*https://doi.org/10.31925/farmacia.2024.6.9* **ORIGINAL ARTICLE**

# OXIDATIVE STRESS: MARKER OF ENDOTHELIAL DYSFUNCTION IN EXPERIMENTAL MODELS OF RATS WITH HYPERHOMOCYSTEINEMIA

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## Abstract

The present experimental study aimed to evaluate the influence of hyperhomocysteinemia on endothelial dysfunction via the assessment of antioxidant activity in an experimental model of rats with hyperhomocysteinemia. Fourteen 6-month-old male Wistar rats weighing 450 - 550 g were allocated into two groups. After 2 weeks of accommodation, both groups received High-Fat diet (HFD) for the entire study duration. The experimental group (G-II) was also administered methionine 1 mg/kg body weight orally daily during the first 20 days. Following model confirmation, G-II received atorvastatin 20 mg/kg body weight by daily gavage for the next 20 days. We evaluated, at the beginning and the end of the experimental study, the values for Homocysteine (Hcy) and parameters of oxidative stress (OS) - total antioxidant status (TAS), glutathione peroxidase (GPX) and superoxide dismutase (SOD). HFD induced significant lipid profile modification, while methionine induced hyperhomocysteinemia by altering the metabolism of Hcy. Atorvastatin treatment rescued both Hcy values and lipid profile in G-II. Our results show that atorvastatin treatment has lipid-lowering properties and antioxidative effects and also reduces the Hcy concentration in this experimental model of rats with hyperhomocysteinemia. Atorvastatin treatment proves promising in alleviating endothelial dysfunction and cardiovascular diseases.

## Rezumat

Scopul acestui studiu a fost estimarea efectului hiperhomocisteinemiei asupra disfuncției endoteliale, prin intermediul evaluării statusului oxidativ, într-un model de rozător cu hiperhomocisteinemie indusă artificial. 14 șobolani Wistar masculi, în vârstă de 6 luni, cântărind între 450 și 550 g, au fost distribuiți în două grupuri. După 2 săptămâni de acomodare, ambele grupuri au primit dieta bogată caloric (HFD) pe întreaga perioadă a desfășurării studiului. Grupul de studiu (G-II) a primit suplimentar metionină 1 mg/kg/zi, pe cale orală timp de 20 de zile. După confirmarea modelului, grupului G-II a fost administrată atorvastatină 20 mg/kg/zi, prin gavaj. Am evaluat, la începutul și la sfârșitul studiului, valorile Hcy și parametrii stresului oxidativ (OS) - statusul antioxidant total (TAS), glutation peroxidaza (GPX) și superoxid dismutază (SOD). Dieta bogată caloric a generat modificări îngrijorătoare asupra profilului lipidic, în timp ce metionina a indus hiperhomocisteinemie prin afectarea metabolismului Hcy. Tratamentul cu atorvastatină a determinat ameliorarea atât a valorilor Hcy, cât și a profilului lipidic. Tratamentul cu atorvastatină are proprietăți hipolipemiante, antioxidante și contribuie la scăderea valorilor serice ale Hcy în acest model de șobolani cu hiperhomocisteinemie. Prin urmare, atorvastatina se dovedește promițătoare în scopul ameliorării disfuncției endoteliale și a evenimentelor cardiovasculare.

**Keywords:** homocysteine, oxidative stress, endothelial dysfunction, obese rat model

## Introduction

Despite significant advances in preventive medicine, cardiovascular diseases (CVDs) remain the leading cause of death worldwide, according to the World Health Organization [1-3]. The Framingham Heart Study remains fundamental in identifying risk factors and predicting cardiovascular events. Risk factors for CVDs include both modifiable and non-modifiable elements, such as age, stress, obesity, dyslipidaemia, hypertension and smoking. Additionally, non-traditional risk factors like atherosclerosis, oxidative stress (OS), elevated homocysteine (Hcy) levels and inflammation are increasingly recognised for their role in CVD development [4].

Endothelial function is one of the most important elements in the physiopathology of CVDs [5]. The early disruption of mechanisms that regulate vascular homeostasis determines endothelial dysfunction (ED) and several adverse effects [6]. The vessel walls become more predisposed to vasoconstriction, lipid

infiltration, leukocyte adhesion, platelet activation and oxidative stress (OS) [7].

Endothelial dysfunction, characterised by impaired endothelium-dependent vasodilation, inflammation, hypercoagulability and leukocyte adhesion, marks the early stage of vascular diseases. These factors contribute to a pro-inflammatory state, initially at a local level, which is thought to be the first stage in forming atheromatous plaque. Additionally, ED plays a key role in atherosclerosis by promoting plaque development and rupture, particularly during the more advanced stages of the disease [8, 9]. Oxidative stress refers to an imbalance between producing reactive oxygen species (ROS) and the body's ability to neutralise or detoxify them using antioxidants. When ROS levels exceed the body's antioxidant defences, oxidative damage occurs, affecting cells, proteins, lipids and DNA. This process is linked to the development of various diseases, including CVDs, neurodegenerative disorders, cancer and aging-related conditions.

Homocysteine (Hcy), a non-proteinogenic α-amino acid, results from methionine demethylation in muscles, the liver and other tissues. The normal values of Hcy plasma levels are considered in the 5 to 15 µmol/L range. Data from literature classifying Hcy values as mildly elevated (15 - 30 µmol/L), moderately elevated (31 - 100 µmol/L) and severely elevated (more than 100 µmol/L) [10]. Therefore, an increase in plasma levels of Hcy was described in a variety of pathological diseases such as B-vitamin deficiency, smoking, chronic alcohol consumption, kidney diseases, hypothyroidism and oncological diseases. Hyperhomocysteinemia (HHcy) impairs the cardiovascular system at several levels. It disrupts endothelial function, promotes vasoconstriction and activates the coagulation cascade by generating ROS. These play a crucial role in the progression of oxidative stress, endothelial dysfunction, inflammation and cell proliferation. Altogether, these processes lay the foundation for the development and progression of CVDs [11, 12].

A recent meta-analysis focused on the role of homocysteine on atherosclerosis development and progression highlights the definite role of HHcy in determining CVD in young and overweight subjects [13]. The involvement of specific nutritional deficiencies - B12 vitamin and folic acid - is demonstrated by a masking phenomenon of the deleterious action of Homocysteine in young individuals with a balanced nutritional profile, as opposed to ageing frail patients. A lower B12 status has been proven to be linked to increased oxidative stress - a sum of elevated pro-oxidative species and decreased antioxidant defence [14]. However, the evidence level is not satisfactory, mainly due to the lack of randomised controlled trials focused specifically on B12 levels as an outcome of altered oxidative status in humans. Despite our study being focused on an experimental model of hyper-homocysteinemia, we believe that more proof regarding the relation between oxidative balance and B12 levels will be useful for future comparative analysis.

Aside from the canonical hypocholesterolaemia actions of statins, these molecules exhibit a wide array of pleiotropic actions, which better explain the beneficial effects of this drug class on endothelial function. Most pleiotropic effects occur through statin interaction with Rho-kinase/ROCK, Rac1 inhibition and activation of Peroxisome Proliferator-Activated Receptor-γ [15]. The strong protective effect of statins on cardio-vascular health is indisputable. It is also reflected through consistently lowering Hcy levels in clinical trials [16]. However, there is no current clear perspective on the relation between lipid profile, B12 and oxidative status in a hyperhomocysteinemic setting following treatment with a statin. Therefore, this study focused on filling this gap in the scientific knowledge.

## Materials and Methods

*Animals*

Fourteen male Wistar rats, aged 6 months (450 - 550 g), were procured from the "Cantacuzino" Institute, Bucharest, Romania and contributed to elaborate experimental studies as we described in a previous study [17]. The rats were housed in CEMEX at "Grigore T. Popa" University of Medicine and Pharmacy, Iași, Romania, in individually ventilated cages and a special condition (facility conditioned at 20 ± 4°C, 50 ± 5% humidity, on 12 h light/12 h dark cycle). They also had access *ad libitum* to water and food. After 14 days of acclimatisation, the animals were included in the experimental protocols.

*Ethical Approach*

The experimental investigation was conducted according to European Directive 2010/63/EU. Therefore, it is approved by the Ethics Committee of the "Grigore T. Popa" University of Medicine and Pharmacy, Iași, Romania, and we have authorisation from the Romanian National Sanitary Veterinary and Food Safety Authority.

*Experimental design and treatment groups*

After 14 days of acclimatisation, all specimens were weighed and distributed according to the following protocol. To evaluate the biochemical modification of the administration of atorvastatin on markers of oxidative stress and Hcy levels, the rats were grouped into two groups of equal count (n = 7). At this stage, the actual day 0 of the study, a pre-mixed water-based solution of L-methionine was prepared to be administrated to all animals in a precise quantity of 1 mg/kg of body weight *per* day in each specimen in group G-II for the next 20 days, to induce the experimental model of hyperhomocysteinemia.

On day 21, also denoted $T_0$ (35 days after the animals arrived in the laboratory), we measured the Hcy level to confirm the experimental model. After that, only the animals in the second group (G-II) received atorvastatin 20 mg/kg body weight *per* day for 20

days - that is, until day 42, also denoted Tend (56 days after the animals arrived in the laboratory). Animals had unrestricted access to water and food.
Atorvastatin (Sortis, Pfizer, no. NM4027) was mixed with distilled water. We administered treatment once a day orally, by gavages, using a sterile dispositive for every rat (16G × 1.1/2, Popper and Sons). The dosage of atorvastatin was chosen in conformity with the recent studies where the authors reported that 20 mg/kg body weight *per* day for at least 20 days is considered effective and safe [18, 19]. All animals were monitored every day regarding food and water consumption and weight (g) was evaluated every week on the same day.

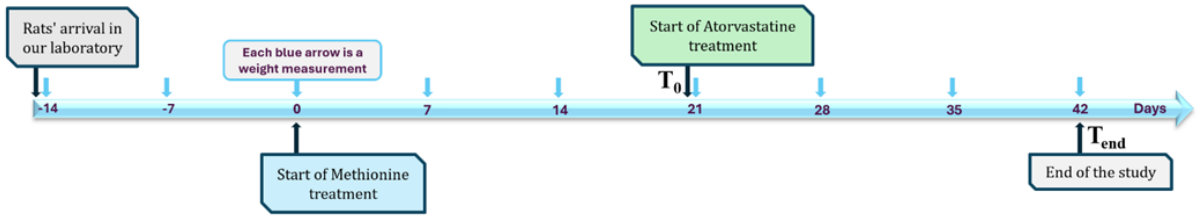


**Figure 1.**
Graphical depiction of our experiment

The high-lipid, high-cholesterol diet employed in our study was processed to order. We chose a higher lipid and cholesterol content since we aimed at inducing and maintaining obesity to serve as a foundation for the hyperhomocysteinemic rodent model [20, 21]. In agreement with previous research, we opted for the following caloric split: 40% fats (mainly from palm oil), 20% protein (mainly casein) and 20% fructose. The rest of the caloric content is ensured by different types of carbohydrates. The whole mix averages at an approximate value of 4 kcal *per* gramme of chow.
*Measurement of biochemical parameters*
All reagents for the experiment and for detecting the biochemistry parameters and plasma Hcy concentration were procured from BioSystems S.A. (Barcelona, Spain) and Merck. We utilised the automated analyser ACCENT-200 (PZ Cormay, Warsaw, Poland) and the manufacturer's indication to validate the biochemistry results.
To assess biochemical parameters at time $T_0$, serum was sampled by puncturing the retro-orbital plexus with a capillary tube. At time $T_{end}$, rats were sacrificed, and the serum was sampled by puncturing the epicardium. A series of biochemical tests were monitored to investigate the potential effects of atorvastatin. Blood samples were collected and centrifuged at 1500× for 15 minutes at 4°C, approximately 30 minutes after collection. The serum was then analysed for various biochemical parameters. Antioxidant activity, including superoxide dismutase (SOD), glutathione peroxidase (GPX) and total antioxidant status (TAS), was assessed using Randox kit procedures. Plasma homocysteine (Hcy) levels were determined using a validated high-performance liquid chromatography (HPLC) method [22]. The chromatographic system consisted of Agilent 1200 HPLC 6520, Binary Pump, Zorbax SB-C 18 (4.6 mm × 250 mm, 5 µm) and UV-VIS detector (DAD). Elution was performed in gradient mode. The sample volume was 20 µL, the flow rate was 1.2 mL/min, the wavelength was 355 nm, and the column temperature was maintained at 25°C.
*Statistical analysis*
Biochemical measurements were analysed using the Q-Q plot and, under certain circumstances, the Shapiro-Wilk test to assess the normality of the distribution of values since this study involved small sample sizes. For each parameter, we calculated the test statistic and the associated p-value. The null hypothesis in this scenario is the sample values follow a normal distribution. Accordingly, a p-value less than 0.05 indicates the data does not follow a normal distribution. Subsequently, for parameters that were normally distributed ($p > 0.05$), parametric tests (independent samples t-test) were applied. For parameters that were not normally distributed ($p \leq 0.05$), non-parametric tests (Mann-Whitney U) were used to highlight statistically significant differences between groups. Where correlations were not expected to go in a certain direction, we employed 2-tailed analysis; otherwise, the one-tailed tests were used. Values are expressed as mean value ± standard deviation unless otherwise specified.

## Results and Discussion

In the present experimental studies, after inducing the experimental model of HHcy, we evaluated the effects of atorvastatin on lipid profile, pro-oxidant indicators and levels of Hcy. In the studied group, analysing the body weight during the four weeks of the experiment, it can be observed that the weight is the same between experimental groups, which confirms the experimental models of obesity (Table I).
Regarding the average water intake across all individuals from a single group, Table II shows that the average values of water amounts are relatively different between groups (Group I – with a minimum value of 18 mL and a mean of 25.86 mL/24 h). Group II – with values oscillating between mean 31.86 mL/24h and minimum 20 mL/24 h). The two distributions in this comparison

appeared normal on the graphical plot, allowing for a t-test to be performed.

After inducing hyperhomocysteinemia following the protocol described before, blood samples were collected on day 21 ($T_0$-where the control group G-I is only obese with HFD, while G-II is obese, HHcy and starts atorvastatin treatment) and at the end of the experiment to compare the initial and final values (week 6 *versus* $T_0$). Firstly, we confirmed that statin treatment in obese rats can improve the lipid profile. Focusing on the control G-I group, we observed that at time $T_0$, these specimens had significantly higher concentrations of total cholesterol (Col) and low-density lipoprotein-cholesterol (LDLc), together with a lower level of high-density lipoprotein-cholesterol (HDLc). These are the consequences of the high-lipid, high-cholesterol diet. This result agreed with a previous report showing that a continuous high fat could induce dyslipidaemia in rats [23].

**Table I**

Body weight (g) assessment in the groups examined during the experiment. Weight distributions were parametrical in all instances

| Time/Weight | G-I (g) | G-II (g) |
|---|---|---|
| **Week 2** | 482.4 ± 27.5 | 542.0 ± 16.1 |
| **Week 3** | 506.4 ± 33.1 | 563.5 ± 12.1 * |
| **Week 4** | 531.1 ± 27.5 | 584.1 ± 8.3 * |
| **Week 5** | 562.8 ± 21.6 * | 587.8 ± 11.6 |
| **Week 6** | 570.4 ± 15.8 | 590.4 ± 6.6 |

* statistically significant differences ($p \leq 0.05$, CI = 95%) between two time points are assumed only if the t-value is higher than the value for the two-tailed test with a 95% confidence interval, 12 (2n - 2) degrees of freedom = 2.179. Here, we compared values registered in consecutive weeks inside each group, but no comparison was performed between groups at the same time. As such, the * symbol encountered in a certain week and group denotes a significant weight difference compared to the previous week

**Table II**

Water intake in the experiment

| Water (mL) / Group | G-I | G-II | Independent two-sample t-test |
|---|---|---|---|
| **Mean for experiment** | 25.86 ± 2.26 | 31.86 ± 7.22 * | 5.075 * |

* statistically significant differences ($p \leq 0.05$, CI = 95%) between similar groups between the baseline and final phase of the experimental study are assumed only if the t-value is higher than the value for the two-tailed test with a 95% confidence interval, 12 (2n - 2) degrees of freedom = 2.179

The experimental group G-II also displayed values for LDLc and Col high enough to be classified as dyslipidaemia. Atorvastatin treatment led to a statistically significant decrease in LDLc and Col and an increase in HDLc (Table III). The magnitude of these changes is higher than the differences seen in the control G-I. These results are in line with the literature and present guidelines. Aside from their traditionally reported effect of cholesterol-lowering, statins also provide additional benefits - included under the umbrella of pleiotropic effects - including cardio protection, plaque stabilisation, increased nitric oxide (NO) bioavailability, antifibrotic and anti-inflammatory properties [24-26]. Current guidelines stress the tremendous benefits brought by the initiation of statin therapy, which far outweigh any potential intolerance [27]. Practitioners should not follow these guidelines blindly since there is a potential danger in lowering LDLc to dangerously low levels, as well as there are some harmful pleiotropic effects: newly onset diabetes, liver damage and muscle wasting [27, 28].

**Table III**

The effect of atorvastatin on lipid profile in our experiment was determined initially and, finally, the experiment after treatment

| t | Group | LDLc (mg/dL) | HDLc (mg/dL) | Col (mg/dL) |
|---|---|---|---|---|
| **$T_0$** | **G-I-In** | 61.71 ± 16.10 | 62.86 ± 3.83 | 247.29 ± 29.75 |
| | **G-II-In** | 94.43 ± 4.198 | 60.14 ± 7.60 | 275.43 ± 35.27 |
| **$T_{end}$** | **G-I-Fin** | 49.71 ± 4.27 [a] | 57.43 ± 8.10 [a] | 237.14 ± 40.78 [a] |
| | **G-II-Fin** | 49.57 ± 11.19 [a] | 63.29 ± 4.64 [a] | 216.14 ± 35.40 [a] |

a = statistically significant differences ($p \leq 0.05$) in the same study group between the baseline and final phase of the experimental study

It is interesting to note the values of HDL-cholesterol in group G-II, which, despite the apparent similarity to the naked eye, barely hit statistical significance. It is important to note that statistical significance may mean nothing in the medical field unless absolute efficacy is available. In this case, the treatment with atorvastatin to improve HDLc values remains on the side of questionable proof. On the other hand, LDLc in G-II displayed a dramatic drop following atorvastatin treatment. There was also a significant decrease in value in G-I, which only underwent the High-Fat diet. Plotting the two effects yields the treatment arm as more significant.

Our experiment showed that Hcy levels decreased compared with values on day 21 (Table IV). There is a hypothesis that lipid-lowering agents influence the values of Hcy plasma levels; therefore, a link between dyslipidaemia and levels of Hcy has been proposed, but it is still disputed.

**Table IV**

The effect of atorvastatin on TAS, GPX and SOD was determined initially and finally after the experiment

| t | Group | TAS (mmol/L) | GPX (U/L) | SOD (U/mL) | Homocysteine (µmol/L) |
|---|---|---|---|---|---|
| $T_0$ | **G-I-In** | 2.73 ± 0.11 | 2392.64 ± 305.32 | 1.71 ± 0.16 | 26.57 ± 4.99 |
| | **G-II-In** | 4.52 ± 0.60 | 8415.60 ± 3495.83 | 2.29 ± 0.34 | 33.14 ± 2.85 |
| $T_{end}$ | **G-I-Fin** | 2.81 ± 0.25 | 1181.35 ± 158.39 [a] | 1.98 ± 0.55 | 24.14 ± 4.67 |
| | **G-II-Fin** | 3.39 ± 0.22 [a] | 8822.86 ± 437.86 [a] | 3.66 ± 1.24 [a] | 23.14 ± 4.67 [a] |

a = statistically significant differences ($p \leq 0.05$) in the same group between the baseline and final phase of the experimental study
-In and -Fin denote values registered at the beginning and the end of the study, respectively

Hcy can affect HDLc metabolism by inhibiting enzymes or molecules in HDLc particle formation [4, 29]. Several data from the literature demonstrate the importance of Hcy level and report that HHcy was associated with different cardiovascular diseases, and the mechanisms are widely varied. The clinical studies report a non-inflammatory component related to elevated Hcy, demonstrated by the onset and progression of carotid plaques without highly sensitive CRP [30]. The more traditional mechanisms, such as the production of reactive oxygen species, direct impairment of endothelial nitric oxide synthase and aberrant activation of Toll-like receptor 4 – TLR-4, the inflammatory arm of HHcy – should not be neglected [31].

There are direct mechanisms through which atorvastatin resists the harmful effects of HHcy – one would include these under the vague pleiotropic umbrella. Oxidative stress is inhibited through cessation of overexpression of Nox4 mRNA in endothelial cells, leading to normalisation of the activity of the nicotinamide diphosphate hydrogenase enzyme [32]. Aside from oxidative stress, endothelial stress is inhibited by excessive activation of AMP-dependent protein kinase in *in vivo* specimens [33]. Moreover, apoptosis is also inhibited by antagonization of p38-MAPK expression [32].

In a small clinical study on renal transplant recipients, the authors have shown that atorvastatin treatment determined a mathematically robust reduction in Hcy plasma concentration, the significance of which was maintained after multivariate adjustment. However, the relevance of this in daily practice is unclear due to the minimal absolute difference in means [34]. Another study focused on patients with dyslipidaemia and investigated the effect of therapeutical dosages of either atorvastatin or simvastatin on the extent of the increase in Hcy plasma levels. While atorvastatin was the more efficient statin on the whole range of Hcy values, there was no influence on the Hcy values after administering the treatment [35]. These results agree with other small studies on this subject, which evaluate the impact of statin treatment on Hcy levels [36, 37].

Regarding animal models, the beneficial effects of statins extend to other models of metabolic diseases. In the case of diabetic rats, we have recently proven that atorvastatin does reduce Hcy levels and suppresses oxidation [38]. The impact of statins in experimental models is confirmed by the rescue of serum Hcy and several genes responsible for cognitive functions in a wild type of mouse exposed to dietary hyperhomocysteinaemia [39]. At present, the effect and importance of statin treatment under hyperhomocysteinaemia settings are unclear. As such, we highlight the discrepancies in the literature and stress the need for more research targeting the culprit of this study, homocysteine.

The last point of this experiment is to evaluate the impact of treatment with a statin in the case of HHcy. Following methionine treatment, based on the levels of the Hcy, we classified the HHcy as moderate, based on the accepted definition. The evidence from the literature suggests that serum Hcy values have been associated with changes in oxidative stress, mainly by binding to the N-methyl-D-aspartate receptor [40]. This, in turn, leads to increased calcium influx, propensity to apoptosis and oxidative species generation. Oxidative stress has been associated with cardiac and vascular damage, causing hypertension and atherosclerosis, and SOD is considered one of the most important intracellular antioxidant defence mechanisms. A recent study concluded that the upregulated activity of biochemical pathways that increase the rate of ROS utilisation and intensify changes in lipid peroxidation and antioxidant protection may exacerbate oxidative stress in patients with obesity and metabolic syndrome [41]. The association between HHcy and oxidative stress is not limited to patients with pre-existing metabolic syndrome. The relation is valid and independent of other cardiovascular risk factors in the case of women voided from the protective effect of oestrogens [42]. SOD is an important endogenous antioxidant enzyme that is the first line of defence against reactive oxygen species. A clinical study observed that SOD is a marker of cardiovascular changes in hypertensive patients with hypertension and associated diabetes mellitus, as changes in serum values were correlated with alterations in vascular structure and function [43]. This conclusion could be explained by the fact that patients who associate HTA and DZ cannot efficiently remove superoxide anion from the circulation and, therefore, suffer an increase in ROS-induced vascular damage. Thus, there is an inversely proportional relationship: low SOD level is associated with significant vascular injury. There is an important involvement of homocysteine in determining the activity of SOD. DNA methylation, and consequently, extracellular expression

of SOD is significantly suppressed by high levels of Hcy [44].

Another research reported that low SOD activity values in rat liver were not compensated by changes in serum GPx values [45]. Experimental studies using non-specific markers of oxidative injury demonstrated a reduction in SOD and GPx activity inversely correlated with blood pressure in new-diagnosed and untreated hypertensive subjects compared to healthy subjects [46]. The mechanism employed by HHcy in reducing the activity of GPx is an altered methylation of DNA, very similar to impairment of SOD [47]. Because the glutathione and GPx constitute the primary antioxidant defence system, it is plausible to assume that a correct determination of plasma GPx would accurately reflect the antioxidant status. In our groups, no significant changes in GPx activity parameters were evident.

The results of our research confirmed existing data from previous studies on HHcy-induced changes in oxidative stress. As can be seen in Table IV, in the obese and HHcy groups, a decrease in TAS values and an increase in serum SOD values can be observed.

It is widely accepted that HHcy can disturb the anti-oxidant balance, generate reactive species and decrease the TAS. In our study, we observed that in the group treated with statin, an increase in oxidative stress parameters attracted an increased level of SOD. Also, we observed decreased levels of TAS.

However, an experimental study reported that 20 mg/day of atorvastatin for 8 weeks increased the SOD and GPx activities [48]. Moreover, the authors of another experimental data showed that simvastatin is more efficient than atorvastatin in the case of the model of HHcy rats and can ameliorate the marker of oxidative stress after 4 weeks of therapy [23]. In the case of obese rats, simvastatin ameliorated ROS by increasing the levels of antioxidant enzyme activities [7].

Table V identifies the correlations between serum homocysteine parameters and TAS values. We can observe inversely proportional, moderate correlations in group G-II, which were determined initially and finally in the experiment.

As expected, Table VI shows statistically significant inverse proportional correlations between serum Hcy and GPx values.

Considering the discrepant values registered in the study between Homocysteine and SOD, moderate inverse correlations can be identified in Table VII.

**Table V**

Correlation between Homocysteine levels and TAS **

| **Group** | Spearman`s rho correlation (ρ) **homocysteine and TAS** | | **n** |
|---|---|---|---|
| | ρ | *p* | |
| **[a] G-I-In** | 0.214 | 0.645 | 7 |
| **[b] G-II-In** | 0.709 | 0.074 | 7 |
| **[c] G-I-Fin** | 0.893 | 0.268 | 7 |
| **[d] G-II-Fin** | **-0.487** ** | **0.007** | 7 |

Correlation is significant at the 0.01 level (2-tailed). -In and -Fin denote values registered at the beginning and the end of the study, respectively

**Table VI**

Correlation between homocysteine levels and GPX *

| **Group** | Spearman`s rho correlation (ρ) **homocysteine and GPX** | | **n** |
|---|---|---|---|
| | ρ | *p* | 7 |
| **[a] G-I-In** | 0.360 | 0.427 | 7 |
| **[b] G-II-In** | **-0.775** * | **0.041** | 7 |
| **[c] G-I-Fin** | 0.505 | 0.248 | 7 |
| **[d] G-II-Fin** | **-0.847** * | **0.016** | 7 |

Correlation is significant at the 0.05 level (2-tailed)

**Table VII**

Correlation between Homocysteine levels and SOD *

| **Group** | Spearman`s rho correlation (ρ) **homocysteine and SOD** | | **n** |
|---|---|---|---|
| | ρ | *p* | |
| **[a] G-I-In** | 0.857 | 0.014 | 7 |
| **[b] G-II-In** | **-0.837** * | **0.019** | 7 |
| **[c] G-I-Fin** | 0.071 | 0.879 | 7 |
| **[d] G-II-Fin** | -0.559 | 0.192 | 7 |

Correlation is significant at the 0.05 level (2-tailed)

In our studies, we observed a significant correlation between Hcy levels and markers of oxidative stress.

Obesity, age, dyslipidaemia and HHcy are considered to be risk factors for early-stage endothelial dysfunction

and the progression of atherosclerosis. Early treatment with statin can improve the modifiable risk factors.

In conclusion, our experimental research described the experience of treatment with statin in the case of HHcy rats and confirmed the hypothesis that HHcy disturbs oxidative stress. Also, statin treatment improves the lipid profile in obese rats and can enhance oxidative stress, endothelial function and the prevention of cardiovascular events.

It is essential to discuss the limitations of this research. The study was conducted on small groups of obese animals and HHcy, which may affect the statistical data and conclusions. Secondly, we only assessed one statin dose and one statin therapy in this group. Based on the available literature, we opted for a single dosage because this dosage is an efficient and safe dose and has not determined toxicity in animal models. Moreover, the following period in this experiment was relatively short – after the initiation of statin – which could undermine the statistical findings presented. The absence of a third group to control for the influence of transitory administration of methionine may play a role in our limited ability to assign more beneficial roles to treatment with atorvastatin. Lastly, certain confounding metabolic factors could be related to hyperhomo-cysteinemia – *e.g.*, reduced glucose tolerance. The statistical power may be diminished if we have incorporated measurements for these types of "cryptogenic" variables.

## Conclusions

Our findings confirm the importance of monitoring homocysteine values as a risk factor for cardiovascular disease. The disparate findings regarding statin treatment's impact on Hcy metabolism require caution when noticing an abnormally high value. There is specific evidence that atorvastatin exhibits the highest efficacy in reducing cardiovascular risk under hyperhomocysteinaemia, but the inconclusive results warrant the need for more clinical research focused on homocysteine. Whether this molecule is more indicative of impending cardiovascular disorder rather than a direct cause remains a research subject.

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## Conflict of interest

The authors declare no conflict of interest.

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