity, and insulin resistance [14, 15]. Studies have indicated that testosterone levels are lower in obese and diabetic men, and treating low testosterone levels in men with diabetes might improve insulin sensitivity and glycaemic control [14, 16]. It has been suggested that high blood glucose levels may result from increased oxidative stress damage to Leydig cells responsible for testosterone production in the testes [15]. Oxidative stress, involving an imbalance between oxidants like reactive oxygen species (ROS) and antioxidants, may cause damage to Leydig cells, reducing testosterone production, as well as causing damage to spermatozoa, defects in sperm, sperm DNA fragmentation, and potential varicocele and testicular torsion cases in men. Thus, increased oxidative stress in obese and diabetic men may cause physical and mental damage by reducing testosterone levels, and may lead to infertility by decreasing sperm count and quality, especially in young men [16, 17].

The aim of this study was to compare serum testosterone levels and oxidative stress marker levels (TAS: total antioxidant capacity, TOS: total oxidant capacity, OSI: oxidative stress index and IMA: ischaemic modified albumin) among control, obese, T1DM, and T2DM rat models. We investigated changes in serum oxidative stress parameters in these disease models and aimed to reveal their relationship with serum testosterone.

Material and methods

Animals

Twenty-eight male Sprague Dawley rats (160–180 g, 4 weeks old) were housed at room temperature (21–22ºC), 60% ± 5% humidity, and 12:12 light/dark. This experimental study was approved by the Burdur Mehmet Akif Ersoy University Animal Experiments Local Ethics Committee. Feed (standard rat chow or high-fat diet [HFD] on a case-by-case basis) and water were provided ad libitum.

Experimental procedure

Rats were randomly distributed into 4 groups, each containing 7 rats. The groups were as follows:

1. Control group: The rats were fed standard rat chow (5 weeks), and a single dose of citrate buffer was administered intraperitoneally (*i.p.*) after overnight fasting for one week before the experiment.
2. Obesity group: The rats were fed HFD (5 weeks), and a single dose of citrate buffer was administered *i.p.* after overnight fasting for one week before the experiment [18, 19].
3. T2DM + HFD group: The rats were fed HFD (5 weeks), and a single dose of streptozotocin (STZ, 30 mg/kg) was administered *i.p.* after overnight fasting one week before the experiment. STZ was dissolved in 0.01 M citrate buffer (pH 4.5) solution [19, 20].
4. T1DM group: The rats were fed standard rat chow, and 25 mg/kg STZ was administered *i.p.* for 5 consecutive days in the second week [19, 20].

HFD was locally prepared in our laboratory by pulverising normal rat pellet diet and mixing it with tallow in a 1:0.5 ratio. The mixture was solidified by freezing and pelleted.

At the conclusion of the experiment, rats were euthanised under ketamine (40 mg/kg) and xylazine (5 mg/kg) anaesthesia. During necropsy, blood samples were collected from each animal for biochemical analyses. Serum samples were obtained by centrifugation at 4000 rpm for 10 min and stored at –80°C until analysis.

Biochemical analysis

Firstly, all samples were kept at room temperature until defrosting. The samples were then vortexed. Biochemical analyses included serum glucose, albumin, triglyceride, total cholesterol, high-density lipoprotein (HDL) and low-density lipoprotein (LDL) cholesterol, alanine aminotransferase (ALT), and aspartate aminotransferase (AST) levels, which were determined using an autoanalyser (Beckman AU5800; Beckman Coulter Diagnostics, Brea, CA). Serum total testosterone levels were measured using the chemiluminescence method on a Beckman Coulter AccessDxI800 autoanalyser with commercial kits.

TOS measurement

This method was developed by Erel [21]. This method is based on the detection of the coloured compound formed by the oxidation of ferrous ion-iron compounds to ferric ion in acidic medium. The colour intensity determined spectrophotometrically is related to the total amount of oxidant molecules in the sample. Hydrogen peroxide is used as calibrator. Results are given in μmol H_2O_2 equiv/litre.

TAS measurement

Again by Erel [22] in this method, antioxidants in the sample cause a colour change in ABTS. This colour change is recorded at 660 nm. The results are given as micromolar trolox/litre.

Oxidative stress index

This is obtained by the ratio of TOS level to TAS level. It can be expressed as oxidant capacity corresponding to unit antioxidant capacity [23].

IMA measurement

The decreased cobalt-albumin binding capacity (IMA level) is a colorimetric method developed by Bar-Or et al. [24]. Briefly, it is based on the detection of the amount of $CoCl_2$ added to the sample that is not bound to albumin by dithiothreitol. The results were expressed as absorbance units (ABSU).

Statistical analysis

The between-group comparison of numerical variables was performed using a Kruskal-Wallis test, while post hoc comparisons were made using a Mann-Whitney U-test. Repeated measured ANOVA was performed for serum glucose and weight. Correlation analysis utilised Spearman's rank test, and the association between categorical variables was evaluated using a chi-square test. A significance level of $p < 0.05$ was considered.

Results

The blood glucose levels and weights of the rats included in the study were measured periodically (every 5 weeks). Blood glucose remained stable throughout the follow-up period in the control group, and the weight of the rats in this group increased in parallel with normal development. Blood glucose

Table 1. *Changes in weight and glucose of the experimental groups*

	1 week	2 weeks	3 weeks	4 weeks	5 weeks	Difference (%)*	p
Weight [gr]							
Control group	300 ± 13	328 ± 16	348 ± 14	371 ± 10	391 ± 12	30	< 0.01
Obesity group	260 ± 11	293 ± 13	314 ± 18	350 ± 17	370 ± 22	42	< 0.01
T1DM group	277 ± 21	317 ± 24	332 ± 27	342 ± 29	359 ± 32	30	< 0.01
T2DM + HFD group	301 ± 25	336 ± 27	365 ± 25	394 ± 30	419 ± 33	39	< 0.01
Glucose [mg/dL]							
Control group	85 ± 7	94 ± 5	87 ± 7	94 ± 6	96 ± 6	13	0.06
Obesity group	85 ± 4	84 ± 5	87 ± 10	96 ± 6	96 ± 11	13	0.05
T1DM group	75 ± 5	91 ± 8	280 ± 38	305 ± 42	296 ± 35	294	< 0.01
T2DM + HFD group	76 ± 6	85 ± 7	92 ± 5	95 ± 10	345 ± 44	353	< 0.01

The control group had physiological weight gain, and their blood glucose levels remained stable. In the obesity group, blood glucose levels remained stable, but there was an average 42% weight gain from the beginning of the experiment. Weight change in the T1DM group was similar to that in the control group. Blood glucose, on the other hand, increased after the second week of sequential administration of STZ. In the T2DM + HFD group, there was a 39% weight gain from the beginning of the experiment, and blood glucose increased after the rapid dose of STZ was administered one week before the experiment. *Percentage difference between the first week and the fifth week. Repeated ANOVA test. T1DM — type 1 diabetes mellitus; T2DM — type 2 diabetes mellitus; HFD — high-fat diet; STZ — streptozocin

remained stable in the obesity group, while there was an increase in body weight; blood glucose increased together with weight gain in the T2DM + HFD group; and blood glucose increased in the T1DM group (Tab. 1).

We assessed liver function with serum ALT, AST, and albumin. Additionally, for the serum lipid panel, we analysed total cholesterol, HDL cholesterol, LDL cholesterol, and triglyceride values. We could not detect any significant difference between the groups in terms of serum albumin levels ($p = 0.246$). Serum ALT and AST levels were found to be significantly higher in the T1DM and T2DM + HFD groups, with the highest levels observed in the obesity group, compared to the control group ($p < 0.01$ for ALT and AST). As expected, lipid parameters were elevated in the obesity and T2DM + HFD groups, compared to the control and T1DM groups ($p < 0.01$ for all of the lipid parameters).

Serum testosteron levels were lower T1DM and T2DM + HFD groups,compared to the control and obesity groups ($p < 0.01$). There were no statistically significant differences between control and obesity groups. When oxidative stress parameters were analysed, TOS levels were highest in the T2DM + HFD group, followed by the T1DM group, the obesity group, and the control group ($p < 0.01$). No significant difference was found between the obesity group and the control group in terms of TOS levels (post-hoc analysis). Regarding TAS levels, the order observed was control group > obesity group > T2DM + HFD group > T1DM group ($p < 0.01$). Although OSI showed a trend of T2DM + HFD group > T1DM group > obesity group > control group ($p < 0.01$), there was almost statistical significance in T2DM + HFD group > T1DM group ($p = 0.06$). IMA levels were elevated only in the T2DM + HFD group (Tab. 2, Fig. 1).

The correlation analysis revealed consistent correlations, indicating the success of our experiment and biochemical analysis. Correlation analyses showed that serum ALT levels were positively correlated with AST, HDL cholesterol, total cholesterol, LDL cholesterol, OSI, TOS, and triglycerides, while they were negatively correlated with TAS. Serum AST levels exhibited positive correlations with cholesterol, HDL cholesterol, LDL cholesterol, triglycerides, TOS, and OSI levels. Cholesterol was positively correlated with LDL, triglycerides, TOS, and OSI levels. There was a positive correlation between LDL and triglycerides and TOS. There was a positive correlation between IMA and LDL TOS and OSI. OSI was negatively correlated with TAS and positively correlated with TOS and triglycerides. Finally, testosterone was positively correlated with TAS and negatively correlated with TOS and OSI (Tab. 3).

Discussion

In this study, we examined the relationship between oxidative stress and testosterone in experimentally induced obese, T1DM, and T2DM rats. We periodically measured the weights and blood glucose levels of the experimental and control groups (Tab. 1). At the end of the 5-week experiment, we observed significant differences through repeated ANOVA analysis for changes in the rats' blood glucose and weight (Tab. 1). As expected, we found elevated serum lipid panel tests in the obesity and T2DM + HFD groups. We also found elevated ALT and AST levels in the obesity, T1DM, and T2DM + HFD groups compared to

Table 2. *Biochemical parameters*

Parameter	Control group (n = 7) (a)	Obesity group (n = 7) (b)	T1DM group (n = 7) (c)	T2DM + HFD group (n = 7) (d)	p
Albumin [g/L]	26.9(25.6–29)	26.5 (24.2–27.6)	25 (24.1–26.7)	31 (27.3–35.2)	0.246
ALT [U/L]*	31 (21–38)	77 (64–87)	60 (46–67)	58 (54–66)	< 0.01
AST [U/L]**	97 (86–119)	184 (152–205)	169 (130–182)	172 (157–226)	< 0.01
Total cholesterol [mg/dL]***	38 (33–47)	74 (63–85)	43 (38–53)	94 (70–137)	< 0.01
HDL cholesterol [mg/dL]#	22 (17–26)	38 (31–41)	25 (22–30)	41 (33–52)	< 0.01
LDL cholesterol [mg/dL]##	13 (10–15)	30 (23–33)	11 (10–17)	37 (28–65)	< 0.01
Triglyceride [mg/dL]###	36 (28–69)	107 (92–192)	61 (50–76)	340 (247–498)	< 0.01
Testosterone†	8 (6–9)	7 (1–12)	0.94 (0.8–2)	1.2 (0.89–3)	< 0.01
TOS†† [μmolH_2O_2 Equiv./L]	3.4 (2.5–4.2)	4.8 (3.6–5.4)	5.31 (4.4–6.6)	13.5 (10–21)	< 0.01
TAS††† [μmol trolox/L]	2.5 (2.3–2.7)	2.1 (1.9–2.1)	1.2 (1.18–1.3)	1.9 (1.3–2)	< 0.01
OSI +	1.4 (1–1.5)	2.4 (1.6–2.6)	4.3 (3.2–5.2)	7.8 (4.6–11.6)	< 0.01
IMA++ [ABSU]	0.368 (0.328–0.454)	0.336 (0.327–0.554)	0.344(0.307–0.389)	1.594 (0.465–3.245)	< 0.01
***Post hoc* comparisons**					
*b > a; p < 0.01	b > c; p = 0.01	b > d; p = 0.01	c > a; p < 0.01	d > a; p < 0.01	
**b > a; p < 0.01	b > c; p = 0.02	c > a; p < 0.01		d > a; p < 0.01	
***b > a; p < 0.01	b > c; p < 0.01	d > c; p < 0.01		d > a; p < 0.01	
#b > a; p = 0.01	b > c; p = 0.01	d > c; p = 0.01		d > a; p < 0.01	
##b > a; p = 0.04	b > c; p = 0.01	d > c; p < 0.01		d > a; p = 0.01	
###b > a; p < 0.01	b > c; p < 0.01	c > a; p = 0.04	d > a; p < 0.01	d > b; p < 0.01	d > a; p < 0.01
†a > c; p < 0.01	a > d; p < 0.01	b > c; p < 0.01	b > d; p < 0.01		
†† a < c; p < 0.01	a < d; p < 0.01	b < d; p < 0.01	c < d; p < 0.01		
††† a > b; p < 0.01	a > c; p < 0.01	a > d; p < 0.01	b > c; p < 0.01	d > c; p < 0.01	
+ a < c; p < 0.01	a < d; p < 0.01	b < c; p = 0.02	b < d; p < 0.01	c < d; p = 0.06	
++ d > a; p < 0.01	d > b; p < 0.01		d > c; p < 0.01		

ALT — alanine aminotransferase; AST — aspartate aminotransferase; HDL — high-density lipoprotein; LDL — low-density lipoprotein; TAS — total antioxidant capacity; TOS — total oxidant capacity; OSI — oxidative stress index; IMA — ischaemic modified albumin

the control group, with significant correlations between ALT and AST levels and lipid panel and oxidative stress tests in the entire study group. Analysing oxidative stress markers, we found that TOS levels were highest in the T2DM + HFD group, significantly higher than the control group. Although TOS levels in the T1DM group were higher than in the obesity group, the difference lacked statistical significance. Similarly, although TOS levels were higher in the obesity group compared to the control group, there was no statistical significance. We discovered a significant correlation between TOS levels and ALT, AST, cholesterol, LDL, HDL, and triglycerides, indicating a relationship between oxidative stress, increased liver synthesis capacity, and non-alcoholic fatty liver disease. TAS levels were highest in the control group and lowest in the T1DM group. The only significant difference in TAS levels was between the T1DM and T2DM + HFD groups. OSI (TOS/TAS) expresses oxidant capacity per unit antioxidant capacity, signifying that increased oxidative stress surpasses antioxidant defence. In our study, the highest OSI level was in the T2DM + HFD group, followed by the T1DM group, the obesity group, and finally the control group. While comparing the T1DM and T2DM + HFD groups in terms of OSI, although T2DM + HFD was higher, it was almost statistically significant (p = 0.06). However, OSI was positively correlated with liver enzymes and lipid panel tests. The T2DM + HFD group, with the highest IMA level, did not significantly differ from the other groups and correlated only with LDL, TOS, and OSI tests.

The main findings of our study revealed that serum testosterone levels were lower in the T1DM and T2DM + HFD groups compared to the control

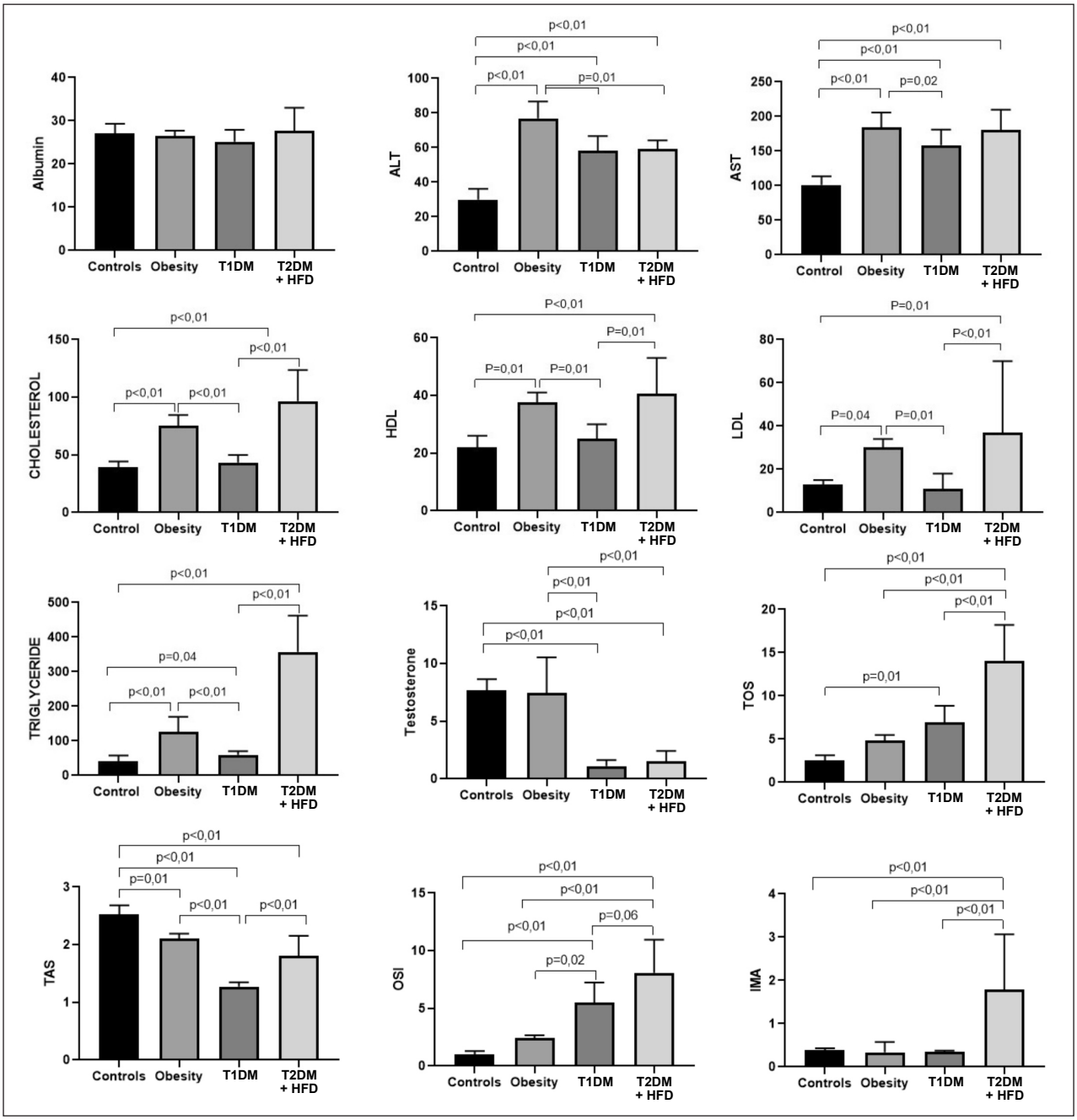

Figure 1. *Distribution of biochemical parameters and post hoc comparisons: there was no significant difference between the groups in terms of serum albumin levels. Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels were highest in the obesity group and in the type 2 diabetes mellitus (T1DM) and type 2 diabetes mellitus plus high-fat diet (T2DM + HFD) groups, compared to the control group. Lipid parameters were higher in obesity and T2DM + HFD groups, compared to the control and T1DM groups. Serum testosterone levels were lower in the T1DM and T2DM + HFD groups compared to the control and obesity groups. The highest total oxidant capacity (TOS) levels were found in the T2DM + HFD group > T1DM group > obesity group > control group. No significant difference was found between the obesity group and the control group in terms of TOS levels. In terms of total antioxidant capacity (TAS) levels, it was observed that the control group > obesity group > T2DM > T2DM + HFD. In oxidative stress index (OSI), it was found as T2DM + HFD group > T1DM group > obesity group > control. Ischaemic modified albumin (IMA) levels were found to be high only in the T2DM + HFD group. HDL — high-density lipoprotein; LDL — low-density lipoprotein*

and obesity groups. There were no statistically significant differences between the T1DM and T2DM + HFD groups. In our correlation analysis, we observed a positive correlation between testosterone and TAS and a negative correlation between testosterone and TOS and OSI.

Obesity is a metabolic disorder characterised by excessive body fat accumulation or inappropriate dis-

Table 3. *Correlation analysis*

Parameters	r	p	Parameters	r	p
ALT & AST	0.604	< 0.01	ALT & HDL	0.602	< 0.01
ALT & CHOL.	0.600	< 0.01	ALT & LDL	0.533	< 0.01
ALT & OSI	0.377	0.06	ALT & TAS	–0.396	0.05
ALT& TOS	0.406	0.04	ALT &TRIG	0.482	0.01
AST & HDL	0.648	< 0.01	AST & CHOL.	0.633	< 0.01
AST & LDL	0.560	< 0.01	AST & OSI	0.400	0.05
AST & TRIG	0.608	< 0.01	AST & TOS	0.462	0.02
CHOL. & OSI	0.537	< 0.01	CHOL. & LDL	0.920	< 0.01
CHOL. & TRIG	0.925	< 0.01	CHOL. & TOS	0.642	< 0.01
LDL & TRIG	0.843	< 0.01	LDL &TOS	0.474	0.01
IMA & TOS	0.484	0.01	IMA & LDL	0.569	< 0.01
OSI & TAS	–0.829	< 0.01	OSI & IMA	0.388	0.04
OSI & TRIG	0.581	< 0.01	IMA & TRIG	0.549	< 0.01
TESTO & TAS	0.662	< 0.01	OSI & TOS	0.974	< 0.01
TESTO & OSI	–0.629	< 0.01	TAS & TOS	–0.723	< 0.01
TESTO & TOS	–0.626	< 0.01			

ALT — alanine aminotransferase; AST — aspartate aminotransferase; CHOL — cholesterol; OSI — oxidative stress index; TOS — total oxidant capacity; HDL — high-density lipoprotein; LDL — low-density lipoprotein; TRIG — triglycerides; IMA — ischaemic modified albumin; TAS — total antioxidant capacity; TESTO — testosterone

tribution of body fat due to environmental and genetic factors. A high intake of carbohydrates and saturated fatty acids, especially trans fats, increases oxidative phosphorylation, glyceraldehyde autooxidation, protein kinase C activation, activation of polyol and hexosamine pathways, and oxidative stress [25–28].

Obesity and a diet causing obesity have been shown to induce many changes, especially in adipose tissue.

Experimental studies have demonstrated that obesity and the oxidative stress triggered by it cause abnormal metabolism, proliferation, and differentiation in adipose cells. Increased ROS formation and low antioxidant defence in adipocytes cause abnormal postprandial metabolism, hyperleptinaemia, and chronic inflammation [29, 30]. In our study, although not statistically significant, we found that TOS levels were higher in the obesity group compared to the control group and lower than in the T1DM group, but statistically significantly lower than in the T2DM + HFD group. We found that OSI levels in the obesity group were significantly higher than in the control group. These results show that oxidative stress increases in obesity and exceeds the antioxidant defence system.

Obesity is the most common cause of insulin resistance and T2DM. However, the mechanisms by which increased body fat accumulation and circulating fatty acids cause insulin resistance in obesity have not been clearly determined [29]. Many molecular mechanisms have been proposed to explain the relationship between obesity and T2DM. One of these is the damage caused by increased oxidative stress in obesity on insulin receptors in muscle and liver. Studies have shown that increased intracellular oxidative stress affects many kinases and phosphatases involved in the insulin signalling pathway. Furthermore, attenuation of hydrogen peroxide production by genetic engineering of catalase overexpression in mouse muscle mitochondria maintains insulin sensitivity despite HFD, indicating that the degree of insulin sensitivity is functionally linked to cellular redox status [30–34]especially in obesity states. However, sustained nutrients overflow may dysregulate this function resulting in adipocytes hypertrophy, AT hypoxia, inflammation and oxidative stress. Systemic inflammation may also contribute to the disruption of AT redox equilibrium. AT and systemic oxidative stress have been involved in the development of obesity-associated insulin resistance (IR). In our study, the development of T2DM as a result of obesity increased TOS levels almost 4-fold compared to the control group and almost 3-fold compared to the obesity group. Similar increases were observed in OSI and IMA levels. These results suggest that obesity and diabetes, along with diabetes-related complications, increase oxidative stress.

While there is a gradual progression from obesity to T2DM in general, the autoimmune reaction in TI-DM is triggered at an earlier age in response to environmental factors not fully elucidated in genetically

predisposed individuals [11, 12, 35]. The autoimmune process in pancreatic beta cells is characterised by infiltration of macrophages and dendritic and T cells in the islets of Langerhans. Biopsies of mouse and human pancreatic islet cells with T1DM have shown that pro-inflammatory cytokines (interferon gamma, tumour necrosis factor alpha, interleukin 1 beta), immune cells, and free radicals are the main factors inducing apoptosis [36–39]. It is not known whether inflammation in T1DM causes oxidative stress in pancreatic cells or whether triggered cellular oxidative stress causes inflammation. In our study, we found that TOS levels were significantly higher in the T1DM group compared to the control group. Although TOS levels were higher in the T1DM group than in the obesity group, there was no significant difference. TOS levels in the T1DM group were lower than in the T2DM + HFD group. The reason for this may be that the already increased oxidative stress in obesity is further increased by the addition of diabetes and its complications. Interestingly, the T1DM group had the lowest serum TAS levels. In obesity and T2DM, antioxidant vitamins stored in adipose tissue and lipoprotein HDL with high antioxidant capacity may be the main reasons for this.

In obesity, the balance of energy metabolism hormones such as adiponectin [40], ghrelin [41], leptin [42], and many other hormones is disturbed. One of these hormones is testosterone. The interaction of the hypothalamic-pituitary-gonadal (HPG) axis with leptin may alter serum testosterone levels by affecting gonadotropin-releasing hormone (GnRH), follicle-stimulating hormone (FSH), and luteinising hormone (LH) release. Ghrelin receptors are reported to be present in the testis, and ghrelin may play a key role in testosterone production [43]. Serum adiponectin level has been shown to be negatively correlated with both testosterone and ROS production [44, 45].

All these metabolic hormones either directly or indirectly decrease the androgens in men. The complex crosstalk between these hormones is disrupted in obesity, causing a major disruption in the hormonal milieu, which in turn affects male reproductive function. Although there is a body of evidence highlighting the complexity and multifactorial effects of obesity on specific male reproductive functions, the relationship between obesity and semen parameters is still debated [46, 47]. In our study, we did not find a significant correlation between the testosterone levels of obesity rats and the control group. However, serum testosterone levels of the T1DM and T2DM + HFD groups were significantly lower than those of the obesity group.

DM has been shown to affect many functions of the male reproductive system, including erectile dysfunction, ejaculatory dysfunction, and impaired semen quality. DM-induced sexual dysfunction has been extensively studied in men. In the male reproductive system, DM-induced complications such as neuropathy, vascular endothelial dysfunction, disruption of the hypothalamic–pituitary–gonadal axis, increased germ cell apoptosis in the testes, impaired energy metabolism, and altered expression of glucose transporter proteins significantly affect testosterone levels. Decreased testosterone concentrations have been reported in animal models of DM such as STZ [48, 49], alloxan [50, 51], and nicotinamide + STZ [52] and in BB rats [53]. Decreases in 3β-hydroxysteroid dehydrogenase (HSD) and 17β-HSD activities were detected during testosterone synthesis stages in the testis of the STZ-induced T1DM rat model [54]. However, no significant difference was found in testosterone levels in a T2DM model in Otsuka Long Evans Tokushima Fatty (OLETF) rats compared to the control group [55].

Increased oxidative stress has been shown to be the main cause of dysfunction of the male reproductive system in both T1DM and T2DM rats. In both T1DM and T2DM animal models, there is a significant decrease in antioxidant enzymes and an increase in testicular lipid peroxidation. Studies using STZ-induced T1DM rats have shown a significant decrease in antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) activities and a significant increase in malondialdehyde (MDA) levels [56, 57]. In studies conducted in alloxan-induced T1DM rats, a significant decrease in superoxide dismutase, glutathione peroxidase, and catalase (antioxidant enzymes) activities and a significant increase in the amount of thio-barbituric acid-reactive substance in the testis were reported [50].

In another study using STZ-induced T1DM rats, it was reported that there was no significant change in testicular SOD, CAT, GPx, and MDA (antioxidant enzymes) activities even after 1 and 8 weeks [58]. In our study, we found that serum testosterone levels were significantly lower in both the T1DM group and T2DM + HFD groups compared to the control and obesity groups. Serum testosterone levels showed a strong positive correlation with serum TAS levels, while serum TAS and OSI levels showed a strong negative correlation. These results, like those of other studies, supported that the main reason for decreased serum testosterone levels in T1DM and T2DM are increased oxidative stress in these diseases.

Conclusion

Obesity and diabetes affect the male reproductive system as well as many metabolic pathways. DM has been shown to have negative effects on male fertility, sperm

quality, semen components, sperm motility, and sperm DNA damage. This condition not only causes infertility but may also cause mutation by damaging sperm DNA, and it may also damage embryonic development stages [59]. As we have shown in our study, increased oxidative stress in diabetes is one of the important factors that damage the male reproductive system. In particular, the strong correlation of serum TAS and TOS levels with serum testosterone levels is important evidence for this. In diabetic men, testosterone treatment and antioxidant treatment options specific to pancreatic beta cells have emerged [16, 56]. Combining testosterone with antioxidant therapy in diabetes may be promising. Of course, further molecular and clinical studies are needed to test this hypothesis.

Conflict of interest

The authors did not declare any conflict of interest

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None.

Data availability statement

Our study data are available.

Ethics statement

This experimental study was approved by Burdur Mehmet Akif Ersoy University Animal Experiments Local Ethics Committee.

Authors' contribution

H.Y.E.: conceptualisation, data curation, methodology, project administration, supervision, writing-review, and editing. R.A.: data curation, validation, visualisation, and methodology. M.K.: validation and visualisation. G.A.: formal analysis and supervision. O.A.: formal analysis and validation.
O.O.: methodology and data curation. R.C.C.: formal analysis and supervision. U.D.: conceptualisation and supervision.

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