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Antioxidant enzyme activities in rabbits under oxidative stress induced by high fat diet

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Abstract

Introduction: The aim of this study was to investigate whether the type and form of oil (raw/non-oxidised (N) or post-frying/oxidised (O)) consumed in high-fat diets affect the oxidative status of an organism, as observed by malondialdehyde (MDA) concentration as an oxidative factor and antioxidant enzyme activity. **Material and Methods:** Fats in the diet came from rapeseed oil (R) and olive oil (O). **Results:** The applied diet caused a decrease in MDA concentration (μmol/L) in serum in group RN from 2.94 ± 0.87 to 1.76 ± 0.13, in group ON from 2.45 ± 0.62 to 1.50 ± 0.10, and in group OO from 2.70 ± 1.16 to 1.84 ± 0.36. Meanwhile, MDA concentration (mmol/L) increased in blood haemolysate in group RO from 0.15 ± 0.07 to 0.22 ± 0.03 and in group OO from 0.17 ± 0.02 to 0.22 ± 0.02. The observed changes caused a response of the enzymatic antioxidant system in both models, especially followed by an increase in activities of total superoxide dismutase and its mitochondrial isoenzyme in all experimental groups, while its cytosolic isoenzyme activity increased only in ON and OO groups. Increased activity of glutathione peroxidase (GPX) in groups RN and RO and of catalase (CAT) in groups ON and OO was observed. Significant differences in responses to the different types and forms of oils were probably caused by the different oxidative stability of the studied oils. **Conclusion:** This diet disturbed the body's oxidative status; however, during the six-month study the enzymatic antioxidant system remained effective.

Keywords: rabbit, oil, antioxidant enzymes, MDA, oxidative stress.

Introduction

The proper amount and form (raw/non-oxidised) of vegetable oils with an optimal ratio of omega 3 to omega 6 fatty acids are essential aspects of a well-balanced diet and factors for the sufficient development of an organism. However, consumption of excessive amounts of fats (even vegetable) in either non-oxidised or oxidised form in a high-fat diet (HFD) may be an oxidative stress inductor, which in turn may damage proteins, lipids, and carbohydrates and causes many diseases (2, 10, 20). Nevertheless, due to different amounts of unsaturated fatty acids and non-enzymatic antioxidants in the composition of oils, consumption of one oil may promote oxidative stress to a greater or lesser extent than consumption of another (5, 6, 21).

To determine the oxidative status of an organism, it is necessary to determine the relationship between oxidant and antioxidant parameters in the body. Since lipid peroxidation is one of the most predominant and recognised consequences of intensified generation of free radicals, oxidative stress can be evaluated based on concentration of malondialdehyde (MDA), the main product of lipid peroxidation (4, 9). The largest role in the antioxidant activity of a body is played by the enzymatic system, which includes the following enzymes: total superoxide dismutase (SOD) and its isoenzymes: cytosolic (CuZnSOD) and mitochondrial (MnSOD), catalase (CAT), and glutathione peroxidase (GPX). SOD is the only enzyme decomposing hydrogen peroxide (H_2O_2) to the superoxide anion radical (O_2^-). This reaction requires the combined

effects CAT and GPX which decompose H_2O_2, CAT doing so at high and GPX at low concentrations of H_2O_2 (18).

In addition to enzymatic antioxidative factors, the protective system also comprises non-enzymatic antioxidants soluble in water and fats, such as vitamin E, β-carotene, and vitamin C, polyphenols or other compounds such as squalene. They are delivered through a suitable diet and improve the body's enzymatic antioxidant system (7, 23). One way of supplying these components is consuming vegetable oils.

The aim of the study was to investigate whether the type and form of oil (raw or oxidised) consumed affect the oxidative status of an organism, as observed by the MDA concentration and antioxidant enzyme activity.

Material and Methods

Preparation of oil samples. Rapeseed oil and olive oil were purchased from a local supermarket. For oil oxidation, 1 L of the oil was heated to 180ºC for six hours (mixed, with air access). The extent of oxidation was determined by the peroxide (PV) and iodine (JV) values as suggested by the Polish Committee for Standardisation (15) and by a chromatographic method to measure the content of fatty acids (FA) (26).

Animals. The study was conducted under the guidelines of the Animal Care Committee of the University of Silesia. It was assigned the approval number KNW-022/LKE-1-25/08 and lasted six months. In total, 32 male Chinchilla rabbits (b.w. 2,800 ± 200 g) were obtained from the Centre for Experimental Medicine of the Medical University of Silesia in Katowice. The animals were housed individually in stainless steel metabolic cages under a 12 h light/dark cycle. They were fed 80 g of proper fodder/kg b.w./24 h and allowed water *ad libitum*. The animals were weighed weekly throughout the duration of the experiment. The fodder was provided once daily. Feed intake was assessed by evaluating the leftover feed. The amount of additives was calculated by the weight of the animal and modified during the experiment.

The rabbits were divided into four equal groups according to the following scheme:

RN – rabbits fed basal diet (BD) supplemented with 10% non-oxidised rapeseed oil;

RO – rabbits fed BD supplemented with 10% oxidised rapeseed oil;

ON – rabbits fed BD supplemented with 10% non-oxidised olive oil;

OO – rabbits fed BD supplemented with 10% oxidised olive oil.

The diets for each group were prepared weekly and stored in a refrigerator at 4ºC. The BD was composed of protein, carbohydrate, and fat providing respectively 24%, 69%, and 7% of the total energy content of the diet. Groups fed BD with 10% oils received respectively: 18% energy from protein, 39% from carbohydrate, and 42% from fat. Basal diet was from the Morawski Feed Mill, Kcynia, Poland.

Sample collection. At the end of the study period the rabbits were sacrificed under anaesthesia with a mixture of 50 mg/kg b.w. of ketamine, 0.1 mg/kg b.w. of fentanyl, and 0.1 mg/kg b.w. of droperidol administered intramuscularly following a 12 h fasting. The livers were collected for biochemical study after isotonic saline rinsing.

Biochemical examination. The concentration of MDA was determined in serum (shown in results as S-MDA) and blood haemolysate (shown in results as L-MDA). The activities of SOD, MnSOD, and CuZnSOD were determined in serum and are denoted in results by S-SOD, S-MnSOD, and S-CuZnSOD. The activities of CAT, GPX, and glutathione reductase (GR) were determined in blood haemolysate and have the descriptors L-CAT, L-GPX, and L-GR in the results. Serum was obtained by centrifuging the blood at 3,000 rpm for 10 min at 4ºC. On the day the blood was taken, blood haemolysate was prepared from centrifuged blood cells after centrifugation as mentioned above. The sediment of erythrocytes was rinsed three times with saline and then haemolysed with deionised water. Determination of the haemoglobin (Hb) concentration was carried out in 10% haemolysate, using Drabkin's cyanomethaemoglobin method (22). The Oyanagui method was used to determine the activities of SOD, MnSOD, and CuZnSOD (EC 1.15.1.1) (13). Enzymatic activity was expressed in nitrite units per millilitre (NU/mL). In this method one nitrite unit (NU) means 50% inhibition by SOD of nitrite ion production. The activity of CAT (EC 1.11.1.6) was determined by the kinetic method described by Aebi (1). Glutathione peroxidase (EC 1.11.1.9) activity was measured by the kinetic method according to Paglia and Valentine (14). The activity of GR was determined according to Richterich (16). Data for all activities are shown in international units/g Hb (kIU/gHb). The MDA concentration was determined spectrofluorimetrically in serum (S-MDA) and blood haemolysate (L-MDA), using its reactivity with thiobarbituric acid according to Ohkawa *et al.* (12). Data were reported as μmol/L in serum and mmol/L in haemolysate.

Statistical analysis. Statistical analysis was performed using STATISTICA 10.0 PL software (StatSoft, Poland). Data were presented as mean value ± standard deviation and their normality was checked with the Shapiro-Wilk test, based on data histogram and quantile-quantile plots. Homogeneity of variance was tested by the Levene test. In all cases, data distributions met normality assumptions. In order to check statistical hypothesis, two-way ANOVA was used with contrast analysis as post-hoc test. The results are presented in figures showing means with confidence intervals. $P < 0.05$ was taken to be statistically significant.

Results

The results are shown in Table 1 and Figs 1–8.

Oxidised rapeseed oil characterisation. Obtained results were reported in a previous publication (24). We noted an increased content of palmitic and oleic acids and a decreased content of linoleic and linolenic acids after oxidation at 180ºC for 6 h. During rapeseed oil oxidation, PV increased 106 times and JV decreased by about 2%, and during olive oil oxidation PV increased about 18% and JV decreased about 3%.

Figures 1 and 2 show changes between profiles of MDA concentrations in serum (S-MDA) and haemolysate (L-MDA) during the experiment. Table 1 gives statistical significance levels (p) for differences between S-MDA and L-MDA concentrations among groups Q and D receiving HFD with different vegetable oil additions.

There were differences between groups RN and ON (Q3) and RO and OO (Q4) for S-MDA ($P < 0.001$) and between groups RN and RO (Q1), RV and ON (Q3), and RO and OO (Q4) for L-MDA ($P < 0.01$). We also observed a significant decrease in S-MDA concentrations in the RN (D_1), ON (D_2), and OO (D_4) groups at the end of the experiment in comparison to the first day ($P < 0.05$): in the RN group from 2.94 ± 0.87 µmol/L to 1.76 ± 0.13 µmol/L; in the ON group from 2.45 ± 0.62 µmol/L to 1.50 ± 0.10 µmol/L,; and in the OO group from 2.70 ± 1.16 µmol/L to 1.84 ± 0.36 µmol/L. We also noted a significant increase in L-MDA in the RO (D_3) and OO (D_4) groups ($P < 0.01$): in the RO group from 0.15 ± 0.07 mmol/L to 0.22 ± 0.03 mmol/L, and in the OO group from 0.17 ± 0.03 mmol/L to 0.22 ± 0.02 mmol/L. Other changes were not statistically significant.

MDA concentration

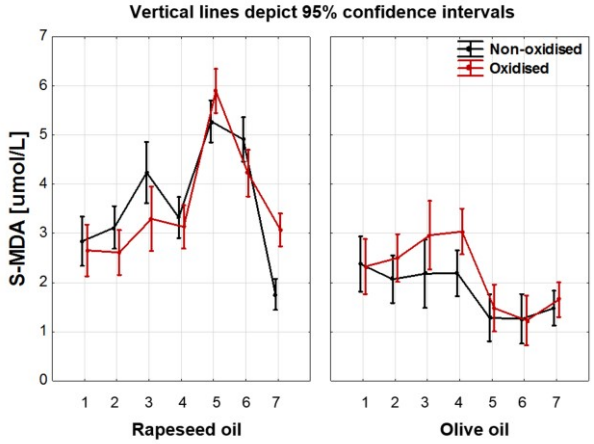

Fig. 1. Concentration of MDA in serum in groups receiving non-oxidised and oxidised rapeseed oil and olive oil during experiment

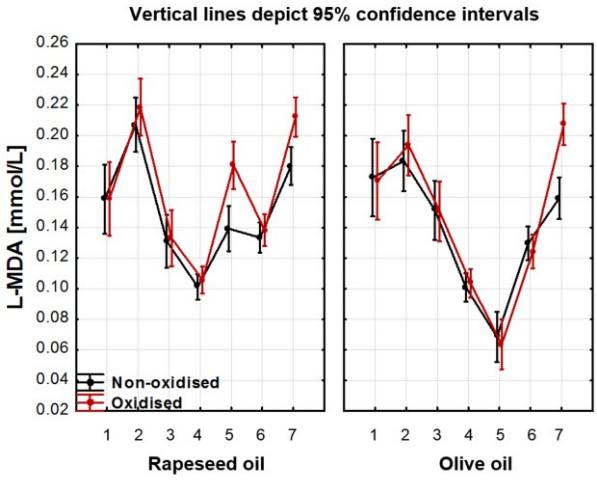

Fig. 2. Concentration of MDA in haemolysate in groups receiving non-oxidised and oxidised rapeseed oil and olive oil during experiment

The activities of antioxidant enzymes and MDA concentration

Table 1. Statistical significance levels for differences between groups receiving high fat diets with different vegetable oils

Parameter	Comparison between oxidised and non-oxidised oil				Δ_{7-1} Comparison between the last (seven months) and first (one month) values			
	Q_1	Q_2	Q_3	Q_4	D_1	D_2	D_3	D_4
S-MDA	0.67	0.13	**<0.001**	**<0.001**	**<0.01**↓	**<0.05**↓	0.21	**<0.05**↓
L-MDA	**<0.01**	0.14	**<0.01**	**<0.001**	0.07	0.31	**<0.001**↑	**<0.01**↑
S-SOD	0.52	0.90	**<0.001**	**<0.001**	**<0.05**↑	**<0.001**↑	**<0.01**↑	**<0.001**↑
S-MnSOD	0.86	0.88	0.38	0.37	**<0.001**↑	**<0.001**↑	**<0.001**↑	**<0.001**↑
S-CuZnSOD	0.85	0.89	**<0.001**	**<0.001**	0.09	**<0.05**↑	0.76	**<0.05**↑
L-CAT	0.68	**<0.05**	**<0.05**	0.47	**<0.01**↓	**<0.05**↑	0.61	**<0.01**↑
L-GR	0.99	0.79	**<0.01**	**<0.01**	**<0.05**↓	0.62	0.18	**<0.05**↓
L-GPX	0.49	0.62	0.25	**<0.05**	**<0.001**↑	**<0.001**↓	**<0.001**↑	**<0.001**↓

Q1 – comparison between oxidised and non-oxidised rapeseed oil
Q2 – comparison between oxidised and non-oxidised olive oil
Q3 – comparison between non-oxidised rapeseed oil and non-oxidised olive oil
Q4 – comparison between oxidised rapeseed oil and oxidised olive oil
D1 – change through time (seven months *vs* one month) for non-oxidised rapeseed oil
D2 – change through time (seven months *vs* one month) for non-oxidised olive oil
D3 – change through time (seven months *vs* one month) for oxidised rapeseed oil
D4 – change through time (seven months *vs* one month) for oxidised olive oil

SOD, MnSOD, and CuZnSOD activities

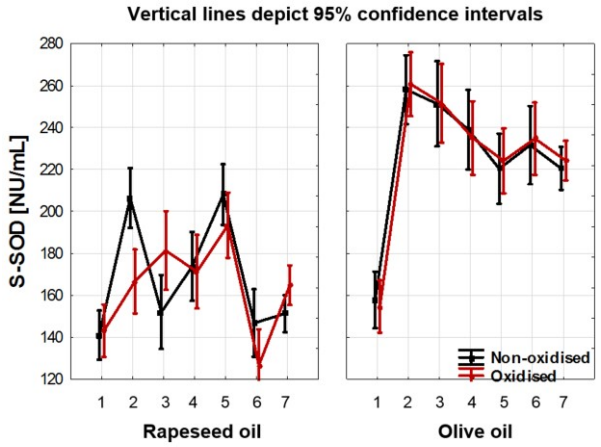

Fig. 3. Activity of total superoxide dismutase in serum in groups receiving non-oxidised and oxidised rapeseed oil and olive oil during the experiment

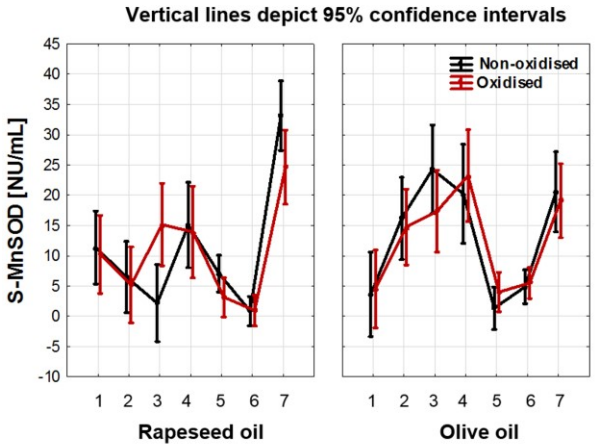

Fig. 4. Activity of superoxide dismutase mitochondrial isoenzyme in groups receiving non-oxidised and oxidised rapeseed oil and olive oil during the experiment

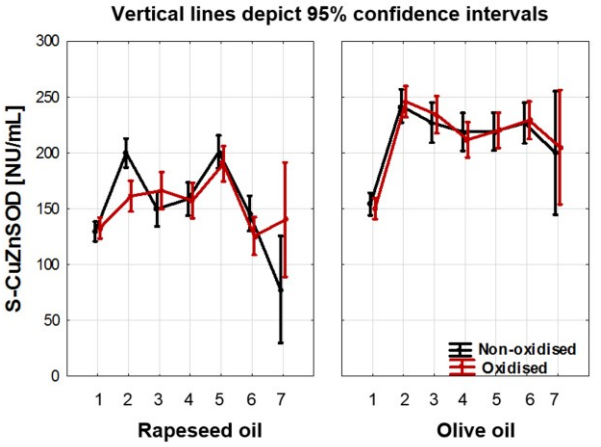

Fig. 5. Activity of superoxide dismutase cytosolic isoenzyme in groups receiving non-oxidised and oxidised rapeseed oil and olive oil during the experiment

CAT, GPX, and GR activities

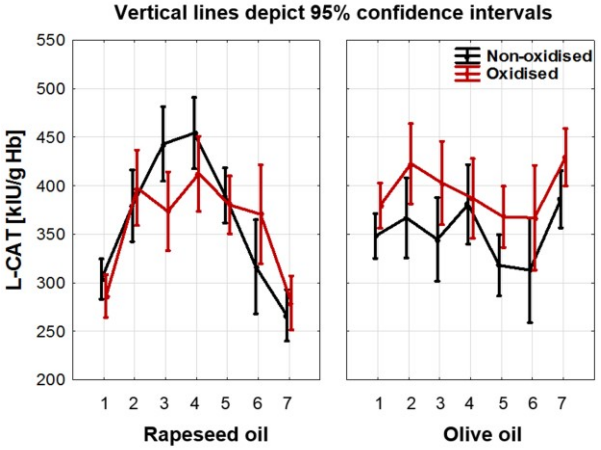

Fig. 6. Activity of catalase in haemolysate (L-CAT) in groups receiving non-oxidised and oxidised rapeseed oil and olive oil during the experiment

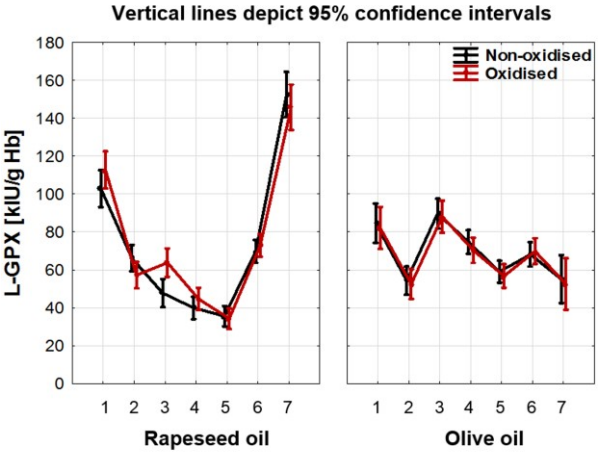

Fig. 7. Activity of glutathione peroxidase in haemolysate (L-GPX) in groups receiving non-oxidised and oxidised rapeseed oil and olive oil during the experiment

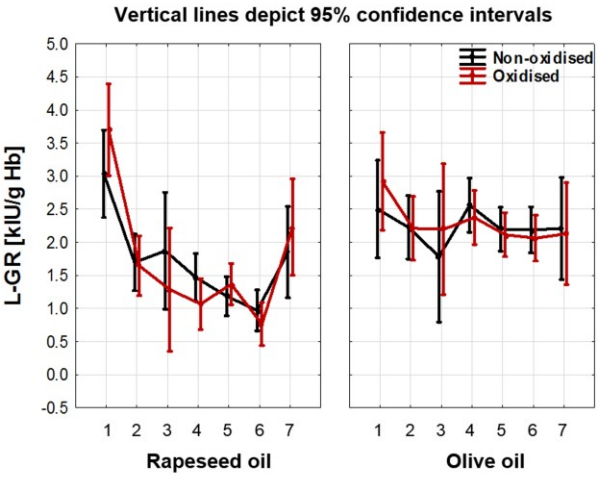

Fig. 8. Activity of glutathione reductase in haemolysate (L-GR) in groups receiving non-oxidised and oxidised rapeseed oil and olive oil during the experiment

Figures 3, 4, and 5 present changes in S-SOD, MnSOD, and CuZnSOD activities in rabbit serum during the experiment. Table 1 gives statistical significance levels (p) for differences of SOD, MnSOD, and CuZnSOD activity among groups Q and D receiving HFD with different vegetable oil additions. There were differences between groups RN and ON (Q3) and RO and OO (Q4) for SOD and CuZnSOD activity ($P < 0.001$). A significant increase in SOD and MnSOD activity in all groups ($D_{1\text{-}4}$; $P < 0.001$) was also observed at the end of the experiment in comparison to the first day of the experiment. The results which follow are all in NU/mL. For SOD activity in the RN group the increase was from 131.43 ± 18.56 to 142.31 ± 14.30, in the ON group it was from 159.94 ± 12.00 to 218.37 ± 8.36, in the RO group from 134.50 ± 11.28 to 170.16 ± 11.76, and in the OO group from 153.00 ± 12.69 to 225.49 ± 1.71. For MnSOD in the RN group the value rise was from 7.02 ± 0.65 to 35.27 ± 4.9, in the ON group from 3.49 ± 0.32 to 23.9 ± 6.72, in the RO group from 4.70 ± 0.26 to 25.14 ± 9.19, and in the OO group from 4.58 ± 0.38 to 14.67 ± 6.14.

In the case of CuZnSOD only in the ON and OO groups (D_2, D_4; $P < 0.05$) was there activity increase: in the ON group from 156.45 ± 10.66 to 194.48 ± 1.81 and in the OO group from 148.41 ± 11.75 to 210.82 ± 7.83.

Figs 6, 7, and 8 present changes in profiles of CAT, GPX, and GR activity in blood haemolysate during the experiment. Table 1 gives statistical significance levels (p) for differences in CAT, GPX, and GR activity among groups Q and D receiving HFD with different vegetable oil additions. There were differences between groups ON and OO (Q2) and groups RN and ON (Q3) for CAT activity ($P < 0.05$). For GR activity, there were differences between groups RN and ON (Q3) and groups RO and OO (Q4) ($P < 0.01$). For GPX activity there was only a significant difference between groups RO and OO (Q4) ($P < 0.05$). Data for all activities reported in the following are shown in international units/g Hb (kIU/gHb). A significant decrease was observed in CAT activity in the RN group at the end of the experiment (265.80 ± 21.57) in comparison to the first day (298.83 ± 35.31), while an increase in CAT in the ON and OO groups was observed: in the ON group from 350.57 ± 33.44 to 386.97 ± 32.32, and in the OO group from 372.12 ± 18.49 to 419.97 ± 16.08. A decrease was noted in GR activity in the RN and OO groups ($P < 0.05$): in the RN group from 2.85 ± 1.36 to 2.04 ± 0.81, and in the OO group from 2.79 ± 1.45 to 2.02 ± 0.48. For GPX activity, a decrease was witnessed in the ON and OO groups ($P < 0.001$): in the ON group from 87.09 ± 5.75 to 58.66 ± 3.52 and in the OO group from 81.37 ± 5.04 to 53.13 ± 1.54. An increase in GPX for the RN and RO groups was observed: in the RN group from 104.54 ± 20.83 to 156.85 ± 6.83 and in the RO group from 119.19 ± 17.93 to 161.76 ± 18.86.

Discussion

This article is focused on the putative action of the reactive oxygenated species (ROS) in producing oxidative injury/oxidative stress induced by a high fat diet (HFD) rich in oxidised and non-oxidised popular edible vegetable oils. This is a very important issue as oxidative stress, defined as a perturbation of cell redox balance and an excessive production of ROS that cannot be counteracted by the action of antioxidants (enzymatic or non-enzymatic), is the reason of many diseases. This is because oxidative stress induces structure modifications and function modulation in lipids, proteins, and nucleic acids. The imbalance between the oxidant species and the antioxidant defence system may trigger specific factors responsible for oxidative damage in the cell: over-expression of oncogenes, generation of mutagen compounds, and promotion of atherogenic activity or inflammation. This may lead to cancer, neurodegeneration, cardiovascular diseases, diabetes or kidney diseases (3, 11, 17). On the other hand, oxidative stress is a normal phenomenon in the body. Under normal conditions, the physiologically important intracellular levels of ROS are maintained at low levels by various enzyme systems participating in the *in vivo* redox homeostasis. Therefore, oxidative stress can also be viewed as an imbalance between the prooxidants and antioxidants in the body. This balance is maintained by the presence of natural antioxidants and antioxidant enzymes such as superoxide dismutase, catalase, and glutathione peroxidase. The enhancement of lipid peroxidation or a decrease in antioxidant protection present in metabolic disorders or resulting from bad lifestyle (*i.e.* consumption of HFD) can induce many dysfunctions and diseases.

At the end of the conducted experiment, a significant decrease in serum MDA concentration was observed in rabbits fed basal diet (BD) with addition of non-oxidised rapeseed oil (group RN), non-oxidised olive oil (group ON), and oxidised olive oil (group OO). In case of non-oxidised olive oil consumption, a low concentration of MDA was observed throughout the whole time of the experiment, whereas in the case of oxidised olive oil consumption, the decrease was observed from the 4th month of the experiment. In case of non-oxidised rapeseed oil consumption, until the 5th month of the experiment an increase in serum MDA concentration was observed, but in the 6th month the concentration of MDA decreased rapidly. In the 6th month of the experiment there were no changes observed in the group fed BD with addition of oxidised rapeseed oil (group RO).

Meanwhile, in the 6th month of the experiment, a significant increase in haemolysate L-MDA concentration in RO and OO groups was observed, despite the fluctuations and low concentrations of L-MDA observed from month two to month five. It proves that a diet rich in oxidised oils disturbs the oxidative

homeostasis of an organism and results in increased lipid peroxidation. However, in the long term some stabilisation in blood serum can be expected, but not in erythrocyte. Our previous research on rats' liver and blood confirmed this observation. The concentration of MDA determined in liver homogenates and in blood plasma increased statistically significantly after consumption of a diet rich in oxidised oils (24, 25). Similar results were obtained during our other experiment conducted on rabbits (26). The animals were fed a balanced diet with addition of rapeseed oil oxidised for seven days at 120ºC, and erythrocyte MDA concentration increased significantly at the end of the experiment. But after six weeks a decrease in the L-MDA concentration was observed. Izaki *et al*. (8) reported an increased concentration of MDA in rats exposed to oxidised rapeseed oil derived from frying fish paste. The authors connected this observation with an increased content of arachidonic acid and docosahexaenoic acid in lipids isolated from the liver of the animals, as well as a marked drop in the α-tocopherol concentration in the oil examined. Tabatabaei *et al.* (19) also noted that the consumption of vegetable oil oxidised for 48 h at 180°C caused a significant increase in the MDA concentration in rat blood serum.

The obtained results proved that consumption of HFD with the addition of oxidised and non-oxidised oils influenced oxidative status, most likely due to the increase in lipid peroxides concentration. This caused disturbances in the activity of antioxidant enzymes and MDA concentration, both in serum and erythrocytes. A statistically significant increase in the activity of total SOD and MnSOD in all experimental groups was also observed. Moreover, a significant increase in CuZnSOD activity in rabbits fed non-oxidised and oxidised olive oil was found. Analysis of the results leads to the conclusion that consumption of not only oxidised oils, but also non-oxidised oils in higher amounts (unbalanced diet), may cause disruption of antioxidant enzymatic systems. The increased activity of antioxidant enzymes testifies that during oxidative stress induced by HFD, this enzyme protects blood as well as mitochondria. In this experiment we also observed changes in the activity of CAT and GPX, which varied depending on the applied oils. The increased activity of GPX in RN and RO groups evidenced that GPX was the enzyme that decomposed H_2O_2 formed as a result of the SOD activity, while in ON and OO groups this role was played mainly by CAT, as we observed an increase in its activity in these groups at the end of the experiment. This proves the differences between rapeseed oil and olive oil caused by different fatty acid composition and probably by different content of non-enzymatic antioxidant. These differences are in turn the consequences of different oxidative stability of the oils used. We also observed a decrease in GR activity in the RN and OO groups.

In our previous experiment we observed that total SOD activity determined in erythrocytes was not affected by non-oxidised or oxidised rapeseed oil, with the exception of the 6th week of the experiment, when SOD activity decreased in the group receiving oxidised oil (25). A similar observation applies to GPX activity, despite the fact the experiment was conducted under different conditions (26). Similar results were obtained in our other experiment conducted on rats (25). We observed an increase in total SOD and CuZnSOD activities and a decrease in MnSOD activity in serum of rats fed oxidised rapeseed oil. We also observed an increase in CAT and a decrease in GPX activities in the groups fed basal diet with the addition of oxidised oil. These results varied from the ones obtained in this study.

The conducted experiment shows that the consumption of HFD with the addition of oxidised oils and raw/non-oxidised oils disturbs the body's oxidative status. The diverse results obtained for the oils used in this study arise from the different compositions of those oils and hence from their different oxidative stability. This study also leads to the conclusion that both type and amount of oil, as well as the form in which it is consumed (raw or fried) affect our health. It should be noted that consumption of fried vegetable oils, especially in large amount, disrupts the oxidative status of the body and may be a cause of many metabolic disorders. However, it seems that during long-term consumption of such a diet, the body is capable of adapting to the special conditions and the enzymatic antioxidant system may still be effective.

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Animal Rights Statement: The experiments on animals were conducted in accordance with local Ethical Committee laws and regulations as regards care and use of laboratory animals.

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