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Effects of antioxidant diet on the RBC indices in fructose-induced obesity rat model

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Abstract: *The oxidative stress is one of the known and discussed culprits for development and progression of the disturbances in Metabolic syndrome (MetS). In our study, we are investigating the effects of antioxidant additives as additional treatment and prophylaxis in a model of MetS.*

We are using a fructose-induced metabolic syndrome rat model to examine the effects of antioxidant preparation ZellSchutz. Four experimental groups were formed using male Wistar rats (n = 60). The control group received standard rat chow, the other groups received either ZellSchutz (Z), fructose (F), or fructose and ZellSchutz (ZF).

Our results showed an unexpected increase in the RBC indices in rats with dual treatment. There was a significant increase in hemoglobin concentration, MCHC, and MCV in those animals ($p<0.001$).

The preliminary data shows the important role of antioxidants as supplementary therapy in metabolic syndrome. Further investigations will be carried on to reveal the molecular mechanisms of the observed changes.

Keywords: Metabolic syndrome, Antioxidants, RBC indices, Oxidative stress

Introduction

Metabolic syndrome (MetS) is not a specific disease. It is discussed when a patient is affected by several interconnected metabolic disorders, as well as in the presence of risk factors for cardiovascular disease. By definition, it is a combination of several cardiovascular risk factors with insulin resistance and

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hyperinsulinemia [1]. Over a billion people in the world are now affected by MetS [2]. As this epidemic spreads new therapeutic schemes should be developed. Metabolic syndrome is often associated with oxidative stress, a condition characterized by excessive production or decreased deactivation of reactive oxygen species (ROS). ROS have very controversial role in the body – while they can act as important communication molecules and essential part of the innate defenses of the body, their abundance can lead to cellular damage which is referred to as oxidative stress. This is pathological state is often cited as part of the pathogenesis of diseases like atherosclerosis, diabetes, hypertension, Alzheimer's disease, and cancer.

Aim of the study

In our study, we used rats on a high-fructose diet to examine the effects of antioxidant additive ZellSchutz on oxidative stress and metabolic parameters of MetS. Our aim was to examine the effects of this additive on the red blood cell (RBC) indices in our experimental animals and propose a hypothesis about the synergistic effects of fructose and antioxidants on the lifespan and properties of RBC.

Materials and methods

Animal model

We took 60 male Wistar rats, 3 months old at the start of the experiment, and divided them into four groups: C – control (n = 15),

Z – rats on antioxidant additive ZellSchutz (n = 15). The additive was added in the water at a daily dosage of 428 mg/kg.

F – fructose-fed rats (n = 15). The fructose was added to the water in a daily dosage of 0.5 g fructose per 1 g body mass.

ZF – fructose plus antioxidant additive (n = 15), in the same dosages as groups Z and F, for one month.

The weight of the treated rats was measured weekly to correct the doses. The water intake was also controlled. The experiment was one-month long.

ZellSchutz additive

ZellSchutz Nutritional Supplement by P.M. is an oral antioxidant preparation. According to the manufacturer 100 g of the additive contains 375 mg of vitamin C, 60 mg of vitamin E, 37.5 mg of provitamin A (β-carotene), 200 mcg of selenium, carbohydrates (sucrose) 90.8 g, Energy of 373 Kcal.

The reasons for choosing this nutritional supplement is the content of 4 antioxidant ingredients - vitamin E, C, β-carotene, and selenium in optimal concentrations.

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Blood analysis

After the blood was taken in K3-EDTA impregnated vacutainers it was analyzed in Abbott Cell-Dyn 3500 Hematology Analyzer on veterinary program for rats, and complete blood count (CBC) was obtained.

Statistical analysis

All the results were expressed as a mean ± standard error of mean (SEM). The data were analyzed for normal distribution with the Shapiro-Wilk test. The values obtained for Hemoglobin, MCHC and MCV were analyzed by means of two-way ANOVA ($p \leq 0.05$). Each ANOVA was followed by Tukey post hoc test. The analysis was done with GraphPad Prism 8 software (GraphPad Software Inc.)

Ethics of experimentation

The protocol of the study was approved (№184/2017) by the Ethics Committee of the Bulgarian Food Safety Agency and met the Animals in Research: Reporting In Vivo Experiments (ARRIVE) guidelines.

Results

After a one-month treatment in the previously described manner, CBC was done (Table 1).

Table 1. RBC indices of the treated rats at day 30

Blood Count Parameters	Control	ZellSchutz + Fructose	ZellSchutz	Fructose
RBC ($x10^{12}$)	7.59±0.24	8.38±0.24	7.15±0.54	7.59±0.30
Hemoglobin (g/L)	146.2±0.96	174.3±3.76	150.7±3.52	146.2±4.76
MCHC (g/L)	54.74 ± 0.54	52.56 ± 0.47	54.32 ± 0.71	56.22 ± 1.54
MCV (fl)	353.20±6.53	400.50 ± 2.64	358.3±20.32	365.5±12.56

A two-way ANOVA was conducted to determine if the hemoglobin concentration, MCHC, and MCV were different for groups with different diets. In the analysis of hemoglobin, MCHC and MCV, there were no outliers, as assessed by the boxplot; data was normally distributed for each group, as assessed by the Shapiro-Wilk test (hemoglobin – p = 0.110, MCHC – p= 0.225, MCV – p= 0.980); Data is presented as mean ± standard deviation.

Hemoglobin concentration was statistically significantly different between ZF and the others, $F(3, 42) = 116.5$, $p < 0.001$. Mean ZF hemoglobin concentration was higher (174.3 ± 3.76) compared to the others (C - 146.2 ± 0.96, Z -150.7 ± 3.52, F – 146.2 ± 4.76). Tukey post hoc analysis revealed that the differences between the hemoglobin concentration of ZF vs C (28.80, 95% CI (23.37 to 32.80), $p < 0.001$), ZF vs Z

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(23.55, 95% CI (18.84-28.27), p < 0.001) and ZF vs F (28.05, 95% CI (23.34 to 32.77), p < 0.001) were statistically significant, but no other group differences were statistically significant (Fig. 1).

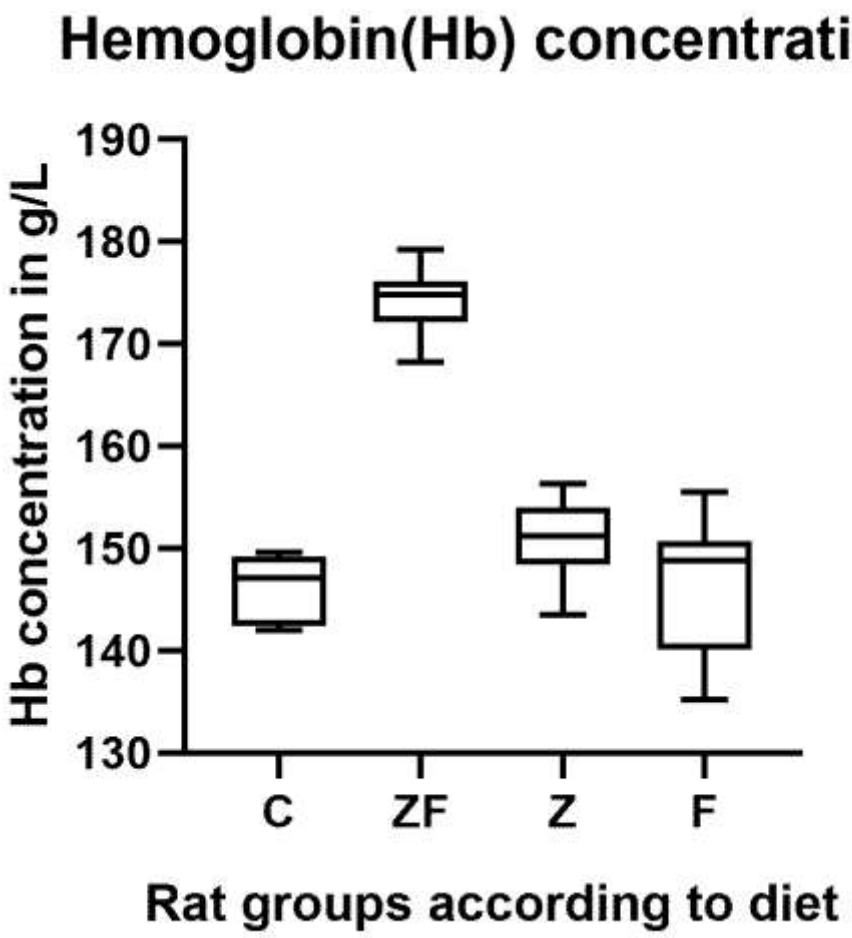


Figure 1. Hemoglobin concentrations of the four different groups on day 30. There is significant increase in the group treated with ZellSchutz and fructose (p< 0.001)

MCHC was statistically significantly different between ZF and the other groups, F (3, 42) = 84.59, p<0.001. Mean ZF MCHC was higher (400.50 ± 2.64) compared to the others (C – 353.20 ± 6.53, Z - 358.3 ± 20.32, F – 365.5 ± 12.56). Tukey post hoc analysis revealed that the differences between the hemoglobin concentration of ZF vs C (47.34, 95% CI (37.64 to 57.05), p < 0.001), ZF vs Z (42.22, 95% CI (32.51 to 51.92), p < 0.001) and ZF vs F (50.53, 95% CI (40.83 to 60.24), p < 0.001) were statistically significant, but no other group differences were statistically significant (Fig. 2).

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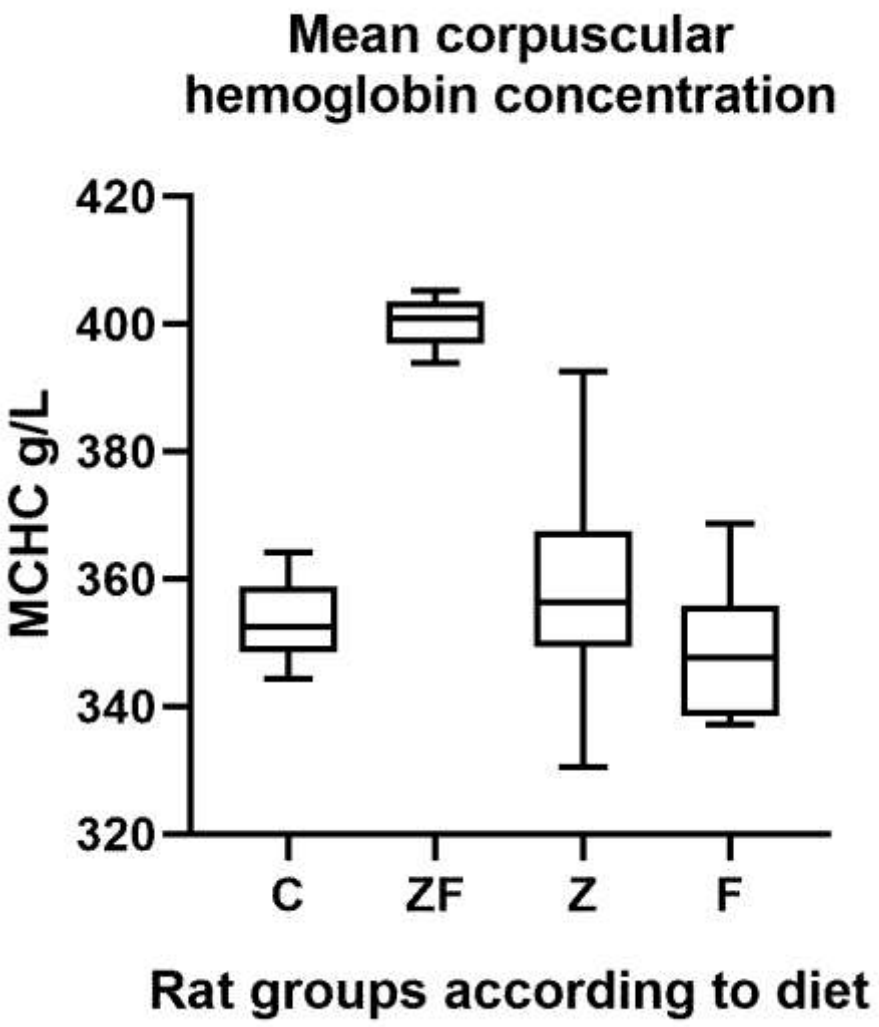


Figure 2. Mean corpuscular hemoglobin concentrations of the four different groups on day 30. There is significant increase in the group treated with ZellSchutz and fructose ($p < 0.001$)

MCV results were statistically significantly different in ZF and F compared to the other groups, $F(3, 42) = 82.38$, $p < 0.001$. Mean ZF MCV was lower (52.56 ± 0.47) compared to the others (C – 54.74 ± 0.54, Z – 54.32 ± 0.71, F – 56.22 ± 1.54). Mean F MCV was higher than that in the other groups. Tukey post hoc analysis revealed that the differences between the MCV of ZF vs C (-2.176, 95% CI (-2.804 to -1.548), $p<0.001$), ZF vs Z (-1.758, 95% CI (-2.385 to -1.130), $p < 0.001$), ZF vs F (-3.660, 95% CI (-4.287 to -3.032), $p < 0.001$), F vs Z (1.902, 95% CI (1.274 to 2.530), $p< 0.001$) and F vs C (1.483, 95% CI (0.856 to 2.111), $p< 0.001$) were statistically significant, but no other group differences were statistically significant (Fig. 3).

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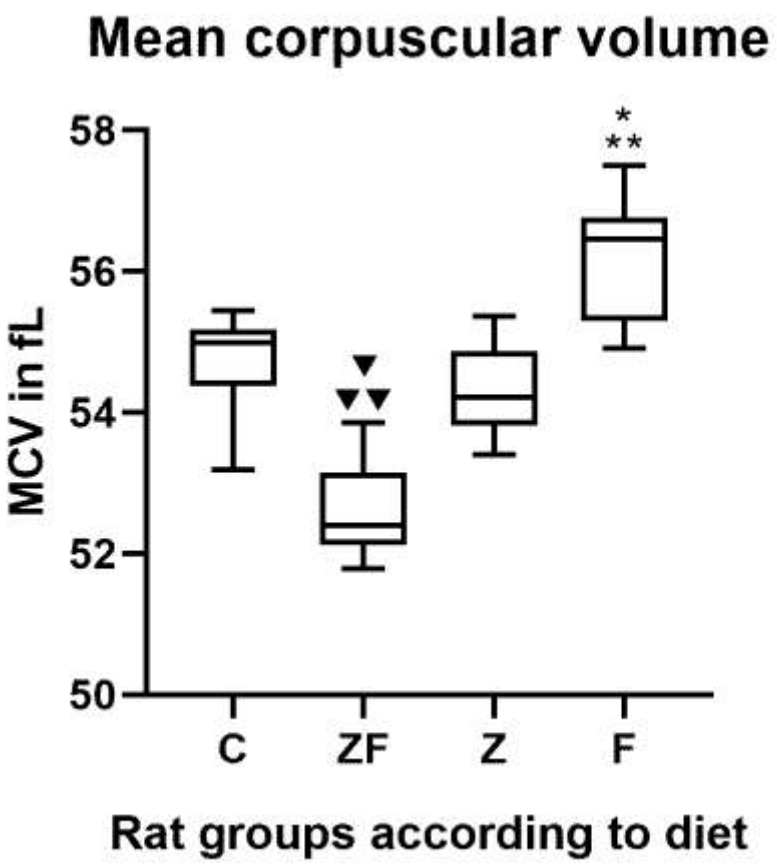


Figure 3. Mean cell volume of erythrocytes of the four different groups on day 30. It is significantly higher in the group treated with fructose vs the others (*, ** - p< 0.001) and significantly lower in the groups treated only with ZellSchutz vs the others (▼, ▼▼ – p<0.001)

Discussion

From our results, it is clear that the rats fed with fructose and antioxidant additive show a significant increase in the RBC indices, compared to the groups on fructose or ZellSchutz alone. While we cannot draw certain conclusions on this early stage of the experiment, we suggest that this can be explained by both the effects of fructose and antioxidants on the RBC life span.

There is data in the literature that shows the effect of hyperinsulinemia on the red blood cell proliferation and increasing of the RBC indices – total RBC count, Hb, MCHC [3]. Some reviews even suggest using those indices as a predictor for the development of MetS [4]. At the same time, a high fructose diet in rats is associated with increased plasma oxidative stress parameters, even after 3 weeks of administration [5, 7]. The increase of oxidative stress was shown by many studies that shorten the lifespan of

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the erythrocytes [7, 8]. Sometimes even the low concentrations of antioxidants (e.g. ascorbic acid) in the body can cause severe shortening of the cell life, and their administration can reduce the cell death. [9, 10] Also in diabetes type 2 erythrocytes mechanical fragility is significantly increased [11]. Our data shows a significant increase in MCV in the F group, which changes can be associated with reticulocytosis and therefore – higher erythrocyte turnover. So we speculate that the net effect of these mechanisms is the cause of the non-significant change in the RBC indices in the fructose-fed group.

In the ZF group, the antioxidants reduce the fragility of the RBCs, probably stabilize the membrane through the β-spectrin repair, as shown in some researches [9] and at the end – extend the lifespan of RBC. Together with the stimulation of the hyperinsulinemia, this can explain the significant increase of RBC indices in the group with dual treatment.

Conclusion

In this pilot study, we are showing the positive and unexpected effect of antioxidant additives on the RBC indices in fructose-induced metabolic syndrome rat model. While the exact mechanisms of this significant increase will be further analyzed in our research, the preliminary data shows the important role of antioxidants as supplementary therapy in metabolic syndrome and hyperinsulinemia.

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