

The Effects of a Single Blood Donation on the Lipid Profile, Iron Storage and Enzymatic Antioxidants

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Abstract

Cardiovascular disease (CVD) is a global illness causing 31% of global mortality. Though many factors contribute to CVD, oxidative stress advances atherosclerosis through several complementary components, such as the initiation of lipid peroxidation by iron. Blood donation may decrease the risk of CVD due to reducing the iron level. Literature reported that blood donors have a lower risk of CVD, possibly due to the lower iron levels. Various effects of blood donation are involved in preventing type II diabetes. However, little is known of the exact mechanism of the benefits of blood donation. In this study, samples were collected from 33 healthy male participants pre- (1 day) and post-donation (1 day, 1, 2 and 3 weeks) and the effect of the blood donation on the iron, lipids and enzymatic antioxidants profiles were assessed. A repeated-measures ANOVA was used for comparing the quantitative variables between the visits. We found that the iron decreased significantly by week 1 (-25.3%). Ferritin decreased significantly at weeks 1, 2, and 3 (-26.3%, -40.3%, -36.7%, respectively). The superoxide dismutase increased significantly at post-donation day 1, weeks 1, 2, and 3 (17.9%, 35.7%, 31.1%, 36.6%, respectively) and in correlation with time [$r(165) = 0.50, P < 0.01$]. Glutathione peroxide decreased significantly at week 1 (-25.0%). Glutathione reductase decreased significantly 1-day post donation (-5.7%) then increased over the next three weeks [$r(165) = 0.3, P < 0.01$]. Finally, the lipids were significantly reduced 24 hours after the donation but not at week 1, 2 and 3. We conclude that blood donation, resulting in a lowered body iron concentration, is an effective way to increase superoxide dismutase and glutathione reductase, which prevent the initiation of lipid oxidation. Our results could be used to advocate for the benefits of blood donation. However, further studies are required to assess the role of blood donation in plaque formation and arteriosclerosis.

Introduction

Cardiovascular disease (CVD) is the most prevalent cause of death globally, accounting for 17.9 million (31%) of global mortality. According to the World Health Organization (WHO), CVDs are heart and blood vessel disorders that include coronary heart disease, cerebrovascular disease, rheumatic heart disease, and other conditions ⁽¹⁾. Males and postmenopausal women are more prone to heart disease than premenopausal women, due to the higher levels of iron stored in these two groups, the failure of the cardiac muscle in patients with iron storage diseases, stored iron increases in males after puberty, and after menopause, women are at the same risk of CVD as men.

Iron has the ability to produce and promote free radical production through physiological or xenobiotic compounds and react with hydrogen peroxide (H_2O_2) to produce extra highly reactive and toxic species, catalyze autoxidation, and initiate lipid peroxidation ⁽²⁾. In 1934, Fritz Haber and Joseph Weiss recognized that the biological systems could produce hydroxy free radicals (OH^\bullet) through an interaction between superoxide $O_2^{\bullet -}$ and H_2O_2 , as shown in the following reaction ⁽³⁾.



Haber–Weiss reaction

H₂O₂, originally come from the respiratory cascade and cellular metabolism, can cross membranes more readily than the -O₂, which derive from mitochondrial NADPH oxidase because of its stability⁽⁴⁻⁶⁾. On a cellular level, the need for a transition metal to convert the H₂O₂ to hydroxyl OH[•] via the Fenton reaction can occur rapidly. The following reaction illustrates the Fenton reaction where the ferrous (Fe₂₊) reacts with H₂O₂ to produce hydroxyl radicals OH[•]⁽³⁾.



Fenton reaction

The generation of the anion superoxide (O₂^{•-}) by oxygen utilization is the first step, which is converted to H₂O₂. The production of hydroxyl radicals OH[•] can result in metal catalyzed oxidation of H₂O₂⁽⁵⁾. Literature considered the oxidative stress as an imbalance between free radical molecules or the generation of reactive oxygen species (ROS) and antioxidant protection mechanisms^(7, 8). ROS can result in cell malformation and the initiation of the atherosclerotic plaques in arteries, a serious CVD. The occurrence of these diseases is due to the oxidation of low-density lipoprotein, cholesterol, and the modification of proteins by ROS⁽⁹⁾.

Blood donation have various beneficial effects on the health status, including the reduction of CVD by maintaining a reduced iron level and subsequently minimizing the oxidation of lipids that are intermediates in the pathogenesis of atherosclerosis^(10, 11). It has been also proposed that this protective effect is a result of the reduction in hematocrit and subsequent blood viscosity that reduces the residency of atherogenic particles and increases the expression of atheroprotective components, such as nitric oxide⁽¹²⁾. Literature also reports beneficial effects of blood donation on the general health status, including CVD, reducing lipid peroxidation, and reducing iron storage. For example, Van Jaarsveld and Pool concluded that donating a unit of whole blood every two weeks three times could prevent atherosclerosis as the blood donation alters substances that contribute to atherogenesis⁽¹³⁾. Other research reported that blood donation in non-smoking men was associated with a reduced risk of cardiovascular events⁽¹¹⁾. In this study, the changes in the lipid profile, iron profile, and enzymatic antioxidants were investigated after donating blood after 24 hours, week-1, week-2 and week-3 in healthy individuals and comparing the results with the baseline 24 hours before the blood donation.

Materials And Methods

Study Design and Variables

This cohort study used repeated measurements with blood donation as the intervention. Participants were monitored before and after the donation for three weeks. The independent variable is blood donation and the dependent variables are iron, lipid and antioxidant profiles.

Study Setting

The study was conducted in the blood donation area, supervised by the Pathology and Laboratory Medicine Department of Prince Mohammad Bin Abdulaziz National Guard Hospital (PAMBAH). The laboratory and its staff are accredited by the College of American Pathologists (CAP), ISO 15189, CBAHI.

Study Participants, Inclusion and Exclusion Criteria

In total, 15 initial volunteers were withdrawn from the study. The reasons female participants were not included were due to possible menstrual cycle hormonal variations affecting metabolic processes ⁽¹⁴⁾, as well as lipid and lipoprotein ⁽¹⁵⁾. The most frequent medical causes for blood donation deferrals are a low hemoglobin and anaemia deficiency ⁽¹⁶⁾. Also, women typically weigh less than men and tend to experience vasovagal reactions and post-donation fatigue ^(17,18).

Table 1: Inclusion/Exclusion Criteria

<u>Inclusion criteria:</u>	<u>Exclusion criteria:</u>
<u>Males from 20 to 50 years</u>	<u>Cancer</u>
<u>Living in Al Madinah Almunwarah</u>	<u>Acute myocardial infarction</u>
<u>Meet blood donation criteria assessed by a physical examination and a questionnaire.</u>	<u>Symptoms of cardiovascular disease</u>
	<u>Taking lipid-related medication</u>
	<u>Malabsorption or intestinal obstruction</u>
	<u>Taking an iron supplement</u>
	<u>Recent blood donation</u>

Sample Size

The participants were categorized in four groups or 12 participants. Each participant was assigned a study number and was provided with scheduled visits' times for the duration of the study. All participants gave informed consent for participation and the study was approved by King Abdullah International Medical Research Center, Institutional Review Board, Riyadh, Saudi Arabia, study number RM19/005/M.

Sample Collection and Processing

Fasting blood samples were collected in two serum separator tubes and two heparin-anticoagulated tubes, labeled with the participant's number and the time of the visit. The first visit was 24 hours before blood donation. Blood donation took place the next day according to the standard blood bank protocol. Participants returned 24 hours, one week, two weeks and three weeks after the blood donation for subsequent sample collection (Table 2). The participants were instructed to maintain their usual lifestyle and food intake throughout the experiment. The samples were collected in the Outpatient Department laboratory, and transferred to the main laboratory.

Table 2: Sample Collection Plan

<u>Visit</u>	<u>Sample</u>
<u>24 h before donation</u>	<u>Sample A. Fasting sample.</u>
<u>Donation of 450 mL of whole blood</u>	
<u>24 h after donation</u>	<u>Sample C. Fasting sample</u>
<u>1 week</u>	<u>Sample D. Fasting sample</u>
<u>2 week</u>	<u>Sample E. Fasting sample</u>
<u>3 week</u>	<u>Sample F. Fasting sample</u>

Specimen Preparation of Analysis

Specimen Preparation for Lipids and Iron Profiles

The specimens were collected in two SST tubes, centrifuged for 5 minutes at 3500 RPM and the serum was transferred in aliquots, one was used immediately for testing the total cholesterol, HDL-C, LDL-C, triglyceride, Iron, ferritin, UIBC. Another aliquoted sample was stored at -28 °C for testing the APO-A, APO-B and glutathione reductase and assayed after all the samples were collected. All previous variables was determined spectrophotometrically by using a Abbott (Architect, c4000). Only the ferritin was determined using chemiluminescent microparticle immunoassay (CMIA) technology (Architect, i1000). Transferrin was estimated by calculation using the Percent saturation= $(\text{Iron} / \text{UIBC}) * 100$ (19) and TIBC was calculated using $\text{TIBC} = \text{UIBC} + \text{iron}$ (20).

Manual Preparation of Superoxide Dismutase

The superoxide dismutase was determined spectrophotometrically. According to the RANSOD kit (cat. No. SD 125) 0.5 mL of whole blood was centrifuged for 10 minutes at 3000 RPM and the plasma was removed. The erythrocytes were washed four times with 3 mL of 0.9% NaCl solution, each cycle with 10 minutes centrifuging at 3000 RPM. The washed packed erythrocytes were resuspended in 2.0 mL cold distilled water, mixed, and left to stand at 4 °C for 15 minutes. The lysate was diluted 25 folds with 10 mM phosphate buffer (pH 7.0).

Manual Prepration of Glutathione Peroxide

The glutathione peroxide was determined spectrophotometrically by using the RANSEL Kit (cat. no. 504), 0.05 mL of whole heparinized blood, diluted with 1 mL of a diluting agent (R3), incubated for 5 minutes at 25 °C, then 1 mL of hemoglobin reagent was added. The samples were assayed within 20 minutes of adding the hemoglobin reagent.

Statistical Package

The descriptive results of the continuous variables are expressed as mean \pm SD or SE. The normal distribution and homogeneity of the variances were evaluated using Shapiro-Wilk and Levene's tests. Repeated-measure ANOVA or the Friedman test was used to evaluate the repeated measures of the quantitative variables depending on the normality of the data. Student's *t*-test or Wilcoxon-signed rank test was used as *Post hoc* evaluation of pair-wise comparisons.

Results

All data are expressed in 95% CI. The mean age was 29.94 - 30.36 years, body mass index (BMI) 29.33 – 29.89 kg/m². According to the WHO categorization of BMI, 21.21% of the sample were normal, 30.30% overweight, 39.39% obese class I and 9.09% obese class II. The BMI and blood pressure (BP: mmHg) were measured at the first visit.

Lipid Profile

The lipid parameters, including the total cholesterol, HDL-C, LDL-C, APOA-1 and APO-B decreased in the post-24h visit after the blood donation (-7.78%, -8.70 %, -7.71%, -5.45%, -7.01%, P <0.001 respectively), but subsequently recovered and remained stable for the last three visits. There was no significant effect on the triglyceride level, at the P <0.05 level, for the four time points and pre-donation point.

Iron Profile

The iron decreased significantly at week-1 after the blood donation (-25.31%, $P < .001$). However, the post-24h, second week and third week samples were not significantly different compared with the pre-24h sample. The ferritin differed significantly in first, second and third weeks (-26.31%, -40.34%, and -36.73%), ($P < 0.001$) after the blood donation. The UIBC level decreased the day after the blood donation (-1.96%). The UIBC increased significantly in week-1, week-2 and week-3 (15.76%, 17.37%, and 18.60%), respectively ($P < 0.001$). Similarly, the blood donation slightly decreased the TIBC level at the post-24h visit (-.52%), but the TIBC increased at the week-1, week-2 and week-3 visits (2.18%, 5.53%, and 8.22%). The transferrin saturation decreased significantly on week-1, week-2 and week-3 (-7.24%, -24.63%, and -23.93%) respectively.

Enzymatic Antioxidants

The superoxide dismutase increased significantly ($P < 0.001$) during the post-24 h (17.96%), week-1 (35.71%), week-2 (31.18%) and week-3 (36.62%). We found a significant correlation between the SOD and the time of visits [$r(165) = 0.50$, $P < 0.01$]. The GPx significantly reduced in week-1 (-25.02%, $P < .001$). The glutathione reductase significantly decreased at the post-24 visit (-5.75%, $P < 0.001$) compared to the pre-24h visit. The glutathione reductase increased slightly above the pre-24h visit, throughout week-1 (1.07%), week-2 (1.75%) and week-3 (1.77%). The GR was positively correlated over time [$r(165) = 0.3$, $P < 0.01$].

Discussion

Lipid Profile

The blood donation significantly decreased the total cholesterol in the post-24h visit (-7.78% $P < 0.001$), which may be due to the amount of blood loss during the donation. The total cholesterol level recovered through the week-1 to week-3 visits, with no significant change compared with the pre-24h visit. Borai et al. also explained that blood donation might temporarily decrease the HDL-C after blood donation, but it would subsequently recover⁽¹⁴⁾. We found that the blood donation decrease the HDL-C significantly the following day (-7.71%, $P < 0.01$). Nevertheless, the HDL-C recovered non-significantly during week-1 (2.66%), week-2 (3.17%), and week-3 (2.63%) compared to the baseline visit. We suggest that blood donation may affect the HDL-C level favorably. HDL-C behaves as an antioxidant protecting against CVD (21, 22).

The effect of the blood donation on the serum LDL-C level vary. Borai et al described that the blood donation have a temporary effect that may lower the LDL-C level after 24 hours following blood donation, with a non-significant increase subsequently⁽¹⁴⁾. Riško et al in 2018 found that the LDL-C is higher in the donor group than the non-donor group⁽²³⁾, and Bani-Ahmad et al reported that repeated whole blood donation elevate the serum LDL-C levels insignificantly⁽²⁴⁾. However, van Jaarsveld and Pool found that donating blood on three occasions with six week intervals may decrease the LDL-C (13). Initially, our

findings indicate that the LDL-C was moderately elevated at the baseline visit. This elevation could be due to the majority (78.78%) of our sample being overweight, and the LDL-C was significantly decreased the next day (-8.70%, $P < 0.01$). This may be due to the amount of blood loss during the blood donation. The level steadily increased compared with the baseline during week-1, week-2, and week-3 (-0.36%, 2.46%, and 2.81%), respectively. Our results support current literature^(13,14). LDL-C in its own original state is not atherogenic, but once oxidized to oxLDL, it can develop atherosclerotic plaque⁽²⁵⁾. To fully understand the effect of blood donation on LDL-C, we recommend additional evaluation of oxLDL.

Triglyceride

Literature agrees regarding the effect of blood donation on triglycerides, specifically that there is no effect on the triglycerides. For example, Riško et al. reported no significant difference in the triglyceride between long-term donors versus non-donor men⁽²³⁾. However, Bani-Ahmad et al demonstrated that donating blood frequently during a year might be associated with an unfavourable effect on the serum triglyceride⁽²⁴⁾. Triglycerides are influenced by a number of hormones, such as insulin, growth hormone and adrenocorticotrophic hormones. In 2016, Borai et al. observed that blood donation stimulates the production of insulin 2-hr post blood donation and the triglycerides decreased insignificantly on week 1 and three months after the donation. Our study found no significant changes in the triglyceride level. The triglycerides decreased insignificantly during week 1, 2 and 3, compared with the pre-blood donation level. We suggest that the reduction of triglyceride is due to the effect of blood donation in lowering growth hormone and the stimulation of insulin.

Apolipoprotein-A

Van Jaarsveld and Pool, in 2002 reported that blood donation elevates the Apo A lipoprotein beneficially. However, our results show that the blood donation significantly decreased the Apo-A post-24h of the blood donation (-5.45%, $P < 0.01$), possibly due to the amount of blood loss during the blood donation. The Apo-A recovered again during the other visits to the baseline level, with no significant changes. ApoA-I and ApoA-II are major apolipoproteins found in HDL-C. An ApoA-I decrease would be expected due to decline of HDL-C post-24h of the blood donation⁽²⁶⁻²⁸⁾.

Apolipoprotein-B

Van Jaarsveld and Pool reported that blood donation would not affect the Apo-B significantly⁽¹³⁾. However, our results show that the blood donation may decrease the Apo-B significantly (-7.01%, $P < 0.01$) 24-h post blood donation. The Apo-B increased again with no significant change during the other visits, compared with the pre-24 h visit. The decline of the LDL-C may reflect the decrease of Apo-B post-24h as the Apo-B is present on the VLDL, IDL, and LDL (29). The amount of blood loss may be responsible for this effect.

Iron Parameters

Iron

The decrease in the iron after blood donation has been investigated. Borai et al. investigated the changes in the iron level, and reported that the iron was not different in the 24-h post blood donation visit. The iron decreased significantly 1 and 2 weeks after the blood donation compared with the baseline level and after three months recovered to the baseline level. We observed that the blood donation may not reduce the iron level immediately the next day (-0.80%). Our results showed that the serum iron decreased significantly in the week-1 visit (-25% $P < 0.01$). However, the iron was still low during week-2 and week-3 (-20.24% and -16.12%), respectively, with no significant difference compared with the baseline visit. Our results are in line with Borai et al. (2016). The Fenton's reaction previously explained that the iron is involved as a free radical. Free transition metals, including iron, would be eliminated by blood donation (30). Free radical production would be reduced and the risk of oxidative damage due to the release of Fe^{3+} from binding proteins might be decreased (31,32). We posit that reducing the serum iron by donating a unit of blood could reduce free radical production and may decrease the effects of oxidative damage.

Ferritin

We observed that the blood donation slightly decreased the ferritin level the day after the blood donation (-3.29 %), and the ferritin decreased in week-1, week-2 and week-3 (-26.31%, -40.34%, and -36.73%), respectively ($P < 0.001$) after the blood donation. A decrease in the ferritin level would be expected due to the low iron level, as ferritin is considered an iron store (30). This observation is in line with literature (14, 23). Ferritin is a protein involved in iron transportation, metabolism and play a role in iron compensation (33). A reduction in the ferritin stores would be expected with a low iron status.

Iron Binding Capacity

The increase in the iron binding capacity following blood donation has been investigated by Riško et al. In the current study, we indicated that the iron binding capacity, after a single blood donation, may become significantly increased post-blood donation. However, Riško et al. reported the comparison between long-term donors versus non-donor men. We observed that blood donation might slightly decrease the UIBC level the day after the blood donation (-1.96%). The UIBC increased in week-1, week-2 and week-3 (15.76%, 17.37%, and 18.60%), respectively ($P < 0.001$). We also observed that the blood donation might slightly decrease the TIBC level the day after the blood donation (-1.52%). The TIBC increased in week-1, week-2 and week-3 visits (2.18%, 5.53%, and 8.22%). It has been reported that the UIBC and TIBC increased in low iron states (Wolf, 2002, Murtagh et al., 2002, Gambino et al., 1997).

Transferrin Saturation

In 2002, Van Jaarsveld and Pool reported that the transferrin saturation decreased in the blood donor group, who donated blood three times with six weeks intervals. However, our results show that the transferrin saturation, after one donation, decreased significantly on week-1, week-2 and week-3 (-27.24%, -24.63%, and -23.93%) respectively. This observation may reflect the low iron status and the increased iron-binding capacity that enter into the circulation. Clinical evidence linked the transferrin saturation to CVD mortality⁽³⁴⁾. Normal transferrin saturation is 20% - 50%, but in iron-overload states such as hemochromatosis, the high iron level increased the transferrin saturation >50%. Regular venesection is frequently a treatment that reduces the saturation to <30% for patients with a high transferrin saturation⁽³³⁾. Blood donation may reduce the transferrin saturation and keep the level <30%, which may protect or decrease the iron overload. We suggest that the low iron and transferrin saturation due to donating a unit of blood would inhibit free radical production and their effects on lipids and other cardiovascular events.

Enzymatic Antioxidants

Superoxide Dismutase

Literature reports the effect of blood donation on the SOD enzyme activity. Yunce et al in 2016 reported an increase in SOD activity at 24h after the blood donation compared with the pre-donation sample (35). Mehrabani et al suggested that repeated donation of whole blood might stimulate the SOD enzyme and increase its activity. However, we found that the blood donation effectively stimulate and continually release SOD during the post-24 h (17.96%), week-1 (35.71%), week-2 (31.18%) and week-3 (36.62%) visits. We also found a significant correlation between SOD and the time of visits [$r(165) = 0.50$, $P < 0.01$]. Copper is an essential component of superoxide dismutase (CuZn-SOD) (36, 37). Iron plays a role in decreasing the Cu/Zn SOD activity⁽³⁸⁾. Increased iron reduces copper absorption and reduce the SOD production⁽³⁹⁾. We suggest that lowering the iron stores after the blood donation could increase SOD, reducing the toxicity of the ROS, which may result in the initiation of CVD. The current study indicated that the plasma SOD level is decreased compared to the European reference range and is similar to Yunce et al. (2016).

Glutathione Peroxide

The effect of the blood donation on the GPx was investigated by Mehrabani et al. We found that the blood donation significantly reduced the GPx in the week-1 visit (-25.02%, $P < .001$). The Mehrabani et al study classified the sample in five groups according to their donation time per year⁽⁴⁰⁾. The reduction in the GPx would be expected due to the low iron in week-1, as the GPx is dependent on iron for its synthesis⁽⁴¹⁾. Literature reported a decreased activity of the GPx enzyme in iron deficiency anemia patients^(42, 43). However, in our research, the GPx improved again during week-2 (-5.95%) and week-3 (-5.67%) with no significant changes to the pre-24h visit.

Glutathione Reductase

We found that the blood donation significantly decreased the glutathione reductase at the post-24 visit (-5.75%, $P < 0.001$), compared to the pre-24h visit. The glutathione reductase increased slightly above the pre-24h visit throughout week-1 (1.07%), week-2 (1.75%) and week-3 (1.77%), and that the GR positively correlate over the time [$r(165) = 0.3$, $P < 0.01$]. The glutathione reductase mainly acts on glutathione to keep it in a reduced status⁽⁴¹⁾. The GR essentially works to supply GP_x with a reductive substrate⁽⁵⁾. We posit that the elevated glutathione reductase levels after blood donation might be reflected by lowering GP_x levels.

Conclusion

In conclusion, our results indicate that the benefits of a single whole blood donation are the reduction in iron content, a risk factor for atherosclerosis. Superoxide dismutase are correlated and significantly increases over the three weeks post-donation. As a result, blood donation could beneficially reduce the oxidative stress by enhancing the production of antioxidants. However, additional studies are required to assess the direct role in lipid oxidation and eventually plaque formation and arteriosclerosis. Our results could be used to advocate for the benefits of blood donation.

Limitations And Recommendations Of The Study

The present study was conducted using a small sample size and confined to males only. The effects of blood donation may be different in females, due to the presence of female hormones and possible differences in the iron status. No generalizations could be made based on the results. The researcher suggests that future studies should include a larger sample size from the same nationality or different nationalities. Fasting glucose, free fatty acid and hormones such as growth hormone, cortisol and adrenocorticotrophic hormone ACTH that have a role in controlling and metabolism of triglycerides should be investigated. In addition, factors related to the role of lifestyle, such as smoking, sleeping and eating patterns should be assessed. Such a scope could add significantly to the body of knowledge in the field. We recommend establishing the reference range for the enzymatic antioxidants in our population. The current research could be used to advocate for the benefits of blood donation. However, further studies are needed to assess the role of blood donation role in plaque formation and arteriosclerosis.

Declarations

Competing interests: The authors declare no competing interests.

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Table 3

Table 3 is available in the Supplementary Files section.

Figures

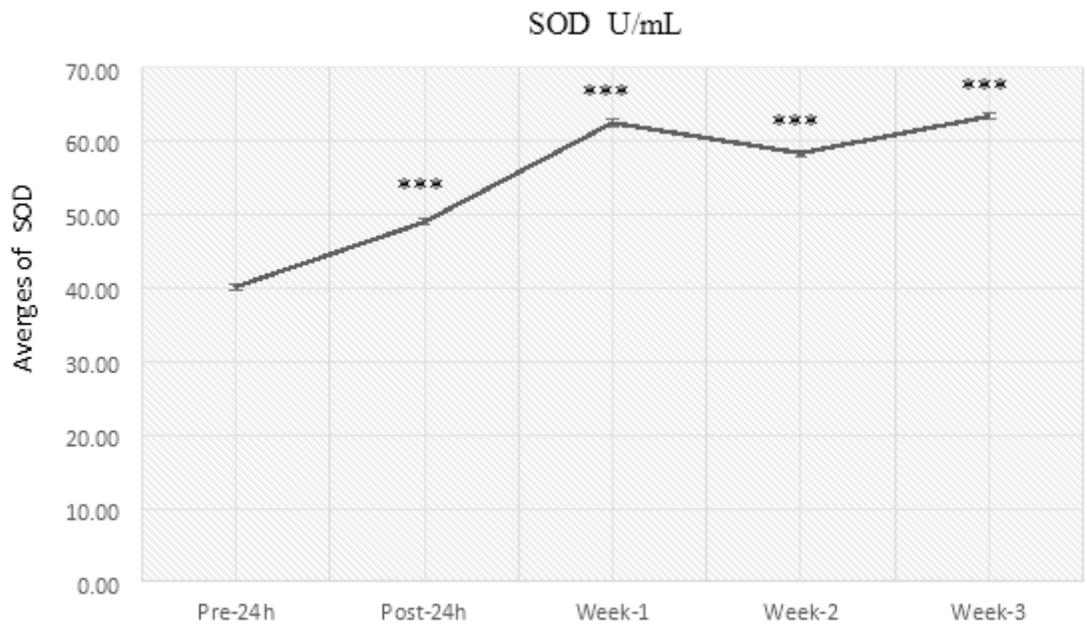


Figure 1

Changes in the mean (standard error of the mean) of superoxide dismutase compared with pre-24h visit

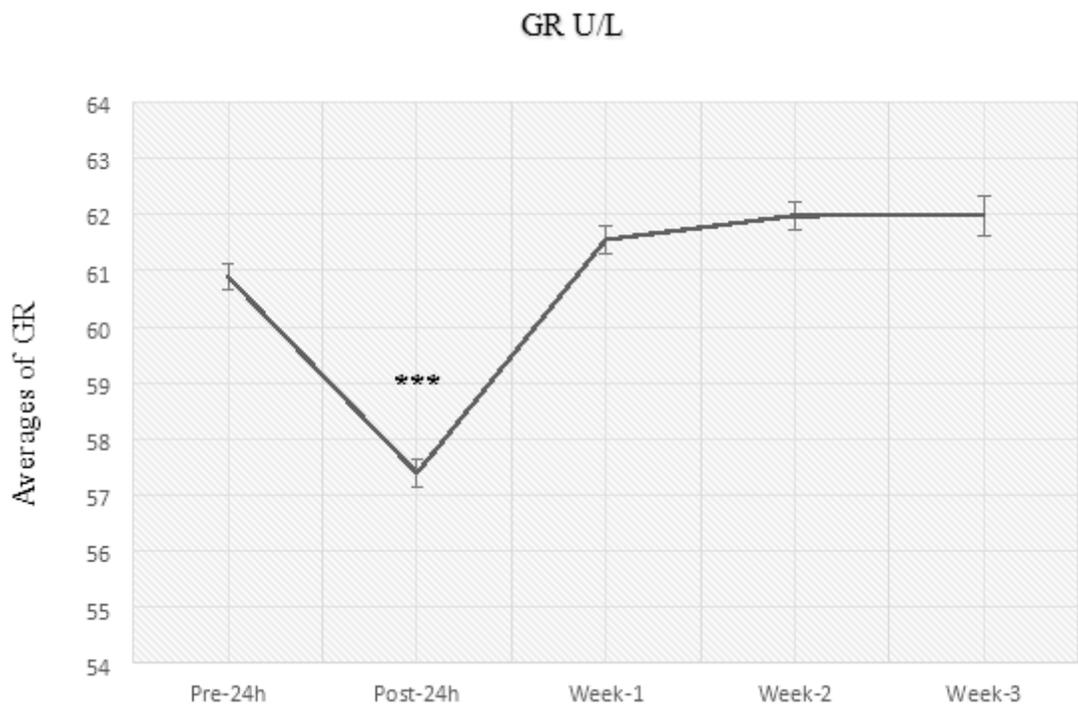


Figure 2

Changes in the mean (standard error of the mean) of glutathione reductase compared with the pre-24h visit

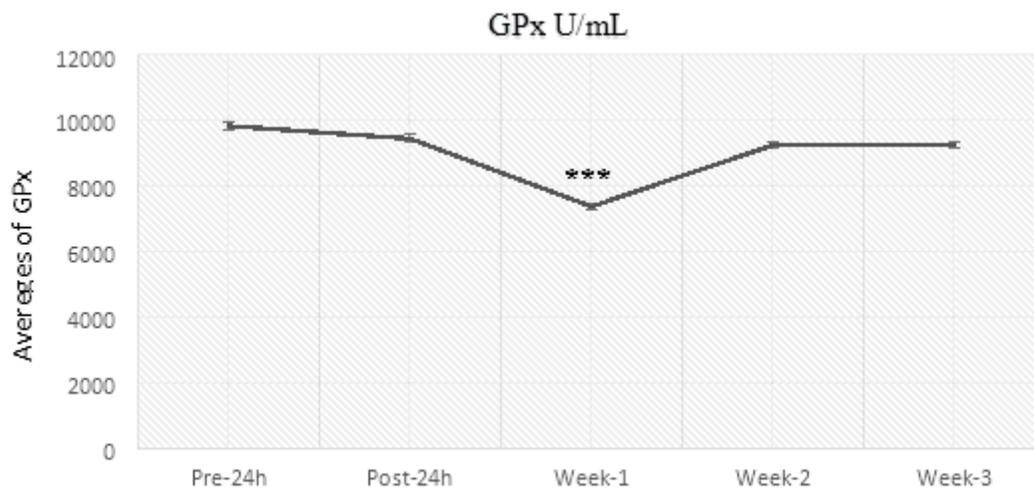


Figure 3

Changes in the mean (standard error of the mean) of glutathione peroxide compared with the pre-24h visit

Supplementary Files

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- [Table3.docx](#)