

Assessment of Cardiovascular-Metabolic Risk in Wistar Rats Fed a Hypercaloric Diet Supplemented with Selenium: A Preclinical Animal Study

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Abstract

Background: Residual cardiovascular risk persists even with adequate serum LDL-c control through statins, possibly due to dysfunctions in serum triglycerides and HDL-c. Elevated TG and reduced HDL-c are associated with metabolic syndrome and cardiovascular diseases. The TG/HDL-c ratio may predict these risks. Selenium, an antioxidant cofactor, may modulate these alterations, especially in diets that dysregulate serum lipid metabolism.

Objectives: To evaluate the effects of selenium supplementation on the cardiometabolic risk (CMR) of Wistar rats exposed to a high-fat hyperglycemic diet (HHD).

Methods: For 11 weeks, 55 female Wistar rats were divided into the following five groups: standard diet (SD), SD + ^{Continuous}Se, HHD, HHD + ^{Late}Se, and HHD + ^{Early}Se. Selenium was supplemented using sodium selenite (1 mg/kg). We performed one-way ANOVA, Kruskal–Wallis, and post hoc tests, adopting a significance level of $p < 0.05$.

Results: Total cholesterol increased in HHD + ^{Late}Se compared to SD ($p = 0.032$) and to HHD ($p = 0.013$). HDL-c increased in HHD + ^{Late}Se compared to SD ($p = 0.004$) and to HHD ($p = 0.008$). LDL-c increased in HHD + ^{Late}Se compared to HHD ($p = 0.008$). TG decreased in HHD + ^{Late}Se ($p = 0.006$) and in HHD + ^{Early}Se ($p = 0.016$) compared to SD. TG/HDL-c ratio decreased in HHD + ^{Late}Se compared to SD ($p < 0.001$) and SD + ^{Continuous}Se ($p = 0.013$); and in HHD + ^{Early}Se compared to SD ($p = 0.005$).

Conclusion: Selenium supplementation showed potential to increase HDL-c and reduce TG, key diagnostic components of metabolic syndrome. It also demonstrated potential to lower the TG/HDL-c ratio, a possible predictor of CMR, in rats fed HHD. Further studies are needed in subjects with high CMR and statin use to better define the clinical response to this therapy.

Keywords: Triglycerides; Metabolic Syndrome; Atherosclerosis; Sodium Selenite; Heart Disease Risk Factors.

Introduction

The serum lipid profile has been widely used for risk stratification of atherosclerotic cardiovascular diseases, such as myocardial infarction, stroke, renovascular disease, and peripheral arterial disease. Several epidemiological studies have established robust associations between elevated serum levels of total cholesterol (TC), low-density lipoprotein cholesterol (LDL-c), and triglycerides (TG), as well as low serum levels of high-density lipoprotein cholesterol (HDL-c) with the incidence of these vascular diseases.¹⁻⁹

Specifically, there is a strong link between elevated serum LDL-c levels and adverse cardiovascular outcomes. In this context, lipid-lowering therapies, such as statins, are widely used to reduce serum LDL-c, with evidence showing that a more aggressive reduction, generally below 70 mg/dL, significantly improves cardiovascular outcomes. However, even after achieving ideal LDL-c serum levels, many patients continue to present a residual risk of cardiovascular events. This phenomenon, known as residual metabolic risk, may be driven by atherogenic mechanisms not fully addressed by simple serum LDL-c reduction, possibly due to residual dysregulation of serum TG and HDL-c.^{1,2,4,7,8-11}

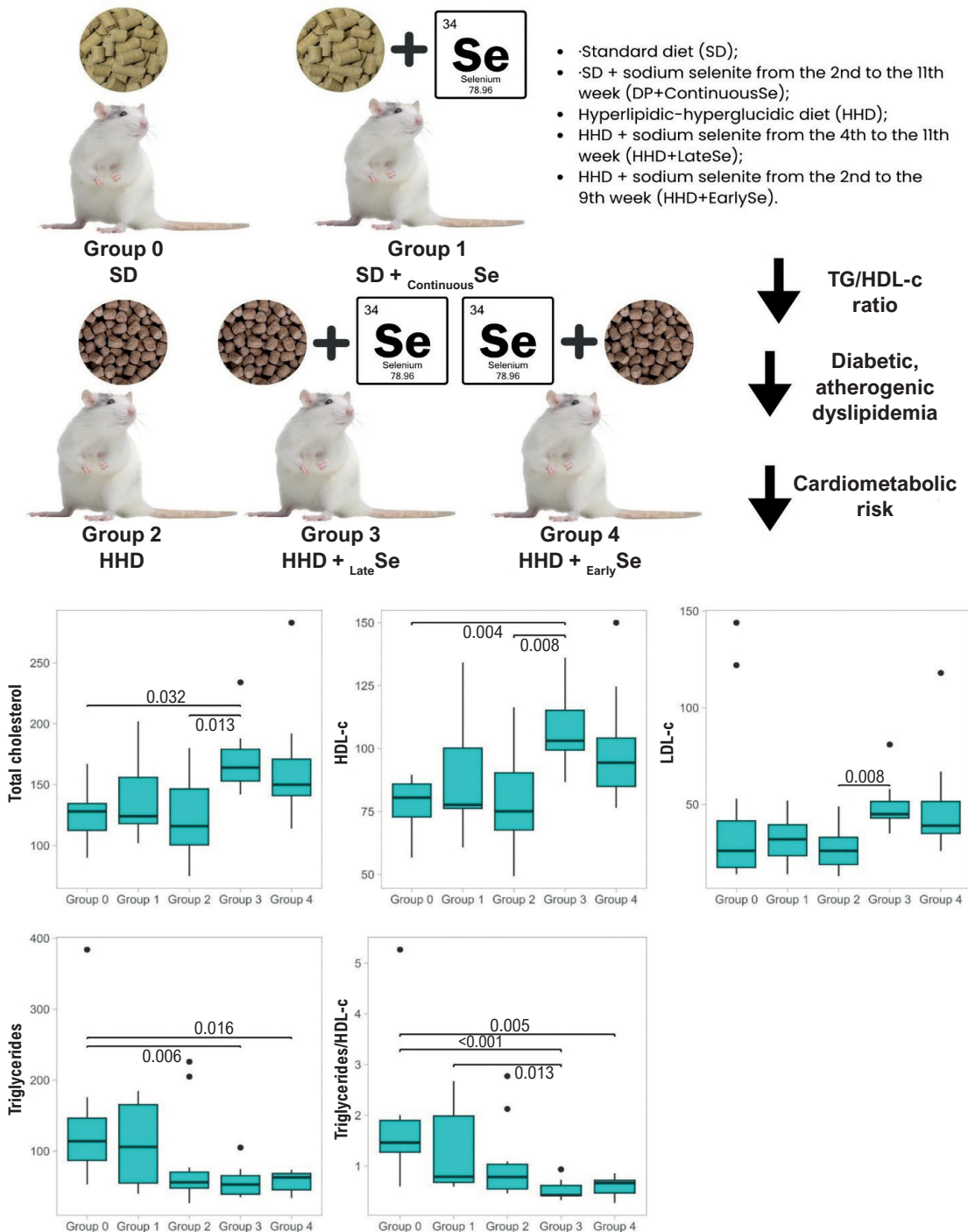
Thus, the TG/HDL-c ratio has emerged as a potentially significant marker of insulin resistance and central obesity, both key components of metabolic syndrome that can increase cardiovascular risk or maintain residual metabolic risk.^{1-3,5-7,12-16} This is also because the TG/HDL-c ratio directly involves two elements of the diagnostic criteria for metabolic syndrome, namely hypertriglyceridemia (TG ≥ 150 mg/dL) and reduced HDL-c (< 40 mg/dL in

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Central Illustration: Assessment of Cardiovascular-Metabolic Risk in Wistar Rats Fed a Hypercaloric Diet Supplemented with Selenium: A Preclinical Animal Study

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Summary of the results with intergroup boxplot representation of serum total cholesterol, HDL-c, LDL-c, and triglyceride levels (mg/dL) across the different experimental groups. Continuous Se: sodium selenite from week 2 to week 11; EarlySe: sodium selenite from week 2 to week 9; LateSe: sodium selenite from week 4 to week 11; HDL-c: high-density lipoprotein cholesterol; HHD: high-fat hyperglycemic diet; LDL-c: low-density lipoprotein cholesterol; SD: standard diet; TG: triglycerides. Source: the authors.

men and < 50 mg/dL in women), both associated with higher cardiovascular risk.^{1,17-20}

Moreover, recent studies highlight the importance of qualitative evaluation of lipoprotein subfractions, as the atherogenic effect of different subfractions can vary considerably. Small, dense LDL-c subfractions are more atherogenic than larger, less dense (floating) subfractions, while larger, less dense HDL-c subfractions are more protective against the development of atherosclerosis. The TG/HDL-c ratio may also reflect the quality of these lipid subfractions. A high ratio is often associated with a predominance of small, dense LDL-c particles and a reduction in large, less dense HDL-c particles, combinations linked to a higher risk of atherogenesis. Conversely, a low TG/HDL-c ratio suggests a predominance of less atherogenic lipoprotein subfractions and a lower cardiometabolic risk (CMR).^{7,10,11,21}

Taking all this into account, it is known that atherosclerosis occurs when LDL-c and TG accumulate in the arterial intima in oxidized form, triggering increased production of reactive oxygen species and promoting vascular inflammation. The enzyme glutathione peroxidase 4 (GPx4), whose function is to reduce reactive oxygen species, plays a crucial role in protecting against oxidative stress and atherosclerotic inflammation. Selenium, an essential mineral, acts as a cofactor for GPx4 and other selenoproteins, conferring significant antioxidant properties.²²⁻²⁵

Studies in animal models have demonstrated that exposure to a high-fat hyperglycemic diet (HHD) is an effective method for inducing dyslipidemia and replicating conditions of metabolic syndrome, obesity, and cardiovascular diseases. These diets, by promoting chronic inflammation and oxidative stress, are useful for studying the pathophysiological and therapeutic mechanisms of atherogenesis.²⁶⁻²⁸

In this context, antioxidant substances such as selenium may act as potential agents in preventing and mitigating the atherosclerotic process and comprehensive or residual CMR.

Therefore, the present study aimed to investigate the effects of selenium supplementation on the lipid profile and CMR in Wistar rats exposed to HHD.

Materials and methods

This work originated from an experimental study on selenium supplementation in the diet of female Wistar rats that evaluated its influence on several variables across five equally sized groups for 11 weeks. The study was approved by the Ethics Committee on Animal Use of the State University of Ponta Grossa (CEUA-UEPG) (Protocol No. 22.000027863-4).

We used female Wistar rats aged 8 weeks at the beginning of the experiment, corresponding to the young adult phase, which is widely adopted in metabolic studies due to hormonal and physiological stability. The exclusive use of females aimed to minimize behavioral and metabolic variability related to sex differences, ensuring greater homogeneity of the sample. This choice followed previous experimental models employing HHDs and selenium supplementation, which demonstrated higher reproducibility and metabolic control in females.^{26,29}

The GPower 3.1.9.422 program was used to calculate the sample size, considering a 50.0% minimum expected difference between groups according to the findings of Shidfar et al.,²⁹ an expected 5% (α) error, 80% test power ($1 - \beta$), and 5 groups. As a result, a sample size of 55 animals was obtained, 11 in each group by simple randomization. The animals were distributed randomly into the following groups (G):

- G0: standard diet (SD);
- G1: SD + sodium selenite from week 2 to week 11 (SD + _{Continuous}Se);
- G2: HHD;
- G3: HHD + sodium selenite from week 4 to week 11 (HHD + _{Late}Se);
- G4: HHD + sodium selenite from week 2 to week 9 (HHD + _{Early}Se).

The study design aimed to evaluate whether selenium exerts a prophylactic effect, when administered early (HHD + _{Early}Se), or a therapeutic effect, when administered later (HHD + _{Late}Se), on the cardiovascular and metabolic outcomes of female rats fed a HHD. The group receiving continuous supplementation (SD + _{Continuous}Se) was included to assess the isolated effects of selenium under standard dietary conditions.

The (HHD + _{Early}Se) group received selenium from the second week of diet exposure, simulating a prophylactic intervention designed to determine whether selenium could prevent or mitigate early lipid and oxidative changes induced by hypercaloric feeding. In contrast, the (HHD + _{Late}Se) group began supplementation at the fourth week, a time point at which previous studies²⁹ have shown the initial establishment of dyslipidemia and hepatic lipid accumulation in high-fat, high-carbohydrate diet models. This group, therefore, represented a therapeutic intervention, aimed at evaluating selenium's ability to reverse pre-existing metabolic disturbances.

The two-week interval between early and late supplementation was defined according to the experimental model proposed by Shidfar et al. (2018),²⁹ who compared zinc and selenium supplementation before and after disease progression in a non-alcoholic fatty liver disease model induced by a high-fat diet. Their findings showed that post-progression supplementation led to greater biochemical and histological improvements, indicating that the timing of micronutrient intervention has a decisive influence on metabolic outcomes.

Before the supplementation phase, animals underwent a one-week adaptation period to the housing conditions and diets. Throughout the experiment, the rats were maintained at the Central Animal Facility of the State University of Ponta Grossa (UEPG) under controlled temperature (22 ± 2 °C), lighting (12-hour light/dark cycle), and free access to food and water, in a noise-free environment illuminated with fluorescent lamps.

The inclusion criteria required animals to be alive and free from any diagnosed diseases. The exclusion criteria applied to any condition that did not meet these inclusion criteria.

The HHD was prepared using standard commercial feed (355 g), 176 g of roasted peanuts, 123 g of micellar casein, 82 g

of corn oil, 88 g of chocolate powder, 176 g of cornstarch biscuits, and water, resulting in an energy value of 4.6 kcal/g, as proposed by Nascimento et al.²⁶ The ingredients were weighed using a precision scale. The peanuts were roasted in an oven at 180 °C for 25 minutes and then ground in a blender along with the feed and the biscuits. Casein was added to the ground ingredients, followed by oil and water. The mixture was then manually molded into units (pallets) with an average weight of 50 g, baked in an oven at 180 °C for 35 minutes and then placed in a refrigerator.²⁶

Sodium selenite (Na_2SeO_3) was diluted in reverse-osmosis water to a final concentration of 1 mg/mL and administered by oral gavage at a dose of 1 mg/kg body weight daily.

The choice of this dose was primarily supported by Ahmadvand et al. (2014),³⁰ who showed that 1 mg/kg of sodium selenite administered intraperitoneally to Sprague–Dawley rats significantly increased glutathione peroxidase and catalase activities in serum, liver, and kidney without toxicity, confirming its antioxidant efficacy and safety.

Although Ahmadvand et al.³⁰ used an intraperitoneal route, the present study opted for oral gavage, as it represents a more physiological and translationally relevant route for evaluating micronutrient absorption and metabolism.

This approach is further supported by Shidfar et al. (2018),²⁹ who administered 2.5 mg/kg of sodium selenite by oral gavage in a high-fat-diet-induced non-alcoholic fatty liver disease model and found significant reductions in serum TG, TC, LDL-C, and hepatic fat accumulation, confirming the metabolic efficacy and safety of oral selenium within this range.

Therefore, the 1 mg/kg oral dose was selected as a safe, biologically active, and literature-supported concentration, suitable for controlled evaluation of selenium's metabolic and cardiovascular effects in a hypercaloric-diet model.³⁰

To ensure that all rats received the same volume daily, those that were not supplemented received only reverse osmosis-treated water. The supplement was administered through a gavage needle daily.

At the end of the 11 weeks of the experiment, the animals were euthanized and subjected to ventricular cardiac puncture for blood collection and subsequently sent to the clinical analysis laboratory for evaluation of the serum lipid profile. The following animal variables were obtained: serum levels (mg/dL) of TG, HDL-c, LDL-c, TC, and the TG/HDL-c ratio for the assessment of CMR.

All experimental procedures were conducted in accordance with the ARRIVE 2.0 Guidelines for reporting animal research, ensuring methodological transparency, reproducibility, and animal welfare.

Statistical analysis

For the statistical analysis, a descriptive analysis of the data was initially performed, considering continuous and categorical variables in relation to the experimental groups. The normality of continuous variables was assessed using the Shapiro–Wilk test. Continuous variables with a normal distribution were presented as mean $\pm$ standard deviation, whereas non-normally distributed variables were expressed

as median and interquartile range. Categorical variables were described as absolute frequencies and percentages, accompanied by their respective 95% confidence intervals.

For group comparisons, a one-way analysis of variance (ANOVA) was employed, followed by the Tukey post hoc test for multiple comparisons in normally distributed data. When the distribution was not normal, the Kruskal–Wallis test was used, followed by the Dunn post hoc test with Bonferroni correction.

All tests were two-tailed, and a significance level of 5% ($p < 0.05$) was adopted. Analyses were performed using the R[®] software (version 4.1.1, R Foundation for Statistical Computing, Vienna, Austria).

Results

The main findings are displayed in the Central Illustration. In the descriptive analysis of the serum variables TC, HDL-c, LDL-c, TG, and the TG/HDL-c ratio, variables with a normal distribution were expressed as mean and standard deviation, whereas non-normally distributed variables were presented as median and interquartile range, as detailed in Table 1. Intergroup comparisons are presented as boxplots in the Central Illustration.

Initially, when comparing the serum levels of biochemical variables between the SD and the HHD groups, no statistically significant differences were observed in serum TC, HDL-c, LDL-c, or TG levels. Despite the lack of statistical significance, the HHD group showed a trend toward reduced TG and a slight increase in HDL-c.

In addition, when comparing the SD and SD + ^{Continuous}Se groups, no statistically significant differences were found in serum TC, HDL-c, LDL-c, or TG levels. Although not statistically significant, the SD + ^{Continuous}Se group exhibited higher TC and HDL-c levels, along with a reduction in TG and LDL-c.

The following results describe the effect of selenium supplementation on serum lipid levels and on the TG/HDL-c ratio, comparing the experimental groups exposed to combinations of the hypercaloric diet and selenium supplementation:

A significant difference in TC concentration was observed among the groups ($p = 0.014$) (Table 1). Multiple comparisons revealed a significant increase in TC levels from the SD group to the HHD + ^{Late}Se group ($p = 0.032$) and from the HHD group to the HHD + ^{Late}Se group ($p = 0.013$). A non-significant increase was also noted from the HHD group to the HHD + ^{Early}Se group (Table 1 and Central Illustration).

For HDL-c, a statistically significant difference was found among the groups ($p = 0.002$) (Table 1). Intergroup analysis showed a significant increase in HDL-c levels from the SD group to the HHD + ^{Late}Se group ($p = 0.004$) and from the HHD group to the HHD + ^{Late}Se group ($p = 0.008$). Additionally, a non-significant increase was observed from the SD group to the HHD + ^{Early}Se group (Table 1 and Central Illustration).

For LDL-c, a statistically significant difference was observed among the groups ($p = 0.003$) (Table 1). A significant increase in LDL-c levels was detected from the HHD group to the HHD + ^{Late}Se group ($p = 0.008$) (Central Illustration).

Table 1 – Descriptive statistics of the continuous variables in relation to group.

Variable	G0 Mean ± standard deviation	G1 Mean ± standard deviation	G2 Mean ± standard deviation	G3 Mean ± standard deviation	G4 Mean ± standard deviation	p value
Total cholesterol (mg/dL)	126.9±23 ^a	140.6±33.3 ^{ab}	122.1±33.2 ^a	169.8±26.1 ^b	161.6±46.8 ^{ab}	0.014*
HDL-c (mg/dL)	77.7±10.3 ^a	89.4±22.6 ^{ab}	79.4±20.4 ^a	107±13.7 ^b	99.1±22 ^{ab}	0.002*
Variable	G0 Median (IQR)	G1 Median (IQR)	G2 Median (IQR)	G3 Median (IQR)	G4 Median (IQR)	p value
LDL-c (mg/dL)	26 (24) ^{ab}	32 (20) ^{ab}	26 (17) ^a	45 (12) ^b	39 (20) ^{ab}	0.003**
Triglycerides (mg/dL)	114 (78) ^a	106 (118) ^{ab}	56 (30) ^{ab}	53 (32) ^b	63 (30) ^b	0.002**
TG/HDL Ratio	1.46 (0.63) ^a	0.79 (1.31) ^{ab}	0.79 (0.48) ^{abc}	0.43 (0.2) ^c	0.67 (0.25) ^{bc}	<0.001**

*ANOVA (one-way) for comparison between groups; **Kruskal–Wallis (non-parametric test) for comparison between groups; IQR: interquartile range. The same letters between groups in the same variable represent nonsignificant differences, whereas different letters represent significant differences in the pairwise comparison. G0: standard diet; G1: standard diet + sodium selenite from week 2 to week 11; G2: high-fat hyperglycemic diet; G3: high-fat hyperglycemic diet + sodium selenite from week 4 to week 11; G4: high-fat hyperglycemic diet + sodium selenite from week 2 to week 9. Source: the authors.

Regarding TC, a significant difference was also observed among the groups ($p = 0.002$) (Table 1). Multiple comparisons showed a significant reduction in TC levels from the SD group to the HHD + ^{Late}Se group ($p = 0.006$) and from the SD group to the HHD + ^{Early}Se group ($p = 0.016$). Moreover, a non-significant decrease was noted from the SD group to the HHD group (Table 1 and Central Illustration).

Finally, when analyzing cardiometabolic risk through the TG/HDL-c ratio, a significant difference was observed among the groups ($p < 0.001$). Post hoc analysis revealed a significant decrease in the TG/HDL-c ratio in the HHD + ^{Late}Se group compared with the SD group ($p < 0.001$), as well as in the HHD + ^{Late}Se group compared with the SD + ^{Continuous}Se group ($p = 0.013$). Similarly, the HHD + ^{Early}Se group also showed a significant reduction compared with the SD group ($p = 0.005$) (Table 1 and Central Illustration).

Discussion

In the present study, no statistically significant differences were observed between the SD and HHD groups regarding serum TC, HDL-c, LDL-c, and TG levels. These findings may suggest that the 11-week exposure period to the hypercaloric diet was insufficient to establish a fully developed dyslipidemic state, although non-significant trends toward reduced TG and slightly higher HDL-c were observed.

Similarly, continuous selenium supplementation in animals fed a standard diet (SD + ^{Continuous}Se) did not produce significant differences compared with the SD group, indicating that, under metabolically balanced and nutritionally adequate conditions, selenium may not meaningfully alter the baseline lipid profile. These results support the hypothesis that selenium's effects manifest primarily under metabolic stress conditions, when lipid metabolism and oxidative balance are disrupted.

Based on this assumption, the significant effects observed in the groups combining the HHD with selenium supplementation (HHD + ^{Early}Se and HHD + ^{Late}Se) may result from the interaction between diet-induced metabolic stress and the antioxidant action of selenium. This association could have enhanced oxidative defense mechanisms and modulated lipid pathways, leading to increased HDL-c, reduced TG, and a lower TG/HDL-c ratio, which are key parameters of cardiometabolic risk.

Regarding each specific serum variable, this study revealed a significant increase in TC in the HHD + ^{Late}Se group compared to the HHD group, indicating that selenium promoted a significant increase in this parameter when combined with HHD. It should be noted that TC depends on HDL-c, LDL-c, and TG levels; consequently, the increase in this parameter may result from significant increases in LDL-c and HDL-c in these groups, which, in isolation, does not reflect possible beneficial or harmful effects of selenium.

These results are similar to those presented in a systematic review by Ju et al.³¹ and observational study by Stranges et al.,³² who verified that high selenium levels were associated with increased TC. However, Lee et al.³³ and Chen et al.³⁴ reported significantly lower TC levels in individuals with higher serum selenium levels. Furthermore, reduced TC was observed by Shidfar et al.²⁹ in rats supplemented with selenium and zinc, as well as in the randomized clinical trial developed by Rayman et al.³⁵ in individuals supplemented with selenium. However, the meta-analysis by Hasani et al.³⁶ found no significant effect of selenium on TC in humans.

Moreover, HDL-c increased significantly in the HHD + ^{Late}Se group compared with the SD group, in addition to an increase close to statistical significance in the HHD + ^{Early}Se group compared with the SD group, which suggests that the mineral had a positive effect on the reverse transport of cholesterol

in rats exposed to a HDD. However, an increase in HDL-c was also observed in the HDD + ^{Late}Se group compared with the HDD group, suggesting the possibility that the administration of selenium can somehow reverse the impacts of a hypercaloric diet on this apolipoprotein.

Similarly, a longitudinal study with a 7.5-year follow-up revealed that participants in the highest quartile of serum selenium had increased levels of HDL-c compared to those in the lowest quartile of this mineral, which corroborates the results found.³⁴ In contrast, a study developed by the National Health and Nutrition Examination Survey (NHANES) revealed that individuals with the lowest percentile of selenium had increased levels of HDL-c when compared to individuals with the highest percentile of this micronutrient.³⁷ Thus, the role of selenium in determining the HDL-c concentration has not yet been well established.

LDL-c was significantly greater in the HDD + ^{Late}Se group than in the HDD group, whereas the HDD + ^{Early}Se group showed an increase that approached statistical significance. These findings suggest that selenium administered in combination with a hypercaloric diet can also contribute to the increase in LDL-c. However, a better assessment of the quality of this high LDL-c requires a deeper analysis of its subfractions with TG and HDL-c, as mentioned in the introduction. That is, although selenium increased this particle, if it was within a reasonable range, the individual might be closer to phenotype A, characterized by a predominance of LDL-c subfractions considered fluctuating and less atherogenic.

The findings related to this phenomenon confirm the findings of Ju et al.³¹ reported increased LDL-c in the population exposed to higher levels of serum selenium. In contrast, Shidfar et al.²⁹ analyzed rats supplemented with selenium and zinc and found a significant LDL-c decrease.

There was a decrease in TG levels in the HDD + ^{Late}Se and HDD + ^{Early}Se groups compared to those in the SD group. This finding might indicate that, despite being exposed to a hypercaloric diet, rats supplemented with selenium present a decrease in TG levels, and this mineral may be an ally in the treatment of hypertriglyceridemia.

Similarly, Shidfar et al.²⁹ developed a study with rats supplemented with selenium and reported decreased TG levels. This was also observed in humans since Hasani et al.³⁶ carried out a meta-analysis with 11 randomized clinical trials and reported decreased levels of serum TG after supplementation with selenium.³⁸ Conversely, a systematic review developed by Tabrizi et al.³⁹ did not find a beneficial relationship between selenium supplementation and TG in patients with metabolic diseases, which was also reported by Omrani et al.⁴⁰ as a result of their clinical trial with hemodialysis patients.

According to Herberg et al.,⁴¹ in humans supplemented with 100 µg of selenium/day, an overall increase in the serum lipid profile was detected. Overall, the effect of selenium on the lipid profile is still not fully understood, and divergences in the literature may be associated with the different dosages of this mineral.

Regarding CMR, the significant decrease in CMR in the HDD + ^{Late}Se group compared to that in the SD group suggests

that even with a hypercaloric diet, selenium had an effect on lipid parameters to the point of decreasing CMR compared to a diet considered standard for those rats. This confirms the findings of Rayman et al.,³⁵ in which fermented selenium supplementation increased HDL-c levels and decreased serum TG levels, suggesting reduced CMR.

There was also a significant decrease in CMR in the HDD + ^{Early}Se group compared to the SD group. These findings suggest the benefit of optimized selenium supplementation in a hypercaloric diet; that is, the inclusion of selenium might reduce the risk of unhealthy diets when it is impossible to carry out more controlled diets with high nutritional value in cases of everyday limitations. In addition, there was also a significant decrease in CMR in the HDD + ^{Late}Se group compared to the SD + ^{Continuous}Se group. These findings suggest a beneficial effect of late selenium on CMR when combined with some substrate present in a HDD that may alter the quality of lipid particles.

With respect to theories about this effect of selenium, one should consider its role in metabolic syndrome, which provokes a pro-inflammatory condition due to a set of metabolic disorders that trigger an increase in various inflammatory parameters.¹⁹ Selenium is known to have a protective effect on these parameters, which might improve the inflammatory lipid profile and have a relevant prophylactic or therapeutic effect on this syndrome.

Furthermore, selenium significantly decreased TG in the HDD + ^{Late}Se and HDD + ^{Early}Se groups compared to that in the SD group, significantly increased HDL-c in the HDD + ^{Late}Se group and showed a nearly significant increase in the HDD + ^{Early}Se group, both of which are related to metabolic syndrome. This phenomenon of decreased TG and increased HDL-c has a positive impact on two of the diagnostic criteria for metabolic syndrome, another finding that corroborates its positive effect on these major disorders, including type 2 diabetes mellitus.

More deeply, in view of the observation of possible prophylaxis (HDD + ^{Early}Se) or therapy (HDD + ^{Late}Se), no significant change was observed among the parameters under analysis. Thus, the results of this study suggest that early or late supplementation of this mineral similarly altered the lipid profile of female rats. This can be explained by the small variation between the number of weeks in the two groups, which made it impossible for selenium to fully exert its physiological effects. This was evidenced in a study by Shidfar et al.,²⁹ in which supplementation lasted 20 weeks, and the difference between the groups treated with selenium was 12 weeks, which enabled greater distinction between the evaluated parameters.

Currently, the standard approach for controlling LDL-c levels typically involves statin therapy, which has been shown to significantly alter cardiovascular outcomes. However, since despite optimal LDL-c control a subset of patients remains at risk for cardiovascular and metabolic events,¹ selenium may play a role in modulating TG and HDL-c levels, potentially increasing HDL-c and decreasing TG. These effects could complement statin therapy synergistically, improving the overall lipoprotein profile and reducing events related to these particles.

In the context of our study subject, Wistar rats are a widely used animal model in cardiovascular research due to their physiological and metabolic similarities to humans.^{42,43} They are particularly useful for studying cardiovascular risk factors, including the effects of diet and nutritional supplements on lipid profiles.⁴²

Our study has several limitations that warrant consideration. Firstly, based on a 50.0% minimum expected difference between groups, an expected 5% (α) error, 80% test power ($1 - \beta$), and 5 groups, the sample size was limited to 55 rats, which may restrict the generalizability of our findings. Additionally, the relatively short intervention period of only 11 weeks may not have provided sufficient time to fully observe the effects of selenium modulation on lipid levels and CMR factors. Furthermore, the study exclusively focused on female rats, potentially limiting the extrapolation of results to male populations or animals with different physiological characteristics. Moreover, the absence of comparison with a group of rats with pre-existing conditions such as diabetes or atherosclerosis prevents a comprehensive assessment of the potential benefits of selenium intervention in the context of pre-existing metabolic or cardiovascular disorders. These limitations underscore the need for further research with larger sample sizes, longer intervention periods, inclusion of diverse populations, and comparison with diseased models to better elucidate the effects of selenium on cardiovascular health.

Finally, we emphasize the need for further studies to fully comprehend the profound impact of selenium on metabolic cardiovascular risk. Future studies could delve into the association between selenium and lipid metabolism biomarkers, along with clinical trials to evaluate selenium's effects on reducing cardiovascular risk, both independently and in conjunction with traditional lipid-lowering therapies such as statins. Randomized clinical trials in human patients with high CMR will be especially relevant to assess selenium's real clinical effectiveness in these patients.

Conclusion

Selenium supplementation showed the potential to increase HDL-c and reduce TG, both of which are components of the diagnostic criteria for metabolic syndrome. It also showed the potential to reduce the TG/HDL-c ratio, a predictor of CMR, in rats fed HHD. We emphasize the need for further studies, especially randomized clinical trials in human patients with high CMR who are taking statins, to assess the effectiveness

of selenium for real clinical improvement in these patients and its broader impact.

Author Contributions

Conception and design of the research and acquisition of data: Salina MVJ, Pironatto VHM, Santos AAM, Gueiber TD, Lima LG, Toledo-Junior AO, Martins CM, Gomes RZ; analysis and interpretation of the data: Salina MVJ, Pironatto VHM, Santos AAM, Gueiber TD, Lima LG, Dechandt EM, Caruso CC, Karpinski PH, Figueiredo TM, Toledo-Junior AO, Martins CM, Gomes RZ; statistical analysis: Salina MVJ, Martins CM; writing of the manuscript: Salina MVJ, Pironatto VHM, Santos AAM, Gueiber TD, Lima LG, Dechandt EM, Caruso CC, Karpinski PH, Figueiredo TM, Toledo-Junior AO, Martins CM, Gomes RZ; critical revision of the manuscript for intellectual content: Salina MVJ, Santos AAM, Dechandt EM, Toledo-Junior AO, Martins CM, Gomes RZ.

Potential Conflict of Interest

No potential conflict of interest relevant to this article was reported.

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Study Association

This study is not associated with any thesis or dissertation work.

Ethics Approval and Consent to Participate

This study was approved by the Ethics Committee on Animal Experiments of the Universidade Estadual de Ponta Grossa (CEUA-UEPG) under the protocol number 22.000027863-4.

Use of Artificial Intelligence

The authors did not use any artificial intelligence tools in the development of this work.

Availability of Research Data

The underlying content of the research text is contained within the manuscript.

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