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EVALUATION OF OXIDATIVE STRESS PARAMETERS AND ANTIOXIDANT STATUS IN PLASMA AND ERYTHROCYTES OF ELDERLY DIABETIC PATIENTS WITH SARCOPENIA

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Abstract: *Objectives:* Oxidative stress may play a role in the pathogenesis of both sarcopenia and diabetes. Although the risk of sarcopenia is increased in people with type 2 diabetes, the relationship between sarcopenia oxidative stress and antioxidant status among the older diabetes population is not well studied. The aim of this present study was to evaluate the relationship between oxidative stress and antioxidant status and sarcopenia in elderly diabetic patients. *Design:* This was a cross-sectional designed study with a control group. A total of 60 type 2 diabetic elderly patients were enrolled in the study (30 sarcopenic and 30 controls). *Measurements:* Comprehensive geriatric assessments and anthropometric measurements were performed. Sarcopenia was diagnosed according to the European Working Group on Sarcopenia in Older People. Skeletal muscle mass was measured using bioelectrical impedance analysis. A handheld dynamometer was used for skeletal muscle strength measurements. Gait speed was measured using a 4 meter walking test. Plasma malondialdehyde (MDA), glutathione peroxidase (GSH-Px) and erythrocyte MDA, GSH-Px, superoxide dismutase (SOD), catalase and xanthine oxidase (XO) measurements were performed. *Results:* While plasma XO was significantly higher in sarcopenic individuals (0.406(0.225-0.775)) compared to controls (0.312(0.112-0.712)) (p=0.006), plasma GSH-Px was significantly lower in sarcopenic individuals (0.154(0.101-0.274)) compared to controls (0.204(0.12-.0312)) (p=0.003). Plasma XO (OR: 2.69 (CI 95% 0.13-52.76, p=0.041) and BMI (OR: 0.6 (CI 95% 0.41-0.89, p=0.009) were independently associated with sarcopenia of diabetes in multivariate analysis. *Conclusions:* Only plasma XO was found to be independently associated with sarcopenia. XO can be important in the pathogenesis of sarcopenia in diabetes. Oxidative stress and antioxidant status might be associated with sarcopenia in diabetic older individuals but this association seems to be mediated by other factors. Further studies are needed on this subject.

Key words: Aged, diabetes mellitus, oxidative stress, sarcopenia.

Introduction

Sarcopenia is a syndrome characterized by generalized and progressive loss of muscle mass and loss of strength, which can lead to physical disability, poor quality of life and poor outcomes (1). There are different definitions of sarcopenia. According to the European Working Group on Sarcopenia in Older People (EWGSOP), the consensus criteria of low muscle mass with low muscle strength and/or function are needed to define sarcopenia (1). The prevalence of sarcopenia varies between different studies according to the sarcopenia definition that is used. According to the definition of the European Working Group on Sarcopenia in Older People (EWGSOP), the prevalence of sarcopenia in elderly people living at home is 1-29% (30% in women), 14-33% for those living in nursing homes (68% in males) and 10% for elderly patients in the hospital (2). Many different and complex factors play a role in the etiology of sarcopenia. Among the pathophysiological causes of sarcopenia are: increase in apoptotic activity of myofibrils, increase in proinflammatory cytokines, decrease in anabolic hormones, increased oxidative stress, changes in mitochondrial function and a reduction in the number of α-motor neurons (3).

Oxidative stress is a condition that occurs when the balance between reactive oxygen radicals that are formed as a result of normal metabolism in healthy individuals and the antioxidant system, which is a defense mechanism of the body, is in favor of radicals. Oxidative stress is an important mechanism that plays a role in cell senescence, and thus cellular destruction, cell damage and cell death in pathophysiological events such as atherosclerosis, diabetes, cancer, chronic inflammatory disease and central nervous system disorders (4). Oxidative stress plays an important role in the pathogenesis of sarcopenia as well. Oxidative stress may interfere with the balance between protein synthesis and breakdown, cause mitochondrial dysfunction and induce apoptosis in skeletal muscle tissue (5).

Type 2 Diabetes mellitus (DM), which is a common chronic disease among older people, is associated with oxidative stress (6). Diabetic individuals lost muscle strength more rapidly compared to non-diabetic individuals in The Health, Aging, and Body Composition Study (7). Sarcopenia is more prevalent among individuals with type 2 DM, and type 2 DM is associated with increased risk of sarcopenia (8). Although risk of sarcopenia in type 2 DM individuals is high, the mechanism

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in this process is not clear. In addition, factors associated with sarcopenia among older people with type 2 DM are not well studied. In this study, we aimed to evaluate oxidative stress and antioxidant parameters in elderly patients with type 2 DM and sarcopenia, and the contribution of these factors to sarcopenia in diabetic individuals.

Methods

Study design and population

This was a cross-sectional case-control study. Individuals who were admitted to the outpatient clinic of the Geriatric Medicine Department of Ankara University School of Medicine were consecutively evaluated for participation in the study. Inclusion criteria were being ≥ 65 years old, diagnosed with type 2 DM and having a body mass index (BMI) <30 kg/m2 in order to prevent recruiting sarcopenic obese subjects. Exclusion criteria were having acute disease, advanced organ failure, active malignancy, cachexia, amputated extremities, extreme pretibial edema, pacemakers, being immobile or bed bound or using antioxidant drugs. All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2008. All patients gave written informed consent. This study was approved by the ethical committee of Ankara University School of Medicine. A total of 60 individuals with type 2 DM (30 type 2 DM with sarcopenia and age/sex matched type 2 DM without sarcopenia) were enrolled in the study.

Comprehensive geriatric assessment

Demographic data, co-morbidities, duration of diabetes, medications and fall history within the last 1 year were recorded. Participants with an education <5 years were categorized as uneducated, the rest were categorized as educated. The Katz index was used to evaluate activities of daily living (ADL). The Lawton instrumental activities of daily living (IADL) was used to assess IADL. Mini Mental State Examination (MMSE) test was used to evaluate cognitive functions. Nutritional status was assessed using the mini nutritional assessment short form (MNA-SF). The MNA-SF is made up of six questions extracted from the full MNA questionnaire. Psychological status of the patients was assessed with the Geriatric Depression Scale Short Form (GDS-SF).

Anthropometric measurements

Height, body weight, BMI, waist circumference and hip circumference of all patients were measured. Height was measured with a height gauge. Body weight was measured with the subjects wearing light clothing using a digital weighing machine. BMI was calculated as weight (kilograms) / height squared (kg/m2). Waist circumference was measured by determining the midpoint between the lower rib and the iliac bone. Hip circumference was measured by determining the widest point on the side of the hip.

Assessment of sarcopenia

Sarcopenia was assessed according to the EWGSOP criteria. Slow gait speed or weak handgrip strength are indications to test for low skeletal muscle mass (1). Four meter gait speed (GS) was measured. Participants walked at their usual speed. A four meter course was clearly marked on the floor. Walking time was measured using a digital stopwatch. The participants were positioned behind the starting line. Measuring time was started at the first movement of the foot and ended when a foot completely crossed the finish line. ≤ 0.8 m/s was accepted as decreased GS (1). Hand grip strength (HGS) was measured with a handheld digital dynamometer (TKK 5101 Grip-D; Takei, Tokyo, Japan). Two measurements were taken from the dominant hand and the highest value was recorded for each patient. Low hand grip strength was defined as < 30 kg for men and < 20 kg for women. Skeletal muscle mass was assessed by bioelectrical impedance analyses (BIA). BIA was performed using the Quadscan 4000 (Bodystat, PO box 50, Douglas, Isle of Man, IM99 IDQ, British Isles) body composition analyzer. The equation that was defined in the study of Jannsen et al. was used to calculate muscle mass (9);

$$\text{Skeletal muscle mass (kg)} = [\text{height}^2(\text{cm}) / \text{BIA resistance (X } 0.401] + [\text{gender X } 0.825] + [\text{age(years) X } - 0.071] + 5.102 \text{ (for gender, female = 0, male = 1)}$$

Resistance value at 50 kHz was used. After calculating skeletal muscle mass, the absolute skeletal muscle mass was obtained from the following formula:

$$\text{Absolute muscle mass} = \text{skeletal muscle mass (kg)} / \text{height}^2 (\text{m}^2)$$

Values less than 8.87 kg/m^2 indicated low skeletal mass for men and values less than 6.42 kg/m^2 indicated low skeletal mass for women (1).

Laboratory analysis

Fasting blood glucose (FBG), creatinine, uric acid, glycolated hemoglobin (HbA1c), vitamin D, lipid profile, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP) levels and hemoglobin levels were evaluated by routine laboratory methods. Glomerular filtration rate (GFR) was calculated according to the MDRD (modified dietary renal disease).

Evaluation of oxidative stress and antioxidant status

Malondialdehyde (MDA) level and Xanthine oxidase (XO) enzyme activity were indicators of oxidant parameters. Superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and catalase enzyme activities were indicators of antioxidant parameters. MDA and GSH-Px were measured in both plasma and erythrocytes. SOD and

catalase were measured in erythrocytes. XO was measured only in plasma. For these measurements, blood samples were taken into different whole blood tubes and stored frozen at -80 ° C after separating the plasma and erythrocytes, in the Ankara University Medical Faculty Medical Biochemistry Department Research Laboratory. On the day of examination, erythrocytes in stored samples were hemolyzed and oxidant and antioxidant parameters were studied in the obtained erythrocyte hemolysates and plasma. The MDA level measurement was performed by a method based on the spectrophotometric measurement of the absorbance at 532 nm of the pink complex formed by MDA and thiobarbituric acid (10).

A method based on the reduction of nitroblue tetrazolium (NBT) compound in the reaction medium was used in the measurement of SOD enzyme activity when the superoxide radical formed by the base xanthine-xanthine oxidase system could not be removed by the SOD enzyme (11). The spectrophotometric method used to measure GSH-Px enzyme activity is based on the fact that GSH and hydrogen peroxide (H2O2) catalyze the conversion of NADPH to GSH-Px in water and GSSG (oxidized glutathione) depending on the amount of GSSG formed (12). Catalase activity was studied using a method based on the spectrophotometric monitoring of the decrease in the absorbance value of H2O2 at 240 nm in the catalase catalyzed reaction (13). The method based on the spectrophotometric measurement of the formation of uric acid of xanthine at 293 nm in the measurement of XO enzyme activity was used (14).

Statistical analysis

Statistical analysis was performed using SPSS program (Statistical Package for Social Sciences) for Windows 22.0 (SPSS Inc, Chicago, IL). Descriptive statistics are presented as mean ± standard deviation, median (minimum-maximum), frequency distribution and percentage. Pearson Chi-square test and Fisher's exact test were used to evaluate categorical variables. The distribution of the variables was examined using visual (histogram and probability plots) and analytical methods (Shapiro-Wilk Test). Student's T-test was used to assess variables with normal distribution between two independent groups. Mann-Whitney U test was used to assess variables without normal distribution between two independent groups. Logistic regression analysis was used in order to evaluate the relationship between sarcopenia, oxidant status and antioxidant status. The relationships between sarcopenia, antioxidant parameters and oxidant parameters were investigated separately. Four models were used. In model 1, age, gender and BMI were adjusted. Diabetes duration, HbA1c and vitamin D levels were adjusted in model 2. Co-morbidities (osteoporosis, dementia, cardiovascular diseases and depression) were adjusted in model 3. Finally, geriatric assessment (ADL, IADL, cognitive function, mood status and nutrition) were adjusted. If all oxidant and antioxidant parameters lost statistical significance before model 4, logistic regression analysis was stopped. Hosmer-Lemeshow test was used for model adaptation. Odds ratio (OR) and confidence interval (CI) 95% are given. Statistical significance level was accepted as $p < 0.05$.

Results

A total of 60 patients were studied. The mean age of the patients was 78.33 ± 5.99. 63.3% of participants were female and 36.7% of participants were male. Demographic and clinical data of sarcopenia and control groups are presented in table 1.

Table 1
Demographic and clinical data of sarcopenia and control groups

Demographic and Clinical Variables	Sarcopenia (n=30)	Control (n=30)	p
Age (year) $\overline{X}$ ±S	79.60±5.95	77.07±5.85	0.102
Sex (%)			
Female	19 (63.3%)	19 (63.3%)	1.000
Male	11 (36.7%)	11 (36.7%)	
Level of Education (%)			
Uneducated	12 (40%)	13 (43.3%)	0.793
Educated	18 (60%)	17 (56.7%)	
Presence of Comorbidities (%)	28 (93.3%)	28 (93.3%)	1.000
Comorbidities (%)			
Hypertension	22 (73.3%)	24 (80%)	0.542
CVS[a] Disease	17 (56.7%)	8 (26.7%)	0.018
Hyperlipidemia	13 (43.3%)	9 (30%)	0.284
Osteoporosis	12 (40%)	9 (30%)	0.417
Depression	4 (13.3%)	3 (10%)	1.000
Dementia	10 (33.3%)	1 (3.3%)	0.003
DM[b] Duration (year) median (min-max)	10 (2-35)	11 (2-45)	0.789
Number of drugs used median (min-max)	6.5 (1-13)	4.5 (2-11)	0.028
Number of patients with falls (within last 1 year) (%)	12 (40%)	10 (33.3%)	0.592
Katz ADL[c] median (min-max)	4 (0-9)	6 (0-6)	0.002
Lawton IADL[d] median (min-max)	5 (0-17)	14 (0-17)	0.001
MMSE[e] median (min-max)	18,5 (0-30)	27 (14-30)	<0.001
MNA-SF[f] median (min-max)	12 (8-14)	13 (10-14)	0.085
GDS[g] median (min-max)	4.5 (0-14)	4 (0-25)	0.435

a. Cardiovascular system; b. Diabetes mellitus; c. Activities of daily living; d. Instrumental activities of daily living; e. Mini mental state examination; f. Mini nutritional assessment short form; g. Geriatric depression scale.

There was a statistically significant difference between sarcopenia and control groups in terms of the presence of dementia and cardiovascular disease and the number of medications used. The percentage of patients with dementia and

cardiovascular disease in the sarcopenia group was significantly higher than the control group. In addition, the number of drugs used by individuals in the sarcopenia group was significantly higher than the control group (Table 1). Patients in the sarcopenia and control groups also showed statistically significant differences in terms of ADL scores of Katz, Lawton IADL and MMSE. The ADL scores of Katz, Lawton IADL and MMSE in the sarcopenia group were significantly lower than the control group (Table 1).

Anthropometric data and components of sarcopenia in the sarcopenia and control groups are shown in table 2.

Table 2
Anthropometric data and components of sarcopenia in the sarcopenia and control groups

Variables	Sarcopenia (n=30)	Control (n=30)	p
BMIa (kg/m^2)	24.43±3.36	27.01±2.56	0.001
Waist circumference (cm)	90.93±9.30	97.00±9.14	0.013
Hip circumference (cm)	98.33±8,74	102.50±8.17	0.061
Absolute skeletal muscle mass (kg/m^2)	7.09±1.11	9.01±1.52	<0.001
Hang grip strength (kg)	16.11±7.24	19.28±6.13	0.073
Gait speed (m/s)	0.43±0.20	0.63±0.10	<0.001

a. Body mass index

Participants with sarcopenia had significantly lower BMI values compared to controls. Waist circumference was also significantly lower in participants with sarcopenia compared to controls. ASM and GS were significantly lower in the sarcopenia group compared to the control group. However, there was no difference for HGS between sarcopenia and control groups (table 2).

Laboratory data, oxidative stress parameters and antioxidant status of sarcopenic participants and control subjects are presented in table 3.

There was a significant difference between sarcopenia and control groups for CRP. There was a statistically significant difference between plasma GSH-Px and plasma XO values in sarcopenia and control groups. Plasma GSH-Px values were significantly lower in the sarcopenia group compared to the control group, whereas plasma XO values were significantly higher in the sarcopenia group compared to the control group. The other laboratory values of participants in the sarcopenia and control groups were similar (table 3).

The independent effect of predicting the possible causal factors from previous comparative analyses was evaluated by multivariable logistic regression analysis and the results are presented in tables 4 and 5. Sarcopenia status was included as the dependent variable. The relationship between sarcopenia and oxidant status was evaluated by logistic regression analysis in table 4. According to the analysis, it was found that only plasma XO (OR: 2.69 (CI 95% 0.13-52.76, p=0.041) and BMI (OR: 0.6 (CI 95% 0.41-0.89, p=0.009) were independent factors related with sarcopenia at the end of model 4. The relationship between sarcopenia and antioxidant status was evaluated by logistic regression analysis in table 5. None of the parameters of antioxidant parameters were found to be significantly related with sarcopenia in model 1. No adjustments were made further.

Table 3
Laboratory data, oxidative stress parameters and antioxidant status of sarcopenic participants and control subjects

Variable	Sarcopenia (n=30)	Controls (n=30)	p
Hemoglobin (g/dL)	12.52±1.63	12.76±1.18	0.522
FBG[a] (mg/dL)	120 (75-384)	124 (84-339)	0.807
GFR[b] median (min-max)	70.0 (21.8-123.0)	79.0 (34.9-141.5)	0.119
Uric Acid (mg/dl)	5.99±1.82	5.66±1.82	0.476
Total Cholesterol, (mg/dL)	178 (112-291)	177 (110-253)	0.605
HDL[c] (mg/dL)	44 (25-88)	18.5 (24-76)	0.261
LDL[d] (mg/dL)	99.5 (63-198)	106.5 (46-160)	0.451
Triglyceride (mg/dL)	147 (57-345)	113 (52-291)	0.723
HbA1c[e] (%)	6.8 (5.4-14.9)	6.7 (5.3-12.7)	0.706
25-OH Vitamin D (ng/ml)	16.6 (5.5-76.0)	15.3 (3.4-67.0)	0.918
CRP[f] (mg/dl)	4.7 (0.1-29.5)	2.2 (0.1-18.4)	0.021
Plasma MDAg	9.52 (0.56-29.68)	9.96 (1.21-30.81)	0.515
Plasma GSH-Pxh	0.154 (0.101-0.274)	0.204 (0.120-0.312)	0.003
Erythrocyte Catalase	44139.6±9576.3	48812.2±10021.7	0.07
Erythrocyte GSH-Px	12.67±2.03	13.48±1.48	0.083
Plasma XO[i]	0.406 (0.225-0.775)	0.312 (0.112-0.712)	0.006
Erythrocyte MDA	906.10±330.58	950.60±293.06	0.583
Erythrocyte SOD[j]	256.78±120.70	287.07±124.92	0.343

a. Fasting blood glucose; b. Glomerular filtration rate; c. High density lipoprotein; d. Low density lipoprotein; e. Hemoglobin A1c; f. C reactive protein; g. Malondialdehyde; h. Glutathione peroxidase; i. Xanthine oxidase; j. Superoxide dismutase

Table 4
Multivariate logistic regression analysis for the relation between sarcopenia and oxidant parameters

MODEL 1			
Variables	**OR[a]**	**%95 CI[b]**	**p**
Erythrocytes MDAc	1	0.99-1.2	0.89
Plasma MDA	0.96	0.9-1.04	0.38
Plasma XOd	5.54	0.33-101.1	0.017
Age	1.03	0.92-1.15	0.54
Gender			
Female	Reference		0.42
Male	0.58	0.15-2.17	
BMIe	0.72	0.56-0.92	0.009
MODEL 2			
Variables	**OR**	**%95 CI**	**p**
Erythrocytes MDA	1	0.99-1.2	0.62
Plasma MDA	0.97	0.9-1.04	0.43

Plasma XO	2.95	0.59-146.1	0.012
Age	1.11	0.96-1.28	0.15
Gender			
Female	Reference		0.29
Male	0.46	0.11-1.93	
BMI	0.68	0.52-0.89	0.006
Diabetes duration	0.94	0.87-1.02	0.17
HbA1[c]	1.09	0.77-1.55	0.61
Vitamin D levels	1.03	0.99-1.08	0.1
MODEL 3			
Variables	**OR**	**%95 CI**	**p**
Erythrocytes MDA	1.01	0.99-1.2	0.542
Plasma MDA	0.94	0.85-1.06	0.15
Plasma XO	4.78	0.23-99.16	0.029
Age	1.14	0.97-1.33	0.1
Gender			
Female	Reference		0.16
Male	0.27	0.046-1.65	
BMI	0.63	0.45-0.88	0.007
Diabetes duration	0.95	0.88-1.04	0.34
HbA1[c]	1.05	0.68-1.68	0.82
Vitamin D levels	1.05	0.98-1.13	0,11
Dementia	17.39	0.96-314.1	0.053
Depression	8.85	0.53-145.4	0.12
Osteoporosis	0.26	0.017-4.19	0.34
CVD[f]	1.64	0.26-10.03	0.59
MODEL 4			
Variables	**OR**	**%95 CI**	**p**
Erythrocytes MDA	1	0.99-1.19	0.91
Plasma MDA	0.93	0.84-1.07	0.15
Plasma XO	2.69	0.13-52.76	0.041
Age	1.02	0.82-1.25	0.8
Gender			
Female	Reference		0.49
Male	0.48	0.06-3.75	
BMI	0.6	0.41-0.89	0.009
Diabetes duration	0.98	0.89-1.07	0.71
HbA1[c]	1.04	0.64-1.65	0.85
Vitamin D levels	1.04	0.97-1.12	0.23
Dementia	8.88	0.26-302	0.22
Depression	19.69	0.8-484.6	0.068
Osteoporosis	0.55	0.01-17.33	0.73
CVD	1.66	0.14-19.03	0.68
ADL[g]	1.25	0.57-2.75	0.57
IADL[h]	0.97	0.75-1.25	0.85
MMSE[i]	0.84	0.68-1.08	0.84
GDS[j]	0.79	0.74-1.24	0.79
MNA-SF[k]	1.3	0.6-2.88	0.49

a. Odds ratio, b. Confidence interval, c. Malodialdehyde, d. Xanthine oxidase, e. Body mass index, f. Cardiovascular disease, g. Activities of daily of living, h. Instrumental activities of daily living, i. Mini mental state examination, j. Geriatric depression scale, k. Mini nutritional assessment short form

Table 5
Multivariate logistic regression analysis for the relation between sarcopenia and antioxidant parameters

MODEL 1			
Variables	**OR[a]**	**%95 CI[b]**	**p**
Erythrocyte GSH-PXc	0.9	0.6-1.35	0.63
Plasma GSH-PX	0.8	0.24-2.73	0.069
Erythrocytes SODd	1.01	0.99-1.08	0.79
Erythrocytes catalase	1	0.78-2.81	0.69
Age	1.02	0.91-1.14	0.69
Gender			
Female	Reference		0.61
Male	0.71	0.194-2.63	
BMI[e]	0.74	0.58-0.94	0.015

a. Odds ratio; b. Confidence interval; c. Glutathione peroxidase; d. Superoxide dismutase; e. Body mass index

Discussion

In the current study, we examined oxidative stress (MDA level and XO enzyme activity) and anti-oxidant status (SOD, GSH-Px and catalase enzyme activities) in diabetic patients with and without sarcopenia. There was a statistically significant difference in plasma GSH-Px and plasma XO values between diabetic patients with and without sarcopenia. Plasma XO was significantly higher in diabetic participants with sarcopenia compared to controls. GSH-Px levels were found to be lower in diabetic sarcopenic subjects. In the literature, both animal and human studies have conflicting results. In the literature, there is evidence that XO enzyme activity increases in aging skeletal muscle. In a study by Ryan et al., XO activity in the gastrocnemius muscle was 65% higher in older rats compared to young rats (15). In addition, inhibition of XO activity reduces oxidative stress and improves skeletal muscle function in aged mice (16). XO inhibition by Allopurinol prevents skeletal muscle atrophy in vitro and improves skeletal muscle strength in individuals with chronic obstructive lung disease (17, 18). Ji et al. showed that GSH-Px activity in older rats increased 2-fold in muscle cytosol and 47% in muscle mitochondria (19). Kumaran et al found that GSH-Px activity in the skeleton in elderly rats was significantly lower than in young rats (20). There was no change in skeletal muscle GSH-Px activity with aging among human subjects in the study of Pansarasa et al. (21).

Although there are no studies specifically evaluating oxidative stress in type 2 DM older patients with sarcopenia, our results and results from the studies above suggest that oxidative stress might play a role in sarcopenia in type 2 DM older patients. Possible pathologic mechanisms of oxidative stress leading to sarcopenia are shifting protein balance negatively, impairing the function of neuromuscular junctions and excitation-contraction coupling and cross-bridge cycling

within the myofibrillar apparatus (22). The other pathological factors especially associated with sarcopenia in diabetics are decreased protein synthesis due to insulin resistance, intramyocellular, intermuscular, intramuscular fat deposition, neuropathy associated with diabetes and poor muscle perfusion (23). Although poor glycemic control was also suggested as a causative factor for sarcopenia among type 2 diabetic patients (24), this did not affect our study since there was no difference in HbA1c and FBG levels between study groups in our study.

Among oxidant and antioxidant parameters, only plasma XO was found to be independently associated with sarcopenia in multivariate regression analysis (tables 4 and 5). The other factor associated with sarcopenia in diabetic patients in multivariate regression analysis was low BMI. Xanthine oxidase can induce free radical generation in skeletal muscle, and inhibition of xanthine oxidase inhibitor may have a role in the treatment of sarcopenia. The xanthine oxidase inhibitor allopurinol was found to prevent this type of damage in several studies (25). In a rat model of cancer cachexia, another xanthine oxidase inhibitor, febuxostat, decreased the loss of lean mass, of which skeletal muscle mass is a component (26). In addition, xanthine oxidase inhibition reduces hyperglycemia induced oxidative stress in skeletal muscle of diabetic mice (27). Xanthine oxidase enzyme seems to be important in the pathogenesis of sarcopenia, especially in diabetic patients, and xanthine oxidase inhibition could be a potential therapeutic approach for sarcopenia of diabetic individuals. Lack of a significant relationship between oxidative stress and antioxidant parameters with sarcopenia other than plasma XO in multivariate regression analysis may be due to the low number of subjects in our study. Secondly, low BMI is strongly associated with sarcopenia. That is why BMI may have attenuated the effect of oxidative stress on sarcopenia in our study. Low BMI and malnutrition are significant risk factors for sarcopenia (28).

There are several limitations to this study. One of the limitations is the small sample of our study. In addition, the design of our study was cross-sectional with case-control groups. For this reason, the causal relationship between oxidant and antioxidant systems and sarcopenia cannot be determined. Finally, BIA was used for muscle mass assessment. Although CT or DEXA are the gold standard assessment tools for muscle mass evaluation, BIA is easier to implement in clinical practice, is inexpensive and is without ionizing radiation (29).

The strength of the study is that there is no other study evaluating oxidant-antioxidant parameters in peripheral blood specimens, specifically in type 2 DM individuals with sarcopenia. Another strength of this study is the use of EGWSOP criteria to define sarcopenia, which is a consensus definition and includes both qualitative and quantitative features of skeletal muscle.

In conclusion, oxidative stress and imbalance between oxidative and antioxidant systems may be an important factor in both type 2 diabetes and sarcopenia. However, larger studies are needed to evaluate this relationship. In addition, prospective studies are needed to evaluate the role of antioxidant therapy for type 2 diabetics with sarcopenia.

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Ethical Standards: We confirm that any aspect of the work covered in this manuscript that has involved human patients has been conducted with the ethical approval of all relevant bodies and that such approvals are acknowledged within in the manuscript.

References

1. Cruz-Jentoft AJ, Baeyens JP, Bauer JM, Boirie Y, Cederholm T, Landi F et al. Sarcopenia: European consensus on definition and diagnosis: Report of the European Working Group on Sarcopenia in Older People. Age Ageing. 2010;39(4):412-23. doi:10.1093/ageing/afq034.
2. Cruz-Jentoft AJ, Landi F, Schneider SM, Zuniga C, Arai H, Boirie Y et al. Prevalence of and interventions for sarcopenia in ageing adults: a systematic review. Report of the International Sarcopenia Initiative (EWGSOP and IWGS). Age Ageing. 2014;43(6):748-59. doi:10.1093/ageing/afu115.
3. Muscaritoli M, Anker SD, Argiles J, Aversa Z, Bauer JM, Biolo G et al. Consensus definition of sarcopenia, cachexia and pre-cachexia: joint document elaborated by Special Interest Groups (SIG) "cachexia-anorexia in chronic wasting diseases" and "nutrition in geriatrics". Clin Nutr. 2010;29(2):154-9. doi:10.1016/j.clnu.2009.12.004.
4. Sorg O. Oxidative stress: a theoretical model or a biological reality? C R Biol. 2004;327(7):649-62.
5. Meng SJ, Yu LJ. Oxidative stress, molecular inflammation and sarcopenia. Int J Mol Sci. 2010;11(4):1509-26. doi:10.3390/ijms11041509.
6. Morley JE, Malmstrom TK, Rodriguez-Manas L, Sinclair AJ. Frailty, sarcopenia and diabetes. J Am Med Dir Assoc. 2014;15(12):853-9. doi:10.1016/j.jamda.2014.10.001.
7. Park SW, Goodpaster BH, Strotmeyer ES, Kuller LH, Broudeau R, Kammerer C et al. Accelerated loss of skeletal muscle strength in older adults with type 2 diabetes: the health, aging, and body composition study. Diabetes Care. 2007;30(6):1507-12. doi:10.2337/dc06-2537.
8. Wang T, Feng X, Zhou J, Gong H, Xia S, Wei Q et al. Type 2 diabetes mellitus is associated with increased risks of sarcopenia and pre-sarcopenia in Chinese elderly. Sci Rep. 2016;6:38937. doi:10.1038/srep38937.
9. Janssen I, Heymsfield SB, Baumgartner RN, Ross R. Estimation of skeletal muscle mass by bioelectrical impedance analysis. J Appl Physiol (1985). 2000;89(2):465-71.
10. Dahle LK, Hill EG, Holman RT. The thiobarbituric acid reaction and the autoxidations of polyunsaturated fatty acid methyl esters. Arch Biochem Biophys. 1962;98:253-61.
11. Durak I, Canbolat O, Kavutcu M, Ozturk HS, Yurtarslani Z. Activities of total, cytoplasmic, and mitochondrial superoxide dismutase enzymes in sera and pleural fluids from patients with lung cancer. J Clin Lab Anal. 1996;10(1):17-20. doi:10.1002/(SICI)1098-2825(1996)10:1<17::AID-JCLA4>3.0.CO;2-I.
12. Paglia DE, Valentine WN. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. J Lab Clin Med. 1967;70(1):158-69.
13. Bergmeyer HU. Methods of enzymatic analysis. 2d English ed. Weinheim New York: Verlag Chemie ; Academic Press; 1974.
14. Hashimoto S. A new spectrophotometric assay method of xanthine oxidase in crude tissue homogenate. Anal Biochem. 1974;62(2):426-35.
15. Lambertucci RH, Levada-Pires AC, Rossoni LV, Curi R, Pithon-Curi TC. Effects of aerobic exercise training on antioxidant enzyme activities and mRNA levels in soleus muscle from young and aged rats. Mech Ageing Dev. 2007;128(3):267-75. doi:10.1016/j.mad.2006.12.006.
16. Ryan MJ, Jackson JR, Hao Y, Leonard SS, Alway SE. Inhibition of xanthine oxidase reduces oxidative stress and improves skeletal muscle function in response to electrically stimulated isometric contractions in aged mice. Free Radic Biol Med. 2011;51(1):38-52. doi:10.1016/j.freeradbiomed.2011.04.002.
17. Derbre F, Ferrando B, Gomez-Cabrera MC, Sanchis-Gomar F, Martinez-Bello VE, Olaso-Gonzalez G et al. Inhibition of xanthine oxidase by allopurinol prevents skeletal muscle atrophy: role of p38 MAPKinase and E3 ubiquitin ligases. PLoS One. 2012;7(10):e46668. doi:10.1371/journal.pone.0046668.
18. Delample D, Durand F, Severac A, Belghith M, Mas E, Michel F et al. Implication of xanthine oxidase in muscle oxidative stress in COPD patients. Free Radic Res. 2008;42(9):807-14. doi:10.1080/10715760802429039.
19. Ji LL, Dillon D, Wu E. Alteration of antioxidant enzymes with aging in rat skeletal muscle and liver. Am J Physiol. 1990;258(4 Pt 2):R918-23.
20. Kumaran S, Savitha S, Anusuya Devi M, Panneerselvam C. L-carnitine and DL-alpha-lipoic acid reverse the age-related deficit in glutathione redox state in skeletal muscle and heart tissues. Mech Ageing Dev. 2004;125(7):507-12. doi:10.1016/j.mad.2004.05.004.
21. Pansarasa O, Bertorelli L, Vecchiet J, Felzani G, Marzatico F. Age-dependent changes of antioxidant activities and markers of free radical damage in human skeletal muscle. Free Radic Biol Med. 1999;27(5-6):617-22.
22. Baumann CW, Kwak D, Liu HM, Thompson LV. Age-induced oxidative stress: how does it influence skeletal muscle quantity and quality? J Appl Physiol (1985).

2016;121(5):1047-52. doi:10.1152/japplphysiol.00321.2016.
23. Buford TW, Anton SD, Judge AR, Marzetti E, Wohlgemuth SE, Carter CS et al. Models of accelerated sarcopenia: critical pieces for solving the puzzle of age-related muscle atrophy. Ageing Res Rev. 2010;9(4):369-83. doi:10.1016/j.arr.2010.04.004.
24. Park SW, Goodpaster BH, Strotmeyer ES, de Rekeneire N, Harris TB, Schwartz AV et al. Decreased muscle strength and quality in older adults with type 2 diabetes: the health, aging, and body composition study. Diabetes. 2006;55(6):1813-8. doi:10.2337/db05-1183.
25. Ferrando B, Olaso-Gonzalez G, Sebastia V, Viosca E, Gomez-Cabrera MC, Vina J. [Allopurinol and its role in the treatment of sarcopenia]. Rev Esp Geriatr Gerontol. 2014;49(6):292-8. doi:10.1016/j.regg.2014.05.001.
26. Konishi M, Pelgrim L, Tschirner A, Baumgarten A, von Haehling S, Palus S et al. Febuxostat improves outcome in a rat model of cancer cachexia. J Cachexia Sarcopenia Muscle. 2015;6(2):174-80. doi:10.1002/jcsm.12017.
27. Bravard A, Bonnard C, Durand A, Chauvin MA, Favier R, Vidal H et al. Inhibition of xanthine oxidase reduces hyperglycemia-induced oxidative stress and improves mitochondrial alterations in skeletal muscle of diabetic mice. Am J Physiol Endocrinol Metab. 2011;300(3):E581-91. doi:10.1152/ajpendo.00455.2010.
28. Agarwal E, Miller M, Yaxley A, Isenring E. Malnutrition in the elderly: a narrative review. Maturitas. 2013;76(4):296-302. doi:10.1016/j.maturitas.2013.07.013.
29. Rubbieri G, Mossello E, Di Bari M. Techniques for the diagnosis of sarcopenia. Clin Cases Miner Bone Metab. 2014;11(3):181-4.