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EFFECTS OF PLANT OIL AND ANIMAL FAT ON THE FREE-RADICALS FORMATION IN THE BLOOD PLASMA AND LIVER OF JUVENILE RAT EXPOSED TO A FAT-RICH DIET

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ABSTRACT

AIM: In the present work, the effects of plant (refined Palm Oil) and animal (Lard) derived fat on the oxidative stress were evaluated, using juvenile male rat model of fat overconsumption. MATERIALS AND METHODS: Juvenile male Wistar Albino Rats were used as model animals. The group "Standard" was fed with standard rodents chow. The group "Palm Oil" received excessive amount of refined Palm Oil, and the group "Lard" was fed with excessive amount of lard. The free-radicals accumulation in the blood plasma and livers of the experimental animals were estimated using the MTT transformation to MTT-formazan. RESULTS AND DISCUSSION: At the end of the experiment, the group "Lard", showed remarkably higher relative increase of the Body weight (BW) and liver compared with the other groups. Palm oil and lard resulted in substantially more free-radicals formed in the blood plasma and liver of juvenile male rats, this effect being stronger in the blood plasma of group "Palm Oil" while in the liver of group "Lard", compared with group "Standard". CONCLUSIONS: Excessive dietary fat resulted in an increased free-radicals accumulation in rat blood plasma and liver. Group receiving Lard remarkably increased in BW.

Key words: Palm oil, Lard, Free-Radicals, Oxidative Stress, Male Wistar Rat.

INTRODUCTION

The overweight and obesity have been associated with chronic diseases such as diabetes, cardiovascular diseases, immune defense, and certain types of cancer (1). Plant derived fat became the main source of dietary fat in the developing countries and within the low-income social groups, while the excessive consumption of animal products due to Country-specific, cultural-specific or increased wealth resulted in an excessive consumption of animal dietary fat (2, 3). In animals and humans, a link between obesity and Oxidative Stress (OS) was observed (4-9). Involvement of the OS in the obesity itself (4), heart disease (6), liver oxidative injury (8), metabolic syndrome (10), arterial hypertension (11), cancer (12) and many more diseases was proved. The relative differences between free-radicals' formation due to excessive consumption of palm oil and lard in a juvenile rat model of obesity, were not evaluated yet. Nutrient stress occurs not only due to malnutrition, but due to nutrient excess, during which the Reactive Oxygen Species (ROS) production exceeds that required for normal physiological responses (13, 14).

In the present work, the effect of two types of Fat-Rich Diet (FRD) on free radicals accumulation in the blood plasma and liver of juvenile male Wistar rats, were estimated. The fat-rich diet FRD1 was used as a model for excessive consumption of plant-derived fat (refined palm oil), while FRD2 being representative for excessive dietary consumption of animal-derived fat (lard). The transformation of 3-(4,5-dimethylthyazolyl-2)-2,5-diphenyltetrazolium bromide (MTT) to MTT-formazan in presence of blood plasma and liver homogenate, was used as a marker for the free-radicals formation. The relative differences among free-radicals levels in tissues, due to the type of FRD (FRD1 or FRD2) were statistically verified using the INSTAT program package.

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MATERIALS AND METHODS

Chemicals and solutions: All chemicals were of finest grade (pro analisi) and supplied by SIGMA-ALDRICH. PBS with pH 7.45, and 0.35 mM Xanthine solutions were prepared using bi-distilled water. Thiazolyl Blue, or MTT (3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide) was dissolved in the PBS. Prior analyses all solutions were sonified for 30 minutes, to remove all gases dissolved. Purified Lard (L) and double-fractioned palm oil (PO) of cooking grade were used for the Fat-rich diets FRD1 and FRD2.

Model animals: Juvenile Wistar rats (28 days old) were separated in three groups (three animals per group), named "Standard" (Std), "Palm oil" ("Palm Oil") and "Lard" ("Lard"). Each animal in "Palm oil" and "Lard" groups received SDD of 1 ml/100gBW liquid fat (palm oil or lard) via gastric tube, for 30 days. Prior administration, the fat portion was heated to a body temperature. The animals were treated in accordance with the General regulations for Treatment of experimental animals, established by the Ethics Committee in the Medical University of Sofia, in agreement with the "Guide to the Care and Use of Experimental Animal Care" (Canadian Council on Animal Care guidelines, 1984)". After 30 days, the animals were decapitated under anesthesia (Uretan, 2 ml/100g BW). Standard rodent's food in pellets and tub water were provided ad libitum.

Tissue extraction and storage: The livers were extracted and washed in ice cold 0.05% BMT saline solution to remove the blood and to prevent interactions with oxygen in the air, and then stored in a freezer (187±1K) for further analysis. Prior investigation, the liver tissue was homogenized using 15wt% tissue in ice-cold PBS (ice bath), then centrifuged at 277K (200XG) for 10 minutes at 277K. The supernatanta was separated and used for further analysis. The blood plasma was separated in EDTA-washed centrifuge tubes by centrifugation for 15 minutes at 293 K (10000XG). After separation the plasma was stored in a freezer at 187±1K. The proteins concentrations were determined spectrophotometrically as described in (15).The following apparatus were used in this investigation: Donau Lab sonic DLs 310T ultrasonic bath, Unipam 302 homogenizer, Zanezki K-24 Centrifuge, and Perkin-Elmer 552 UV-VIS Spectrophotometer with 2 ml quartz cuvettes.

MTT-Assay: One ml glass cuvette contained sample corresponding to 1 mg proteins, 0.20 ml MTT, 0.02 ml Xanthine, and 50mM PBS (pH 7.45). The relative change of the characteristic absorbance of the MTT-formazan (λ=576 nm) was monitored for 5 minutes, in 5 parallel measurements. For the background measurements the supernatanta was omitted. The gross errors were removed using Romanovsky test (16). The effect of the FRD on the free-radicals generation was estimated by presenting the MTT-formazan in a tissue of a group as a percentage of this found in the same tissue of the group Std. Differences among average values were statistically verified using INSTAT Program package.

RESULTS

At the end of the experiment, the total BW and this of the livers of groups "Standard" and "Palm Oil" did not differ **(Figure 1)**.

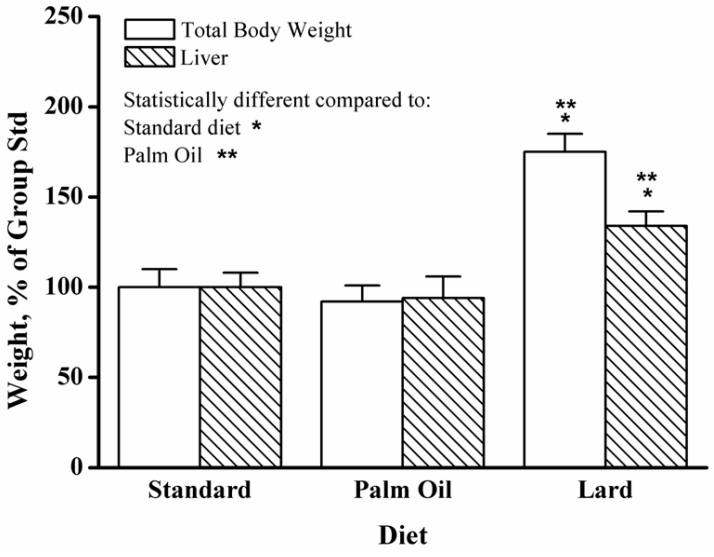

Figure 1. Total Body Weight and weight of the liver of rat exposed to a Standard diet, excessive dietary PO consumption and overconsumption of lard, at the end of the experiment.

These of the group "Lard" were significantly higher than the corresponding values for the "Standard: group. When comparing the total BW gain **(Figure 2)**, it was observed that the remarkable weight gain of group "Lard" compared with group "Standard" took place at the last week of the experiment.

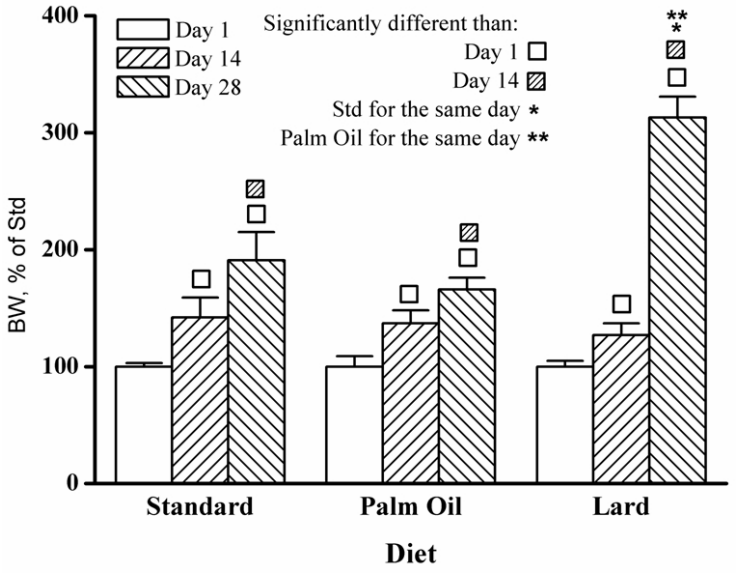

Figure 2. Body Weight gain of groups "Standard", "Palm Oil" and "Lard" during the experiment.

No matter how the BW was affected by the FRD type, in the blood plasma and liver of group "Standard" were found much less free-radicals than these found in corresponding tissues of groups "Lard" and "Palm Oil" **(Figure 3**).

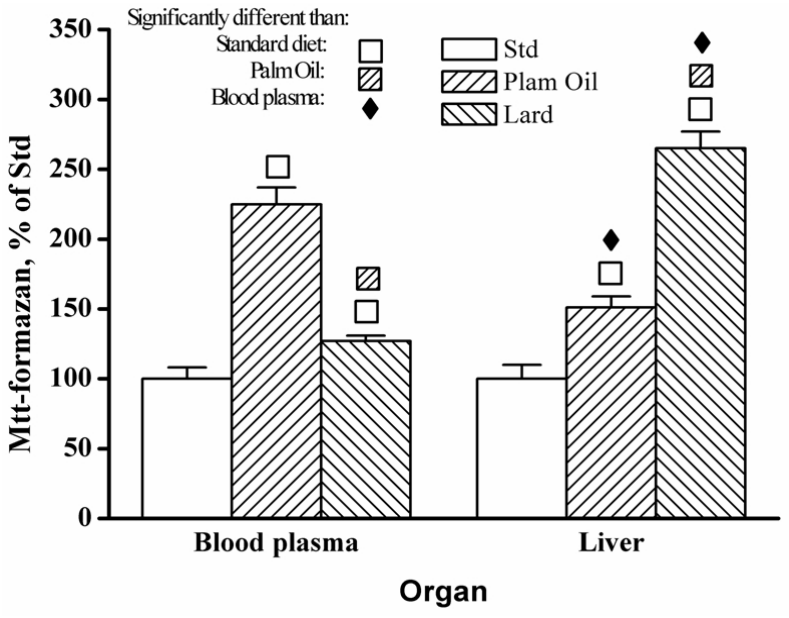

Figure 3. Accumulation of free-radicals in presence of blood plasma and liver tissue of rats exposed to Standard diet, excessive consumption of PO or Lard.

The free-radicals accumulation in the blood plasma was highest in group "Palm Oil", while this in the liver being highest in group "Lard".

DISCUSSION

Our results showed that the excessive dietary refined PO for one month did not affect the

weight gain of rats, while overconsumption of the same amount of lard resulted in a significant weight gain. Both fat-rich diets resulted in higher accumulation of free-radicals in rat liver and blood plasma, compare with the standard diet. As far as the excess of free-radicals is a component of the Oxidative stress, our results confirmed the observed OS due to fat-rich diet in human blood plasma (17-19), and in rat liver (8,20). In the blood plasma free-radicals accumulation decreased in the order PO>L, while in the liver - in the order L>PO. The relatively different effects of palm oil and lard on the free-radical formation in the blood plasma and liver could be related with the relative differences in the content of unsaturated fatty acids in both types of fats after transformation in the living body. It has been observed that after hydrolysis in presence of pancreatic lipase, the iodine value of the palm oil increased, while this of the lard decreased (21). As the iodine value is related with the amount of unsaturated fatty acids in a fat, the higher the iodine value, the higher the content of double bonds in a fat is.

CONCLUSIONS

1. Excessive consumption of palm oil and lard lead to substantially more free-radicals formed in the blood plasma and liver of juvenile male Albino Wistar rats.
2. The fat-rich diet with palm oil lead to more free-radicals formed in the blood plasma, than these in the liver. If lard was the excessive dietary fat, then more free-radicals were detected in the liver, than these in the blood plasma. This difference might be related with the various types of fatty acids that reach the blood plasma and liver.
3. Excessive dietary lard consumption resulted in faster weight gain than the excessive dietary refined Palm oil.

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