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Original Research Article

Positive effects of egg-derived phospholipids in patients with metabolic syndrome

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ABSTRACT

Purpose: Patients with metabolic syndrome (MBS) have an increased risk of all-cause mortality, especially from cardiovascular disease. Egg phospholipids (PL) have been shown to exert a positive impact on cholesterol metabolism and inflammation; eggs are an important source of PL. Our study examined potential effects of egg-yolk-derived PL in non-diabetic patients with MBS.
Methods: The study group consisted of 40 patients with MBS diagnosed according to IDF criteria and divided into an experimental group receiving the PL preparation (n−6: n−3 fatty acids ratio: 1.79) and the comparison group receiving an olive oil preparation, for one month (2012/2013year). The studied dosage was 45 ml (15 ml 3 times per day). It was a randomized, double blinded study.
Results: The waist to hip ratio, GGTP levels, plasma platelet concentrations and flow mediated vasodilation of brachial artery (FMD) significantly improved in the experimental group. A significant decrease in daytime ABPM blood pressure was noticed in both groups.
Conclusions: A phospholipid-enriched diet caused a significant improvement of endothelial vasodilatory function and a significant decrease in waist to hip ratio. A significant decrease in daytime systolic blood pressure were observed in both the phospholipid-enriched and oil-olive group.

1. Introduction

Metabolic syndrome affects approximately 39% of nondiabetic adults in the European and US populations [1,2]. According to the Third Report of the National Cholesterol Education Program, Adult Treatment Panel (NCEP-ATP III) criteria, about 47 million people worldwide suffer from metabolic syndrome, defined as a group of conditions caused by insulin resistance: obesity, hypertension, impaired glucose regulation and dyslipidemia. Nondiabetic, overweight/obese patients with metabolic syndrome have a significantly increased risk of all-cause mortality, especially from cardiovascular disease [1,3].

Current pharmacotherapy addressing separate components of metabolic syndrome seems to be insufficient and so a new primary intervention is needed. Recently, there has been a growing interest in dietary interventions and supplement intake.

Dietary phospholipids were recognized as a source of bioactive lipids that may have widespread effects on pathways related to inflammation, cholesterol metabolism, and high-density lipoprotein (HDL) function [4].

It is possible to enrich hen eggs in n−3 fatty acids by modifying the composition of feed for laying hens [4–7]. N−3 fatty acids [docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA)], have a positive effect on metabolic syndrome components. DHA and EPA lower systolic blood pressure by 3.5–5 mmHg [8,9]. Dietary n−3 fatty acids reduce blood triglycerides [10–12], and change LDL particles from small to larger, less atherogenic [13] and may reduce the risk of primary and secondary heart attack [14,15].

The aim of our study was to evaluate the effects of dietary egg yolk-derived phospholipids (PL) on metabolic syndrome components in nondiabetic patients with MBS. The egg yolk-derived PL used in the study contained n−6 and n−3 fatty acids (ratio 1.79). As a comparator we used olive oil due to its documented positive impact in metabolic syndrome [16].

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In our previous study [17] we documented antihypertensive effects of egg yolk PL in spontaneous hypertensive rats.

2. Materials and methods

2.1. Hens' diet

Eggs were collected from the Lohmann Brown hens, grown on the specialized farm, "Tronina PHW". Birds from the age of 43 up to 45 weeks were fed *ad libitum* with a standard feed mixture NJT-215 (Tasomix, Biskupice Oloboczne, Poland) for laying hens enriched with fish oil (20 g/kg), DHA Gold (15 g/kg, Novus International, Inc., MO, USA) and flaxseed (20 g/kg).

2.2. Isolation of phospholipids from hen egg yolk

Below is the method for preparing the phospholipid mixture with an optimal compound ratio from the egg yolk described in the patent application under number: P 399338 (28.05.2012).

The eggs were subjected to a process of separating the yolks in industrial conditions (OVOPOL Sp. z o.o. (Ltd.) Nowa Sol, Poland), which were then spray dried. Drying was carried out at a temperature of inlet air of 185 ± 5 °C in the drying chamber with an outlet air temperature of 70 ± 2 °C. The extraction of the powder was carried out in a tank equipped with a mechanical stirrer while maintaining the ratio of solvent yolk dilution at 1:6 m:V. The mixing process was carried out for approximately 70 min until a homogeneous mixture was obtained. After the separation of alcohol from the residue using a filter press, the alcohol was evaporated in vacuum of 150 mbar at 50 °C. The crude PL were analyzed to determine purity expressed as acetone insoluble matter, a fatty acids profile, and the content of the basic groups of the PL.

2.3. Determination of the fatty acids profile in the tested preparation and in the olive oil

The PL formulation and the placebo were converted to fatty acid methyl esters (FAME) as follows: 50 mg of the sample were dissolved in 4 mL of 0.5 M methanolic NaOH solution and heated under reflux for 2 min. After that, 4 mL of the BF_3–MeOH (14%) complex were added and the mixtures were heated again, under reflux, for further 2 min. After cooling, the mixtures were extracted with 6 mL of hexane and the organic layers were washed with saturated NaCl solution. Hexane extracts were dried over anhydrous magnesium sulfate and analyzed directly by gas chromatography. To determine the fatty acid profile, chromatographic analysis (GC/MS) was performed with the use of a gas chromatographer (6890N) coupled with a mass spectrometer (GC5973) (Agilent Technologies, Poland). An HP 88 column was used and the flow of carrier gas (He) was at 1.0 mL/min. The injector temperature was set at 230 °C and the detector temperature was set at 240 °C. The temperature program was established within the following time frame: 100 °C/hold 0.5 min then 3 °C/min to 180 °C, hold 17 min and 5 °C/min to 210 °C, hold 45 min. The content of fatty acids in the tested preparation and the placebo is presented in Tables 1 and 2.

All food grade additives used were purchased at Bart Sp. j. (general partnership) Slupno, Poland.

The PL preparation was made by pre-homogenizing all ingredients with the exception of the olive oil. The homogeneous solution was subjected to pasteurization at 60 °C and poured hot into 75 ml bottles.

A homogenized olive oil with added preservatives and a consistency similar to that of the experimental substance was used. The homogeneous solution was subjected to pasteurization at 60 °C and poured hot into 75 ml bottles.

Table 1
The content of fatty acids in the phospholipid preparation compared to olive oil (as a percent of total fatty acid measured).

FAME	Phospholipids formulation/preparation	Olive oil
C14:0	0.23	0.05
C15:0	0.10	0.00
C16:0	19.44	11.65
C16:1	1.09	0.00
C17:0	0.33	0.00
C18:0	7.72	5.83
C18:1	57.80	74.66
C18:2	7.45	6.71
C18:3	1.67	1.10
C20:0	0.30	0.00
C20:2	0.06	0.00
C20:4	0.83	0.00
C20:5	0.36	0.00
C22:6	2.62	0.00
ω−3	4.65	1.10
ω−6	8.34	6.71
ω6/ω3	1.79	6.12
SFA	28.13	17.54
UFA	71.87	82.46
MUFA	58.88	74.66
PUFA	12.99	7.80

UFA – unsaturated fatty acid.
SFA – saturated fatty acid.
MUFA – monosaturated fatty acid.
PUFA – polyunsaturated fatty acid.

2.4. Patients

The study group consisted of 40 patients (26 females and 14 males, mean age 59.85 ± 11 years) with MBS diagnosed in the Department of Endocrinology of the Military Hospital in Wroclaw (Poland) between 2012 and 2013. Exclusion criteria included: diabetes mellitus, any serious concomitant diseases with increased catabolism (such as acute and chronic renal insufficiency, liver failure, neoplastic disease, severe heart failure and respiratory insufficiency), endocrine disorders (Cushing's syndrome, acromegaly, thyroid disorders, polycystic ovary syndrome), infectious diseases, intake of steroids and hypersensitivity to egg proteins. The demographic data of the study group is summarized in Table 3.

MBS was diagnosed according to IDF Consensus definition [18] when a patient had three or more disorders at the same time out of the following five:

1. Obesity, defined as having a waist circumference of 94 cm or more for men and 80 cm or more for women, a WHR (waist to hip ratio) above 0.90 for males and above 0.85 for females, or a body mass index (BMI) above 30.0 kg/m^2.
2. Increased blood pressure, defined as a systolic blood pressure measurement of 130 mmHg or more or a diastolic blood pressure measurement of 85 mmHg or more in ABPM or previously diagnosed and treated arterial hypertension.

Table 2
The composition of the phospholipid preparation compared to olive oil.

Component	Phospholipids formulation/preparation (%)	Olive oil (%)
Phospholipid	25.00	0.00
Olive oil	37.50	99.10
10% glycerol in RO water	35.55	0.00
Alcohol 96%	1.05	0.00
Gallic acid	0.50	0.50
Potassium sorbate	0.20	0.20
Sodium benzoate	0.20	0.20