



Hypolipidemic effect of blends of coconut oil with soybean oil or sunflower oil in experimental rats

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ABSTRACT

Blended oils, consisting of coconut oil with sunflower oil or soybean oil, were prepared (22–24% linoleic acid) to provide higher amounts of PUFA to coconut oil consumers. Animal experiments were carried out to find the effects of coconut oil blends, using weanling rats, by feeding native and blends of oils at 10% level in the diet for 60 days. Serum cholesterol levels were reduced by 5% and 21%, respectively, in rats given blended oils containing CNO/SFO and CNO/SBO while liver cholesterol did not show a significant change when rats were given blends in comparison with rats given CNO. Serum and liver lipid analyses also showed significant change in TG concentration in rats fed blended oils compared with rats given CNO. These studies indicated that the atherogenic potentials of a saturated fatty acid-rich CNO can be significantly decreased by blending with an oil rich in unsaturated lipids in appropriate amounts.

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1. Introduction

Cardiovascular diseases (CVD) have been reported to be a major cause of deaths in several parts of the world and have been found to be unusually high in people originating from the Indian subcontinent. Higher incidence of coronary disease appears to be common in south Asian populations. This presents a challenge for adapting strategies aimed at reducing risk factors for coronary heart disease (Rajeshwari, Nicklas, Pownall, & Berenson, 2005). Nutrition plays an important role as one of the strategies to achieve this goal.

Dietary fats have received considerable attention as modifiers of risk factors for CVD. Coconut oil is an important cooking oil in Kerala and coastal areas of Karnataka in south India. It contains mostly saturated fatty acids and hence is considered to have hypercholesterolemic activity (Meijer, Lemmens, Versluis, Van Zutphen, & Beynen, 1991). Coconut oil is a fat consisting of about 92% saturated fatty acids, among which medium-chain triglycerides contribute 70% of the total fatty acids. The remaining fatty acids are contributed as 6% of monounsaturated fatty acids, and 2% of polyunsaturated fatty acids. Among the saturated fatty acids, coconut oil primarily contains 44% lauric acid, 16% myristic acid, 8% palmitic acid and 8% caprylic acid. Coconut oil primarily contains MCFAs (medium-chain fatty acids) such as lauric acid and caprylic acid.

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Blood lipid levels are positively connected with risk factors for CVD. Lower levels of blood lipids and cholesterol are generally observed in subjects who consume diets containing either low fat or higher amounts of polyunsaturated fatty acids (PUFA) than subjects consuming fats containing saturated fatty acids (Nimal Ratnayake & Sarwar Gilani, 2004).

Number of studies have shown that MUFA-rich oils, e.g. olive, rapeseed, or high oleic varieties of sunflower or safflower oil, are hypocholesterolemic, as are PUFA-rich oils such as soybean, sunflower or safflower oil (Elke, Dörte, Angelika, & Helmut, 1999). Health-conscious individuals normally avoid coconut oil in their diet, primarily due to concern that it is a saturated fat and saturated fat is known to increase blood cholesterol. However, unlike other vegetable oils, coconut oil is chemically very stable and not easily oxidised. It is very resistant to free radical attack and, in combination with other oils, acts as an antioxidant, helping to prevent the oxidation of other oils (Bruce-Fife, 2001). Being a rich source of medium-chain fatty acids (MCFA) (C8:0–C12:0), coconut oil finds application in infant formulae, parenteral and enteral nutrition and in food products used for fat malabsorption cases. MCFA are transported mainly via the portal system and are rapidly oxidised in the liver to provide quick energy (Keys, Anderson, & Grande, 1965). Coconut oil consumption was found to lower body fat deposition, enhance survival rate and reduce tendency to form blood clots, in population studies (Bruce-Fife, 2001). In spite of these advantages, coconut oil is deficient in essential fatty acids. It would be advantageous to modify the fatty acid composition of coconut oil so as to reduce the levels of atherogenic SFA and

increase the level of essential PUFA. This can be achieved by blending coconut oil with PUFA-rich oils. Blending of saturated fats with unsaturated oils has become an alternative approach to give an oil with balanced fatty acids (Reena & Lokesh, 2007).

In the present study, CNO was blended with SFO/SBO to provide essential fatty acids. We now examine the effect on rat serum and hepatic tissue lipids of feeding blends of oils consisting of CNO with SFO/SBO on.

2. Materials and methods

2.1. Chemicals

Edible grade CNO, refined SFO and SBO were purchased from a local market. Cholesterol, dipalmitoyl phosphatidyl choline, and BF_3 (boron trifluoride) in methanol were purchased from Sigma Chemical Corporation (St. Louis, MO, USA). Choline chloride, magnesium oxide, DL-methionine, manganese carbonate, ferric citrate, calcium phosphate, potassium citrate monohydrate, sodium selenite, zinc carbonate, cupric carbonate, potassium sulphate and vitamins were purchased from Himedia Laboratories (Mumbai, India). Ferric chloride and ammonium thiocyanate were purchased from Qualigen Fine Chemicals Ltd., (Mumbai, India). Casein was purchased from Nimesh Corporation (Mumbai, India). All solvents used were of analytical grade.

2.2. Preparation of blended oils

The blends of vegetable oils were prepared after determining the fatty acid compositions of native oils, CNO, SFO and SBO. The oils containing CNO with SFO or SBO were blended in the ratio of 1:1 [v/v]. These oil blends were prepared to provide higher percentages of PUFA to coconut oil consumers.

2.3. Fatty acid composition of oils and tissue samples

Fatty acid composition of native, blended oils and of serum, liver and other tissue samples was determined as per AOCS O.M.No. Ce 2-66 (AOCS, 2002) as their fatty acid methyl esters by using a gas chromatograph (Model GC-14A, Shimadzu Corporation, Kyoto, Japan) equipped with a flame ionisation detector (FID) and fitted to a stainless steel column (3 m length \times 0.55 mm i.d.), packed with 15% diethylene glycol succinate (DEGS) supported on 60–80 mesh chromosorb WAW and operated under the following conditions: column temperature 180 °C, injector temperature 220 °C, and detector temperature 230 °C, nitrogen as carrier gas at a flow rate of 50 ml/min, hydrogen gas at 30 ml/min, and air pressure at 450 ml/min. The individual fatty acids separated were identified by comparing their retention times with reference standards and quantified using a data processor (model CR-4A).

The methyl esters of fatty acids present in the lipid fractions of tissues (serum, liver, heart, adipose) and faecal samples were prepared using BF_3/MeOH (Morrison & Smith, 1963) after saponification of the lipids and the fatty acid composition was determined as indicated above for oils using gas chromatography.

2.4. Experimental animals

Male Wistar rats, weighing 40 ± 3 g, were grouped into five groups (four rats in each group) by random distribution and housed in individual cages, under a 12 h light/dark cycle, in an approved animal house facility at the Central Food Technological Research Institute in Mysore, India. The control animals were given AIN-76 diet, containing native oils (CNO, SFO and SBO), whereas experimental groups received diets containing blended oils of

CNO + SFO and CNO + SBO. In the AIN-76 diet, AIN means American Institute of Nutrition. The committee of AIN have formulated and published purified diet formulas that are well accepted and respected in the scientific community as general nutrition for laboratory rodents. The animals were fed for a total period of 60 days. Animals were given a fresh diet daily, and left-over diets were weighed and discarded. The gain in body weight of animals was monitored at regular intervals. The animals had free access to food and water throughout the study. After 60 days of feeding, rats were fasted overnight and sacrificed under diethyl ether anaesthesia. Blood was drawn by cardiac puncture, and serum was separated by centrifuging at 2000 rpm for 20 min at 4 °C. The liver was removed and rinsed thoroughly with ice-cold saline, blotted, weighed and stored at -20 °C until analysed. Heart and adipose tissues were also removed, washed in ice-cold saline, blotted and stored at -20 °C until analysed.

2.5. Animal diet composition

The following is the composition of the diets used in the study (g/100 g): sucrose 60, casein 20, cellulose 5, mineral mix 3.5, vitamin mix 1, choline chloride 0.2, methionine 0.3, oil 10 (Anonymus, 1977). The experimental protocol was approved by the institutional animal ethics committee.

2.6. Analysis of lipid parameters

2.6.1. Serum lipid extraction

Serum lipids were extracted according to Bligh and Dyer (1959). Methanol and chloroform were added to the serum, separately, in proportions of 2:2 and mixed well, and the extract was filtered using Whatman no.1 filter paper. The filtrate was allowed to settle, and the lower chloroform layer was separated and used for further analysis.

2.6.2. Liver lipid extraction

Liver lipid was extracted by the method of Folch, Lees, and Sloane-Stanley (1956). One gram of liver was homogenised with 1 ml of 0.74% potassium chloride, to which 20 ml of chloroform and methanol (2:1 v/v) were added, and the mixture was homogenised. The mixture was left-overnight and filtered through a Whatman no.1 filter paper; 3 ml of 0.74% potassium chloride were added and mixed well. The mixture was allowed to stand at room temperature. The upper aqueous layer was removed carefully, and then the lower phase was washed with 3 ml of a chloroform:methanol:water (3:48:47 v/v) mixture. The chloroform layer was used for lipid analysis.

2.6.3. Total cholesterol, triglyceride and phospholipid estimations

Serum and liver cholesterol levels were quantified using the method of Searcy and Bergquist (1960). Triglycerides (TG) were estimated by the method of Fletcher (1968), using tripalmitin as reference standard (30–300 μg). Phospholipids were estimated by a ferrous ammonium thiocyanate method (Stewart, 1980), using dipalmitoyl phosphatidyl choline as the reference standard.

2.7. Statistical analysis

The results were analysed by analysis of variance (Fischer, 1970). Data were expressed as means \pm SD. Analysis of variance was employed to evaluate the differences between the groups. A difference of $p < 0.05$ was considered to be significant.

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