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Could the increase in oxidative stress be the reason for the increased polyamine levels in diabetic obese and non-diabetic obese patients?

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ABSTRACT

Putresin, spermine, and spermidine are important polyamines found in all living organisms. In this study, as a first in the literature, we aimed to investigate polyamines levels and their relationship with oxidative stress in obese adults. The study was carried out with 85 obese patients and 29 healthy controls. Glucose, HbA1c, urea, uric acid, CRP, Total antioxidant status and Total oxidant status putrescine, spermine and spermidine levels were analysed. The study found putrescine and spermidine levels in obesity (0.25 ±0.13) (2.29 ±0.79) were found to be significantly lower, respectively, than the control group (0.38± 0.08) (1.80 ± 0.68) ($p<0.05$). It was observed that both OSI and TOS values in the diabetic obese group were statistically higher than both the control group and the non-diabetic obese group. As a result; although polyamine levels are low in obesity, increased oxidative stress in the diabetic obese group caused an increase in polyamine levels.

Keywords: Polyamines, obesity, diabetes mellitus, oxidative stress

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INTRODUCTION

The World Health Organization (WHO) has defined obesity as "excessive fat accumulation that impairs health" [1]. Obesity is a complex and multifactorial disease which affects health negatively. At the same time, it increases the risk of developing metabolic diseases such as Type 2 Diabetes mellitus (T2DM), fatty liver, as well as cardiovascular diseases such as hypertension, myocardial infarction, stroke, and also various cancers. These diseases, which occur on the basis of obesity, constitute 70% of early deaths [1, 2]. According to 2016 data, 39% of adults aged 18 and over worldwide are overweight and 13% are obese. The prevalence of obesity has increased 3 times in the world since 1975 [1].

Obesity is the most important risk factor for T2DM. This is due to the fact that in most cases diabetes occurs on the background of obesity. Common obesity and T2DM are increasingly defined as "diabesity" [3].

Putrescine, spermine, and spermidine are important polyamines found in all living organisms. They can be synthesized endogenously in cells or taken exogenously with food. The precursor molecule is arginine in the urea cycle. Ornithine is synthesized from arginine. Putrescine, formed by decarboxylation from ornithine, is the precursor compound of spermine and spermidine [4] (Figure 1).

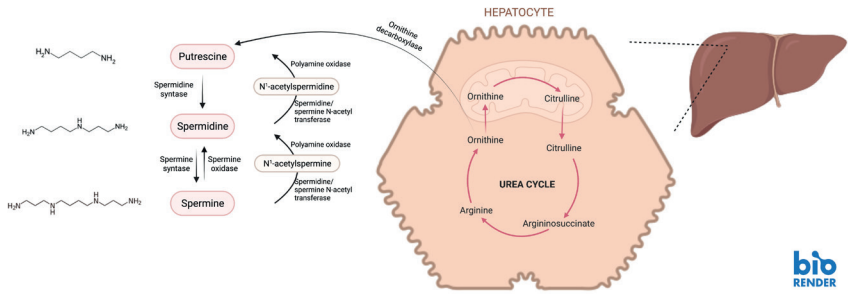

Figure 1. Formation of spermidine and spermine from putrescine.
Figure created with BioRender.com

Polyamines are important for cell growth and differentiation. They are involved in functions such as DNA synthesis and stability, regulation of transcription, ion channel regulation, and protein phosphorylation [5]. Polyamines, which are known to have antioxidant properties, also affect the stabilization of lipids, brain development, nerve growth and regeneration [5, 6].Since polyamines are positively charged at physiological pH, they tend to bind to negatively charged biomolecules such as DNA, RNA, proteins, and phospholipids, acting as polycations. Many studies showed that polyamines perform their functions by binding to biomolecules [7,8]. It was found that polyamines play a role in proinsulin

biosynthesis and insulin secretion [9]. The role of sperm and spermidine in insulin production is provided by stimulating insulin secretion and participating in the proliferation of island cells. In a study conducted in 2003, it was found that spermine and spermidine at physiological concentrations inhibit glucose from reacting with proteins non-enzymatically (glycation) [10].

Obesity is also defined as an increased chronic oxidative stress state. Oxidative stress occurs as a result of the disruption of the balance between oxidants and antioxidants. In obesity, excess free fatty acid induces oxidative stress by causing lipid peroxidation [11, 12].

In this study, we aimed to investigate the level of polyamines in obesity and its relationship with oxidative stress. Our study is the first in the literature to measure putrescine, spermine and spermidine levels in obese adults and to investigate their relationship with oxidative stress.

METHODOLOGY

Ethics committee approval was obtained from the Istanbul Medipol University Non-Interventional Clinical Research Ethics Committee (Decision No: 205). Informed consent was obtained from the patients who applied to Medipol Mega University Hospital for routine examination. The study was carried out with 85 obese patients with a mean age of 18-70 years and 29 healthy controls. According to body mass index (BMI) values, between 18.5 and 24.9 kg/m^2 formed the control group, and values of 24.9 kg/m^2 and above formed the obese group. The individuals, which constituted the study group, were divided into subgroups as diabetic obese (n:29) and non-diabetic obese (n:56) according to the criteria of the American Diabetes Association (ADA) [13].

Being younger than 18 years old or older than 70 years old, smoking, have kidney function disorders, hypertension, heart disease, osteoarthrosis, cancer, polycystic ovarian disease, inflammatory and infectious diseases were adopted as exclusion criteria.

The blood taken from the whole study group was centrifuged at 900 rpm for 15 minutes. If the separated sera could not be studied on the same day, they were aliquoted and brought up to -80°C. Glucose, glycosylated hemoglobin (HbA1c), urea, uric acid, C-Reactive Protein (CRP) levels were measured quantitatively in Cobas-Roche 6000 autoanalyzer using immuno chemiluminescence method. Hemogram test was performed by flow cytometric method on Symex 2000i device.

Total antioxidant status (TAS) and Total oxidant status (TOS) were measured spectrophotometrically [14,15].

Analysis of Total antioxidant assay

ABTS [2,2′-azino-bis(3-ethyl-benzothiazoline-6-sulfonic acid)] (Sigma-Aldrich, Taufkirchen,Germany) reagent is radicalized by hydrogen peroxide (Sigma-Aldrich, Taufkirchen, Germany). When serum is added, antioxidant-sin the serum neutralize existing ABTS radicals. The absorbance is measured at 658 nm.

Analysis of Total oxidant assay

Fe_2SO_4 dissolves in water, releasing Fe^{2+}. Oxidants found in serum enable Fe^{2+} to Fe^{3+} oxidation. TheX-orange (Sigma-Aldrich, Taufkirchen, Germany) reagent used givesa colored complex with Fe^{3+}. The absorbance is measured at 658 nm.

Oxidative stress index(OSI) was calculated using the TOS/TAS x100 formula. Putrescine, spermine and spermidine levels were studied by High performance liquid chromatography (HPLC) method.

Analysis of Putrescine, Spermine and Spermidine

For the measurement of putrescine, spermine and spermidine concentration, firstly, 200 µl of patient serum was centrifuged at 15000xg at 4°C for 10 minutes. 100µl of supernatant was taken and added with 100µl of cold 1.5 M $HClO_4$, and then was stirred at 25°C for 1 minute. Then, 50µl of cold 2 M K_2CO_3 was added and mixed for 10 seconds. It was evaporated with CO_2 gas under vacuum device and then stirred at room temperature for 1 minute. It was centrifuged at 15000xg for 10 minutes at 4°C and 100 µl of supernatant was collected. It was diluted by adding 150 µl of H_2O on it. For HPLC analysis, 700µl of H_2O, 50µl of 1.2% (w/v) benzoic acid and 50µl of sample was added into the vials, respectively. Two injections were made from each sample and their averages were taken. Measurements were performed using an HPLC system (Waters, Milford, MA) equipped with a reverse-phase column (Nova-Pak C18; 3.9 150 mm; 4 m particle size; Waters) and precolumn (Nova-Pak C18; 3.9 20 mm; 4 m particle size; Waters). Fluorescence detection was set to 340 and 450 nm excitation and emission wavelengths, respectively [16].

Statistical Analysis

Statistical analyzes were performed with SPSS (version 17, Chicago, IL, USA) program and Graphpad prism 8.0(San Diego, California, USA). Group distributions for TAS, TOS, glucose, CRP, hemogram, and HbA1c were evaluated using the Kolmogorov-Smirnov test. Student's t test was used for the parameters with normal distribution in the group, and Mann-Whitney U test was used for

the parameters that did not fit the normal distribution.

In subgroup analysis, parametric t-test and one-way ANOVA were used for groups with normal distribution. Mann-Whitney-U and non-parametric analysis of variance Kruskall-Wallis test were used for the variables that did not fit the normal distribution. Pearson correlation analysis test was used for correlation analysis between groups. Statistical significance level was accepted as $p \leq 0.05$.

RESULTS and DISCUSSION

The prevalence of obesity has increased to pandemic dimensions in the last 50 years [2]. Obesity is the second most important cause of preventable death after smoking (sitting is the new smoking)[1,2]. The fact that obesity increases very rapidly, causes many diseases, especially cardiovascular and endocrine, and can be prevented, increases the importance of obesity studies [2].

Oxidative stress arises from an imbalance between free radicals and the antioxidant defense system of the cell. Free radicals, which increase in obesity, have an effect on hypothalamic neurons in the control of hunger and satiety and, accordingly, in the control of body weight [17]. It is known that oxidative stress increases in obesity. In the study of Catoi et al., TAS and TOS examinations were performed in 23 obese and healthy control groups each, and it was observed that TAS levels were lower and TOS values were higher in obese patients compared to the normal-weight healthy group [11]. Serum TAS levels were evaluated in a population-based study in 3042 adult obese adults. In a study conducted with randomly selected 1514 men and 1528 women, it was revealed that obese male participants had 10% lower TAS concentrations than normal-weight men, and female obese participants had 6% lower TAS concentrations than normal-weight ones [11].

In our study, when the obese group and the control group were compared, no statistical difference was observed in TAS values. OSI values were numerically higher in the obese group, while TOS values were statistically higher (Table 1). High OSI values revealed that oxidative stress was increased in our obese study group, which was consistent with the literature.

Table 1. Biochemical parameters of control and obese groups

	Control Group n=29	**Obese Group n=85**	**p**
Putrescine (nmol/mL)	0,39±0,08	0,25±0,13	<0,05
Spermin (nmol/mL)	6,8±1,87	6,47±1,84	>0,05
Spermidine (nmol/mL)	2,29±0,79	1,80±0,68	<0,05
TAS (Trolox Eqv./L)	1,01±0,13	1,03±0,21	>0,05
TOS (mM H_2O_2 Eqv./L)	19,65±2	22,24±5,46	<0,05
OSI (AU)	1,97±0,3	2,25±0,77	>0,05
Glucose (mg/dl)	93,23±9,31	129,3±69,52	<0,05
HgA1c (%)	4,78±0,45	6,24±1,87	<0,05
CRP (mg/L)	1,75±1,73	4,04±3,92	<0,05
Urea (mg/dl)	27,60±7,68	30,87±9,12	>0,05
Uric acid (mg/dl)	4,55±1,56	5,54±1,52	<0,05
Leukocyte	6,84±1,57	7,52±1,57	<0,05
Erythrocyte	4,72±0,44	4,94±0,54	>0,05
Hemoglobin	13,26±1,14	13,78±1,69	>0,05
Lymphocyte	2,1±0,65	2,53±0,71	<0,05

Abbreviations: BMI: Body mass index; TAS: Total antioxidant status; TOS: Total oxidant status; OSI: Oxidative stress index; CRP: C reactive protein; HgA1c: Hemoglobine A1c. p<0,05 was considered statistically significant.

When subgroup analyzes were performed (Table 2), it was observed that both OSI and TOS values in the diabetic obese group were statistically higher than both the control group and the non-diabetic obese group Similar to TOS, glucose and HbA1c OSI was found to be correlated with glucose and HbA1c (Table 3). It was thought that oxidative stress increased in parallel with the increase in blood glucose level and increased oxidative stress increased polyamine levels.

Table 2. Biochemical parameters of control, non-diabetic obese and diabetic obese groups

	Control Group n=29	Obese Group		p	Intergroup Significance[a]
		Non-Diabetic Obese n=56	Diabetic Obese n=29		
BMI (kg/m^2)	22,43 ± 1,79	30,04 ± 4,14	30,92 ± 5,90	<0,05	1-2 1-3
Putrescine (nmol/mL)	0,39±0,08	0,21±0,1	0,33±0,14	<0,05	1-2 2-3
Spermin (nmol/mL)	6,80±1,87	6,23±1,35	6,93±2,49	>0,05	
Spermidine (nmol/mL)	2,29±0,79	1,54±0,52	2,31±0,7	<0,05	1-2 2-3
TAS (Trolox Eqv./L)	1,01±0,13	1,06±0,2	0,98±0,22	>0,05	
TOS (mM H_2O_2 Eqv./L)	19,65±2,0	20,59±2,89	25,41±7,56	<0,05	1-3 2-3
OSI (AU)	1,97±0,36	2,02±0,55	2,69±0,94	<0,05	1-3 2-3
Glucose(mg/dl)	93,23±9,31	99,6±8,98	186,7±95,72	<0,05	1-2 1-3 2-3
HbA1c (%)	4,78±0,45	5,28±0,44	8,11±2,17	<0,05	1-2 1-3 2-3
CRP (mg/L)	1,75±1,73	3,25±2,94	5,56±5,05	<0,05	1-2 1-3
Urea (mg/dl)	27,60±7,70	30,21±8,64	32,16±10,02	>0,05	
Uric acid (mg/dl)	4,55±1,56	5,69±1,54	5,25±1,47	<0,05	1-2
Leukocyte	6,84±1,57	7,69±1,62	7,21±1,45	>0,05	
Erythrocyte	4,72±0,44	4,95±0,55	4,92±0,53	>0,05	
Hemoglobin	13,26±1,15	13,90±1,77	13,55±1,54	>0,05	
Lymphocyte	2,10±0,65	2,55±0,74	2,48±0,67	<0,05	1-2

(Abbreviations: BMI: Body mass index; TAS: Total antioxidant status; TOS: Total oxidant status; OSI: Oxidative stress index; CRP: C reactive protein; HgA1c: Hemoglobine A1c. p<0,05 was considered statistically significantp<0,05 was considered statistically significant. [a]Groups (Groups 1–3) of statistical difference are stated)

Table 3. Correlation matrix

	BMI	Putrescine	Spermidine	Spermin	TAS	TOS	OSI	Glucose	HbA1C	Uric acid	Urea	CRP
BMI	1,00											
Putrescine	-0,24*	1,00										
Spermidine	-0,22*	0,41*	1,00									
Spermin	-0,08	0,05	0,29*	1,00								
TAS	0,18	-0,03	-0,13	0,00	1,00							
TOS	0,19*	0,13	0,07	-0,05	-0,11	1,00						
OSI	0,06	0,08	0,10	-0,06	-0,70*	0,76*	1,00					
Glucose	0,17	0,14	0,18	0,16	0,04	0,45*	0,26*	1,00				
HbA1C	0,25*	0,12	0,16	0,12	0,01	0,44*	0,28*	0,91*	1,00			
Uric acid	0,17	-0,15	-0,25*	-0,02	-0,03	-0,02	0,04	-0,09	-0,08	1,00		
Urea	0,10	0,05	-0,08	0,17	0,05	0,00	0,00	0,06	0,07	0,40*	1,00	
CRP	0,36*	-0,17	-0,05	0,05	-0,04	0,08	0,10	0,37*	0,37*	0,06	0,00	1,00
	BMI	**Putrescine**	**Spermidine**	**Spermin**	**TAS**	**TOS**	**OSI**	**Glucose**	**HbA1C**	**Uric acid**	**Urea**	**CRP**
BMI	1,00											
Putrescine	-0,24*	1,00										
Spermidine	-0,22*	0,41*	1,00									
Spermin	-0,08	0,05	0,29*	1,00								
TAS	0,18	-0,03	-0,13	0,00	1,00							
TOS	0,19*	0,13	0,07	-0,05	-0,11	1,00						
OSI	0,06	0,08	0,10	-0,06	-0,70*	0,76*	1,00					
Glucose	0,17	0,14	0,18	0,16	0,04	0,45*	0,26*	1,00				

(Abbreviations: BMI: Body mass index; TAS: Total antioxidant status; TOS: Total oxidant status; OSI: Oxidative stress index; CRP: C reactive protein; HgA1c: Hemoglobine A1c. $p<0,05$ was considered statistically significant)

In this study, the levels of putrescine, spermine and spermidine, known as polyamines, were found to be lower in the obese group. Putrescine and spermidine levels in obesity) were found to be significantly lower, respectively, than the control group (Table 1). Spermin levels were found to be lower in the obese group compared to the control group (although it was not statistically significant (Table 1). When the correlation analyzes were examined, a negative correlation was found between BMI and Putrescine and Spermidine (Table 3). Our study revealed that in addition to the decrease in polyamine levels in obesity, as obesity level increases, polyamine levels decrease (Table 1).

When the subgroup analyzes were performed, the putrescine and spermidine levels were found statistically lower in the non-diabetic obese group compared to the control group (Table 2). A striking finding was that the putrescine and spermidine levels, in the non-diabetic obese group, were found to be lower when compared to those of the diabetic obese group (Table 2). Spermine levels in the diabetic obese group were also numerically higher than in the non-diabetic obese group (Table 2).

A study in patients with metabolic syndrome with and without type 2 diabetes showed that serum putrescine level was significantly elevated in patients with T2DM compared to those without T2DM and significantly correlated with HbA1c levels [18]. Unlike this study, which associated high polyamine level with hyperglycemia, oxidative stress was found to be correlated with hyperglycemia in our study. It was thought that polyamine levels, which showed antioxidant properties, increased to compensate for the increased oxidative stress. The increase of uric acid, which has antioxidant properties, supports this idea (Table 2)

There are opinions that polyamines prevent oxidative stress by inhibiting the auto-oxidation of metals or by acting as a direct antioxidant. Many studies showed that polyamines greatly modulate the homeostasis of reactive oxygen species. Homeostatis performs this function directly or indirectly by regulating antioxidant systems or by suppressing the production of reactive oxygen species [19].

When HbA1c levels were examined, statistically significantly higher values were found in the non-diabetic obese group compared to the control group. At the same time, statistically significantly lower polyamine levels were found in the non-diabetic obese group (Table 2). This finding suggested that the increase in polyamine levels was due to oxidative stress, not hyperglycemia. Otherwise, we should have seen high polyamine levels in the non-diabetic obese group.

In a study, it was observed that spermine or spermidine, or both were able to protect cells from ROS at normal or supraphysiological concentrations, but spermine was stronger in this regard 20. In our study, it was observed that the increase in spermidine was parallel with the increase in ROS.

In another study conducted on children, it was reported that serum polyamine levels were significantly higher in obese children [21]. The increase in polyamine levels in obese children may be associated with increased oxidative stress with obesity, suggesting that polyamine levels may be different from adults due to physiological growth.

In conclusion, our study is the first in the literature to measure putrescine, spermine and spermidine levels in obese adults. Putrescine, spermine and spermidine levels were found to be lower in non-diabetic obese compared to the control group, but it was observed that polyamines increased with increasing oxidative stress in diabetic obese.

ETHICAL STATEMENT

Our study was approved by Medipol University local ethics committee (Decision No: 205). There are no ethical issues with human or animal subjects.

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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AUTHOR CONTRIBUTIONS

These authors contributed equally.

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