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Original Research

Heating of vegetable oils influences the activity of enzymes participating in arachidonic acid formation in Wistar rats

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ABSTRACT

Dietary intake of lipids and their fatty acids profile influence many aspects of health. Thermal processing changes the properties of edible oils and can also modify their metabolism, for example, eicosanoids formation. The aim of our study was to verify whether the activity of desaturases can be modified by lipids intake, especially by the fatty acids content. The experimental diets contained rapeseed oil, sunflower oil, and olive oil, both unheated and heated (for 10 minutes at 200°C each time before administration), and influenced the fatty acids composition in serum and the activity of enzymes participating in arachidonic acid (AA) formation. The activity of desaturases was determined by measuring the amounts of AA formed in vitro derived from linoleic acid as determined in liver microsomes of Wistar rats. In addition, the indices of Δ^6-desaturase (D6D) and Δ^5-desaturase (D5D) have been determined. To realize this aim, the method of high-performance liquid chromatography has been used with ultraviolet–visible spectrophotometry detection. Diet supplementation with the oils rich in polyunsaturated fatty acids affects the fatty acids profile in blood serum and the activity of D6D and Δ^5-desaturase in rat liver microsomes, the above activities being dependent on the kind of oil applied. Diet supplementation with heated oils has been found to increase the amount of AA produced in hepatic microsomes; and in the case of rapeseed oil and sunflower oil, it has also increased D6D activity.

1. Introduction

Fats are one of the most chemically unstable food components. They easily undergo transformations due to such factors as oxygen, air, temperature, enzymes, water, or heavy metals. They undergo increased oxidation when the oils are used for food preparation. In such cases, self-oxidation and photosensitized oxidation (also known as *photooxygenation*) occur. Heating the oils, especially for a long time, at high temperatures, and with the access of air, increases the oxidation reactions, which lead to the formation of almost 200 products of decomposition. It also results in

Abbreviations: AA, arachidonic acid; ALA, α-linolenic acid; ANOVA, analysis of variance; CLA, conjugated linoleic acid; D5D, Δ^5-desaturase; D6D, Δ^6-desaturase; FAME, fatty acid methyl ester; LA, linoleic acid; OL, olive oil; PGE_2, prostaglandin E_2; PUFA, polyunsaturated fatty acid; RAP, rapeseed oil; SUN, sunflower oil.

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double bond isomerization, cyclization, and the formation of conjugated dienes and fatty acids in *trans* configuration [1]. These compounds change the sensor and nutritional oil characteristics; and above all, they can damage the cell structure, leading to the loss of integrity of intracellular membranes and plasma membrane. They can also exhibit mutagenic and carcinogenic effects as well as accelerate the oxidation of low-density lipoproteins. It has been confirmed experimentally that the lipid oxidation products are easily absorbed in the intestine, and their concentration in serum increases many times after the meal [2].

The investigations performed on animals have shown the influence of oxygenated fatty acids supplied in the diet on the formation and growth of coronary atherosclerosis [3]. Other studies showed that consumption of polyunsaturated fatty acids (PUFAs) heated to 215°C is much more atherogenic than the consumption of the same fatty acids in unheated form [1].

The activity of desaturases is the main factor that controls the conversion of linoleic acid (LA, 18:2, n-6) supplied with food to arachidonic acid (AA, 20:4, n-6). The level of PUFAs in cell structures of mammals is the result of the difference between the type of PUFAs supplied in the diet and their endogenous synthesis, and how they are used to produce cellular components and other compounds that are needed for the proper functioning of the body. Thus, the processes of elongation and desaturation depend on absolute amounts of those fatty acids as well as on the activity of enzymes (mainly desaturases) that play a key role on the pathway of these metabolic transformations. One of the factors that significantly affects desaturase activity is the kind and the composition of the supplied diet [2,4–9]. Many studies showed the influence of low-fat diet, high-fat diet, and PUFA-enriched diet on the activity of Δ^6-desaturase (D6D) [6,10]. The other inhibitors of this enzyme are fatty acids in the *trans* configuration, zinc, calcium, vitamin B_{12}, and folic acid [3,11–13]. The dietary components stimulate also the Δ^5-desaturase (D5D) activity. Among the vitamins that affect the activity of this enzyme, there are vitamin A, vitamin B_{12}, and folic acid [14]. Besides, the deficiency of mineral components such as zinc and calcium leads to a reduction of PUFA levels by inhibiting the desaturation that is catalyzed by the above-mentioned desaturases [12,13].

It is generally considered that the AA derivatives, in particular prostaglandin E_2 (PGE_2), exhibit a stimulating effect on the process of carcinogenesis. Increased PGE_2 level has been found in several types of cancer, for example, breast cancer, colorectal cancer, as well as head and neck cancer. Prostaglandin E_2 regulates cellular immune responses. It can also have a suppressive effect on the ability of antigen presentation by macrophages due to which they lose their defense potential against cancer cells. An increase of PGE_2 notably weakens the immune system and thus increases the invasiveness and metastatic lesions in the neoplastic disease. That is why the activity of desaturases plays an important role in this process [15–17].

It seems that activity of desaturases can be modified by lipids intake, especially by the fatty acids. However, little is known as to how lipid oxidation and lipid oxidation products influence the activity of these enzymes.

The aim of our research work was to examine whether diet supplementation with certain oils varying in terms of fatty acids composition (rapeseed [RAP], sunflower [SUN], olive [OL]), both heated (at 200°C for 10 minutes) and unheated, influences the activity of enzymes participating in the process of AA synthesis. The investigations covered the determination of fatty acids composition in serum, as the indicator of diet modification, and the determination of the amount of AA formed during the incubation period as well as activity of D6D and D5D.

2. Methods and materials

The investigations were approved by the 2nd Local Ethics Commission at Warsaw Medical University, which deals with experiments with laboratory animals. The serum and liver microsomes of male Wistar rats were used in the experiments.

2.1. *Animals*

During the experiments, all animals had constant access to water and food (Labofeed H fodder, produced by Feed and Concentrates Production Plant, A. Morawski, Kcynia, Poland). The ingredient composition of applied fodder is presented in Table 1. Rats were kept in a room of constant humidity and temperature (23°C) in which a 12-hour/12-hour light/dark cycle was maintained.

The rats (n = 48, 60 days old) were randomly assigned to 1 of 6 experimental groups (8 animals per group). After a 10-day adaptation period, starting from 70th day of their lifetime, for the 6 following weeks they were administered RAP, SUN, or OL via gavage in the amount of 0.4 mL/d in both unheated and heated (for 10 minutes at 200°C) forms. Oils were freshly heated each time before administration. Besides, the body weight of rats was assessed every week. The rats were decapitated in the seventh week of the experiment, and

Table 1 – Declared ingredient and nutrient content of the basal diet fed to rats

Protein (g)	210.0		
Fat (g)	39.2		
Fiber (g)	43.2		
Starch (g)	300.0		
Ash (g)	55.0		
Vitamin A (IU)	15000	Vitamin B_6 (mg)	17.0
Lysine (g)	14.5	Histidine (g)	6.0
Vitamin D_3 (IU)	1000	Vitamin B_{12} (μg)	80.0
Methionine (g)	4.1	Arginine (g)	13.0
Vitamin E (mg)	90.0	Pantothenate (mg)	30.0
Tryptophan (g)	3.0	Phenylalanine (g)	10.0
Vitamin K_3 (mg)	3.0	Folic acid (mg)	5.0
Threonine (g)	7.4	Tyrosine (g)	7.8
Vitamin B_1 (mg)	21.0	Nicotinic acid (mg)	133.0
Isoleucine (g)	17.5	Choline (mg)	2750.0
Vitamin B_2 (mg)	16.0	Biotin (mg)	0.4
Valine (g)	11.0		
Calcium (g)	10.0	Potassium (g)	9.4
Iron (mg)	250.0	Cobalt (mg)	2.0
Phosphorus total (g)	8.17	Sodium (g)	2.2
Manganese (mg)	100.0	Iodine (mg)	1.0
Phosphorus saturated (g)	4.5	Chlorine (g)	2.5
Zinc (mg)	76.9	Selenium (mg)	0.5
Magnesium (g)	3.0	Sulfur (g)	1.9
Copper (mg)	21.3		