Research Article

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The activities of GST isozymes in stomach tissues of female obese patients

[Kadın obezite hastalarının mide dokularında GST izozimlerinin aktiviteleri]

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Abstract

Objectives: Obesity has become an important public health problem because of its increasing prevalence and relation with many diseases and mortality. Studies have shown its association with oxidative stress. In this study, the effect of obesity on total amount of thiol and some glutathione S-transferase (GST) isozymes were investigated which could serve as an important criteria in dose adjustment of some certain drugs in obese.

Methods: The gastric tissues removed by gastrectomy operation from 29 morbid obese female patients were analysed for thiol levels and activities of total GST, GSTT1-1 and GSTM1-1. Patients were grouped according to age, presence of hypertension and/or diabetes, and family history.

Results: The average total thiol was 131.22 (± 7.74) nmol/mg protein with no significant differences in between the groups. GSTT1 specific activities were about 20% higher in four groups: with ages over 35 years old, with hypertension, without diabetes and finally without family history, with respect to other groups. The differences between total

GST and GSTM1 activity levels of experimental groups were not significant.

Conclusions: This is the first study to compare activities of GST isozymes and total thiol content in the stomach tissues of obese female patients accompanying some common metabolic disorders, age and family history.

Keywords: diabetes; gastrectomy; GST; hypertension; obesity; total thiol.

Öz

Amaç: Obezite, artan yaygınlığı ve birçok hastalık ve ölümle ilişkisi nedeniyle önemli bir halk sağlığı sorunu haline gelmiştir. Çalışmalar, oksidatif stres ile ilişkisini göstermiştir. Bu çalışmada, obezlerde bazı ilaçların doz ayarlamasında önemli birer kriter olabilen, obezitenin toplam tiyol miktarı ve bazı glutatyon S-transferaz (GST) izozimlerine etkisi araştırılmıştır.

Yöntemler: 29 morbid obez kadın hastadan gastrektomi operasyonu ile çıkarılan mide dokuları tiyol düzeyleri ve toplam GST, GSTT1-1 ve GSTM1-1 aktiviteleri açısından analiz edilmiştir. Hastalar yaş, hipertansiyon ve/veya diyabet varlığı ile aile geçmişi göre gruplandırılmıştır.

Bulgular: Ortalama toplam tiol 131.22 (± 7.74) nmol/mg protein olarak ölçülmüş ve gruplar arasında önemli bir fark gözlenmemiştir. GSTT1 özgül aktiviteleri dört grupta; 35 yaş üstü, hipertansiyonlu, diyabetsiz ve aile öyküsü olmayanlarda, diğer gruplara göre yaklaşık % 20 daha yüksektir. Deney gruplarının toplam GST ve GSTM1 aktivite düzeyleri arasındaki farklar anlamlı değildir.

Sonuç: Bu, bazı yaygın metabolik bozukluklar, yaş ve aile öyküsü ilişkili obez kadın hastaların mide dokularındaki GST izozimlerinin aktivitelerini ve toplam tiyol içeriğini karşılaştıran ilk çalışmadır.

Anahtar Sözcükler: diyabet; gastrektomi; GST; hipertansiyon; obezite; toplam tiyol.

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Introduction

Obesity is a risk factor or determinant for a number of chronic diseases such as diabetes, cardiovascular morbidity and cancer. It, adversely, affects overall health as a result of abnormal or excessive accumulation of fat in the adipose tissue when energy intake exceeds energy consumption over a long period of time [1]. Over the past 50 years, worldwide prevalence of obesity has nearly tripled and it continues to grow at a pandemic dimension.

Obesity is widespread in Turkey compared to most other countries and its prevalence is higher, especially, among women. When the percentages of obesity among female, male and for overall Turkish population in 2011 were compared with those of 2017, there were elevations from 58 to 66% for women, from 52.7 to 62.8% for men, and finally, from 55.4 to 64.4% for whole population [2]. In other words, obesity is a growing problem for Turkey.

Many epidemiological and clinical studies have shown that obesity is associated with changing redox status and increased metabolic risk [3]. Oxidative stress could be both a result and a trigger for obesity [4]. Chronic over-nutrition, feeding with foods containing high levels of carbohydrates, consumption of saturated fatty acids and trans-fatty acids, stimulate intracellular pathways, leading to oxidative stress through a number of biochemical mechanisms [5]. It has, also, been shown that obesity, itself, can induce systemic oxidative stress: in fact, fat accumulation has been shown to increase NADPH oxidase activity and endoplasmic reticulum stress, which leads to increased reactive oxygen species (ROS) production [6].

Other factors contributing to oxidative stress in obesity include abnormal ROS production, hyperleptinemia, chronic inflammation, tissue dysfunction and inadequate antioxidant defense [7]. The detractive effect of obesity on the antioxidant defense system is generated by its effectiveness in lowering the expression of some antioxidant enzymes or altering their activities.

Thiol is one of the important instruments used by living organisms against oxidative stress. It functions both directly by non-enzymatic reactions and indirectly through enzymatic pathways. Total thiol content of the cell was constituted by cysteine residues in proteins and some other low molecular weight thiols such as glutathione, cysteine, homocysteine, cysteinylglycine, gamma-glutamyl cysteine and hydrogen sulfide, in an extent. Any change in total thiol status, in favor of reactive oxygen species, may be the cause of many diseases and it may lead alterations of the

physiological state. The imbalance in total thiol content have been evaluated in some studies and reported as a part of obesity diagnosis and progression [8, 9].

The glutathione S-transferase (2.5.1.18) (GST) superfamily that catalyzes the conjugation of glutathione with electrophiles of both endogenous and xenobiotic origin plays a critical role among detoxification systems. These enzymes are members of phase II system and they contribute significantly to the cellular biotransformation of electrophilic compounds. They protect against the genotoxic and carcinogenic effects of many xenobiotic and endogenous compounds. This enzyme superfamily has been considered and studied in obesity-related oxidative stress metabolism [10]. In most studies to date, polymorphism states of, especially, GSTT1 and GSTM1 have been studied in obese and healthy individuals from different organisms and human populations; but, the activities of those isozymes have, almost, never been studied.

The rate of absorption of drug active components and their fate in metabolism are directly or indirectly related with the activities of phase I and phase II enzyme families among which the glutathione S-transferases play important roles. Consequently, the levels of activities for those GST isozymes are administrative in drug metabolism and bioavailability. For example, warfarin, which is an anticoagulant drug normally used to prevent blood clot formation especially for patients with cardiovascular diseases, is mainly absorbed in stomach. The higher or lower than normal levels of GST activity may play crucial role in warfarin resistance [11]. Besides, there are many studies subjecting the problems in dose adjustment for obese patients. For instance, rifampicin, which is an antibiotic prescribed for tuberculosis, is absorbed in stomach maximum at fasting [12]. Another one is ethambutal which is, also, used for the treatment of tuberculosis. In a study investigating the effects of gastric reduction operations, and the size of the remaining part after the stomach part taken, on absorption of this drug, a 40–60% reduction was reported in those whose stomach was completely reduced [13].

In present study, total thiol content and GST isozyme activities of gastric tissue samples, which were obtained by gastrectomy operation on obese female patients of all age groups, were evaluated. A total of 29 Turkish obese female patients with/without diabetes, hypertension and familial history of obesity were divided into groups and cross-matched with each other for total thiol content and activities of GST isozymes, for the first time.

Table 1: Average age, BMI and total number of samples in experimental groups (SEM: Standard Error of Means).

Experimental groups	Average age $\pm$ SEM	Average BMI $\pm$ SEM	Number of patients
≤ 35	27.46 $\pm$ 1.39	48.21 $\pm$ 1.99	13
> 35	45.94 $\pm$ 1.64	46.08 $\pm$ 1.64	16
HT (+)	42.43 $\pm$ 3.78	44.51 $\pm$ 1.97	7
HT (–)	36.14 $\pm$ 2.37	46.35 $\pm$ 1.24	22
DM (+)	42.00 $\pm$ 2.95	47.63 $\pm$ 2.81	8
DM (–)	36.00 $\pm$ 2.54	45.21 $\pm$ 1.01	21
FH (+)	35.47 $\pm$ 2.72	46.42 $\pm$ 1.62	15
FH (–)	40.00 $\pm$ 3.05	45.33 $\pm$ 1.36	14

Materials and methods

Gastric tissue samples

Gastric tissue samples were obtained from morbid obese (having BMI value over 40) female patients of all ages by gastrectomy operation in Ankara Kecioren Training and Research Hospital, Department of General Surgery. None of the patients reported smoking and metabolic disease other than the subjected ones (hypertension and/or diabetes) (Table 1). Stomach samples were rinsed with homogenization buffer and stored in -80°C freezer immediately after taken from the patients. The samples were transported in cold chain and kept frozen until the day of the experiment.

Homogenization of gastric tissue samples

Onto the thawed and chopped tissues, 1:4 (g:mL) of homogenization buffer (phosphate buffer, 100 mM (pH: 7.4) containing 150 mM NaCl, 1 mM EDTA, 1.4 mM DTT and protease inhibitor cocktail (0.5 mM AEBSF, 0.3 μM Aprotinin, 10 μM Bestatin, 10 μM E-64, 10 μM Leupeptin)) were added and homogenized in ice-dipped Potter-Elvehjem homogenizer with teflon pestle, up and down, six times with 30 s intervals. The homogenates were centrifuged at 12,000 g at $+4^{\circ}\text{C}$ for 30 min. The supernatant was collected, aliquoted and stored in a freezer at -80°C .

Protein determination by Bradford method

Protein concentrations of the samples were measured and calculated by Bradford method. Commercially available Bradford reagent (Sigma–Aldrich, B6916) was used, as it was defined by the supporter; and, all the measurements were taken by ELISA Plate Reader System (Thermo, Multiskan™ FC Microplate Photometer).

Determination of activities of GSTs

Substrates that are specific to certain GST isozymes are often used to detect the specific activities of them. 1-chloro-2,4-dinitrobenzene (CDNB) is a standard model substrate used by almost all GST

Table 2: The composition of reaction media for enzyme activity measurements. The unit of extinction coefficient values (ϵ) were all $\text{M}^{-1}\cdot\text{cm}^{-1}$.

GST enzyme	Substrates (mM)	pH	GSH (mM)	Homogenate (μg)	λ_{max} (nm)	ϵ
Total	1 (CDNB)	7.4	1	3.23–23.60	340	9.6
GSTM1	0.5 (pNBC)	6.5	5	128.75–471.75	310	1.9
GSTT1	0.25 (EPNP)	6.5	0.5	25.75–94.35	360	0.5

isozymes. 1,2-dichloro-4-nitrobenzene (DCNB), *p*-nitrobenzyl chloride (pNBC), ethacrynic acid (EA) and 1,2-epoxy-3-(*p*-nitrophenoxy)-propane (EPNP) are among those specific model substrates and specific to GST M2, GST M1, GSTA4-4 [14] and GSTT1-1 [15], respectively. Three different substrates were used to measure GST enzyme activities in homogenates derived from gastric tissues of female patients: CDNB, pNBC and EPNP (Table 2).

The assay was performed according to Habig's method [16] which was optimized for ELISA Microplate Reader System (Thermo, Multiskan™ FC Microplate Photometer) [17].

The same volume of 10 mM phosphate buffer was added to the blank wells in place of samples. The reaction was initiated by the addition of the enzyme and absorbance values were measured at 20 s intervals for 10 min. In order to obtain consistent results, enzyme activity was measured at least three times for all samples, at different days.

The average rate values were presented by the ScanIt RE4.1 software; and, then, the specific activities were calculated by using Eq. (1):

$$\text{Specific Activity} = \frac{\Delta A / \Delta t}{\epsilon (\text{mM}^{-1} \text{cm}^{-1})} \times \text{DF} \times \frac{1}{\text{mg protein/mL}} \quad (1)$$

$\Delta A / \Delta t$ represents the absorbance change per minute, ϵ is the extinction coefficient of substrates (9.6, 1.9 and $0.5 \text{ mM}^{-1} \text{cm}^{-1}$ for CDNB, pNBC and EPNP, respectively). DF is the dilution factor.

Determination of total thiol

The total thiol content of each sample was determined by the method of Sedlak and Lindsay [18], which was later optimized for the ELISA Microplate Reader system [17]. 10 μL of cytosol was added into 30 μL of 200 mM Tris Buffer, pH 8.2 containing 20 mM of EDTA. Then, 20 μL of 5,5'-Dithiobis-(2-Nitrobenzoic Acid) (DTNB) (2 mM) and 140 μL of methanol were added into each well. For the building of standard curve, reduced GSH was used; a series of dilutions in the final concentration range of (0.5–5 mM) were prepared and added as it was for samples. After incubation for 30 min at 25°C in dark, absorbance values were detected at 405 nm. The total amounts of thiol in the samples were calculated by using the slope value of standard curve and expressed as nmol/mg of protein.

Statistical analysis

The experimental results were assessed for the presence of outliers and the ones out of expected range were discarded. One Way Analysis

of Variance and Tukey's test were performed with Sigmaplot 13.0 licensed software. The normality test (Shapiro–Wilk) and equal variance test (Brown–Forsythe) were applied in/between groups to check whether they passed the tests. The means of groups, standard deviation (SD) and standard error of means (SEM) of the groups were calculated and the standard error of the mean was used to generate error bars ($\pm$ SEM). After the *t*-test, in the group comparisons for the ones that passed the normality tests, single and double-ended *p*-values (one-tailed *p*-value, two-tailed *p*-value) were calculated to determine whether groups were significantly different from each other. $p < 0.05$ was considered statistically significant. The power values of single and double ended tests were, also, calculated as alpha value (Power of performed two-tailed test with alpha) and it was stated whether there was a difference between the groups. For power values greater than 0.800, the difference between the groups was considered statistically significant and acceptable. Mann–Whitney U statistics were used for experimental group comparisons that did not pass the normality test (Shapiro–Wilk); thus, the *p*-value was obtained.

Ethical issues

This research was approved by the ethical review boards (IRBs) under the governorship of the authors' institution of Ankara Kecioren Training and Research Hospital Medical Research Ethics Committee (No: 2012-KAEK-15/[1678] 1800) and authors certify that the study was performed in accordance with the ethical standards as laid down in the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards. All patients included were fully informed via a signed form. The survey forms were only used for research purposes and were kept confidential in compliance with the Turkish laws.

Results

Based on the information obtained from the questionnaires, the patients were grouped according to age, hypertension, diabetes and family history parameters; and, the experimental results were summarized in Table 3.

There was no significant difference in total thiol amount determined for each sample; so, the average values were almost the same in-between experimental groups. Moreover, only 3% difference was observed between the total GST specific activities measured in the stomach tissues of patients grouped according to age criteria. While no significant difference was observed for GSTM1 isozyme activity, it was determined that the average of total amount of thiol for the group above 35 years of age was 3% higher than the group of 35 years old and younger, in contrast to the trend observed in total GST activity. The most obvious difference between the age groups was determined for the average specific activities of GSTT1 isozyme. When the data obtained were evaluated, it was seen that the average of specific activities of GSTT1 isozyme was 20% less in the “over 35 years old” patient group than the “35 years old and younger” group.

Table 3: The results of specific enzyme activities and total thiol measurements arranged for age, metabolic disorders and family histories of obese patients. (SEM: Standard error of means) While CDNB was used as the general substrate to detect total GST activity, pNBC and EPNP were used specifically for GSTM1 and GSTT1 activities, respectively.

		GST specific activity (nmol/min/mg protein) ($\pm$ SEM)			Total thiol (nmol/mg protein) ($\pm$ SEM)
		CDNB	pNBC	EPNP	
Age	≤ 35	65.28 (± 4.60)	1.16 (± 0.10)	64.04 (± 6.88)	129.21 (± 8.70)
	> 35	67.48 (± 3.83)	1.14 (± 0.11)	50.99 (± 7.80)	133.06 (± 8.56)
Hypertension	+	71.08 (± 6.42)	1.20 (± 0.12)	63.92 (± 5.94)	123.68 (± 7.99)
	–	65.04 (± 3.27)	1.14 (± 0.09)	54.46 (± 6.81)	134.03 (± 0.89)
Diabetes	+	62.56 (± 5.51)	1.21 (± 0.11)	48.36 (± 5.27)	139.28 (± 9.01)
	–	67.99 (± 3.45)	1.13 (± 0.09)	60.67 (± 7.41)	128.00 (± 0.97)
Family history	+	64.28 (± 4.06)	1.12 (± 0.10)	51.00 (± 6.50)	133.35 (± 7.42)
	–	68.86 (± 4.23)	1.20 (± 0.10)	64.03 (± 8.83)	129.19 (± 7.74)

With respect to the information obtained from the questionnaires, 7 patients who were reported to have hypertension were grouped as “HT (+)” and 22 patients without hypertension were grouped as “HT (–)”. When the results were evaluated, averages of total GST and GSTM1 isozyme specific activities were 9 and 5% higher in HT (+) obese patients, respectively, compared to HT (–) obese patients. The apparent difference between patients grouped by hypertension status was, again, obtained for GSTT1 isozyme specific activities. In HT (+) obese patients, 17% higher mean of enzyme specific activities was determined compared to that in HT (–) obese patients.

In present study, 8 patients with diabetes were grouped as DM (+) and 21 patients without diabetes were grouped as DM (–). The mean of total GST specific activity was 8% less in DM (+) obese patients than in DM (–) group. On the other hand, the average of specific activities of GSTM1 isozyme were 7% higher in DM (+) obese patients compared to DM (–) ones. The average of GSTT1 isozyme specific activity, for which the most significant difference was measured, was 20% lower in DM (+) compared to DM (–) obese patients. It was determined that the total thiol amount was approximately 9% higher in DM (+) compared to DM (–) group.

Fifteen patients had reported obesity in their family history and they were grouped as FH (+) and remaining 14 patients without obesity in the family history were grouped

as FH (–). When they were calculated, averages of specific activities of total GSTs, GSTM1 and GSTT1 isozymes in FH (+) obese patients were 7, 11 and 20% less, respectively, compared to FH (–) obese patients. When the obtained data were evaluated, it was determined that the mean of total thiol amount was approximately 3% higher in FH (+) obese patients compared to FH (–).

Discussion

Obesity is reported as highly associated with systemic oxidative stress and it causes direct or indirect changes on many metabolic pathways including antioxidative defense system. The present study evaluated specific activity changes in GSTs and fluctuations in total thiol content in stomach tissue, with respect to different parameters of patients.

The differences in the mean values of each couple (age $\leq 35 / > 35$, hypertension +/–, diabetes +/– and family history +/–) were not great enough to reject the possibility that they were due to random sampling variability, for all parameters checked. This might be sourced by the sample sizes and inter-individual differences in each group compared. Nevertheless, the mean values were, still, used in evaluation of the experimental results while recognizing the need to use larger sample groups in future studies to provide higher statistical power.

The data provided by studies on total thiol concentration in human stomach is very rare. Czczot et al. [19] reported a total thiol value of about 2,600 nmol/mg of protein in peritumoral gastric tissue samples. Hoppenkamps et al. [20] measured it as an average value of 47.2 nmol/mg protein, in healthy stomach tissue samples. We determined the same parameter as about 131.22 nmol/mg protein in gastrectomy samples of female obese patients. We concluded that the total thiol content of stomach tissues of obese patients were, somehow, higher with respect to healthy individuals; however, well-below than that of cancerous/peritumoral samples. The higher than normal total thiol levels measured in the tissue samples of obese patients could be considered as a natural consequence of the high oxidative stress created by the current situation and resulting increased antioxidant metabolism [21, 22].

In the study conducted by Loguercio et al. [23] in the Italian population, it was reported that total GST enzyme activities measured in gastric mucosa samples of healthy individuals were decreased, depending on the age. This situation does not exactly match the results obtained in the current study; however, it should be noted that our samples were collected from obese patients from a different

population. On the other hand, in a study completed by Caira et al. [24], a significant increase was detected in the expression of all GST isozymes in obese individuals, in the proteomic study of liver biopsy samples taken from healthy and obese individuals; and, in another study being performed on human subjects, increased GST activity with aging was reported in Indian population [25]. This situation can be interpreted as, above all, the changes observed in healthy individuals for GST activities may not occur in the same way in obese individuals, and in different organs or tissues.

It was previously stated that GSTT1 expression was affected not only by aging but also some other factors; so, its specific activity and expression levels might be completely different from those of the other GSTs [26]. Although it was tissue specific, in Italian population, low expression of GSTT1 in elderly population was reported [27]. A similar situation was, also, reported by Xu et al. [28] as significantly decreased mRNA and protein expression of GSTM1 and GSTT1 in aged rats.

Antioxidants could be useful in the management of essential hypertension to prevent progressive deterioration and target organ damage. Although there are reports of lowered GST expression related with hypertension [29], there are, also, studies clearly demonstrate the increased activity of GST in hypertensive patients [30, 31]. Furthermore, despite it was not statistically significant, the mean of total thiol amount in HT (+) obese patients was approximately 8% less than that in HT (–) patients.

Glutathione S-transferase activity was markedly lower in the obese-diabetic mice when compared to the lean animals [32]. For studies done on human samples, similar results were reported [33, 34] in accordance with our results.

Besides environmental factors are important, genetics plays considerable role with the heritability estimated as 40–70% for BMI variation [35]. The body fat accumulation in monogenic and polygenic form of obesity provides the evidences for strong genetic background [36]. In our sampling group, the patients with family history had higher BMI values but, somehow, lower GST activities with respect to the others. This outcome of the present study is hard to compare with literature, because, the published scientific works are, mostly, focused on the changes in antioxidant status of blood and liver; but, not the stomach, in relation to family history of obesity.

Conclusion

The information about GST activity can help to predict a patient's ability to respond to certain drug treatments [37].

Therefore, like many other members of antioxidant system, the long and short term follow-up of GST isozymes might be important in the prognosis of patients who use a large number and variety of drugs due to obesity and related metabolic diseases. Although drug absorption in the stomach is usually a minor player in the total absorption of a drug dose, there are some important and widely-used drugs such as Rifampicin, Thiopental, Secobarbital, Antipyrine, Warfarin, Theophylline, Phenytoin, Misoprostol, Captopril, Cinnarizine, Chlordiazepoxide whose primary region of absorption is stomach; i.e. they are either completely or substantially absorbed at stomach. For years, many studies have been concentrated on the abnormal body mass as the reason for the inefficient dosage of such drugs at obese [38]. However, abnormal activity levels of phase I and phase II enzymes along the gastrointestinal tract should be taken into consideration. In order to adjust the doses of those and similar drugs passing through the stomach wall, it is useful to examine total GST activity, and especially, activities of GSTM1 and GSTT1 isozymes that have more important place in others and, also, have specific substrates that allow reliable measurement of their specific activities by spectrometric methods, at this tissue [39, 40].

The present study provided pioneer data of specific activities of some GST isozymes in company with total thiol amount in stomach tissues of female obese patients grouped according to age, hypertension, diabetes and family history.

A statistically weak, positive correlation can be mentioned for all three substrates. However, at this point, it can be suggested that the number of patients and interindividual differences were limiting factors. It has been found that hypertension increased all measured GST activities and diabetes decreased them, except GSTM1. In addition, it was found that all GST activities measured in patients with obesity in their family and with possible genetic background were lower compared to the other group. Furthermore, GSTT1 activity has been shown to decrease with increasing age. Moreover, it was found that the total thiol amount would, probably, close to the upper limit in all groups, which was above the values reported in the literature for healthy tissues.

Obesity may contribute to the members of oxidant-antioxidant balance of body in different ways; and, the present study might also put another dimension with the fact that it might also intervent those metabolism oddly in different organs and/or tissues. Besides, the fact that there is little information in the literature about the long-term metabolic effects of the use of antihyperlipidemic drugs containing some active substances which are also effective inhibitors of GST, like clofibrate; and, the lack of studies on the changes in protein profiles of gastric tissue and on the

measurements of enzyme activities make it much more difficult to further interpret the experimental results. Given these realities, the new studies with higher sampling groups should be done on activities of GST isozymes which could serve for diagnostic and prognostic purposes in the future.

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