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Hypocholesterolemic effects of low calorie structured lipids on rats and rabbits fed on normal and atherogenic diet

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ABSTRACT

The hypocholesterolemic effects of two low calorie structured lipids (SL1 and SL2) containing essential fatty acids, prepared by lipase catalysed interesterification of ethyl behenate respectively with sunflower and soybean oils were studied in rats and rabbits. The feeding experiment conducted on rats as well as rabbits, fed on normal and atherogenic diet containing 10% of SL1 and SL2 (experimental) and sunflower oil (control) indicated no adverse effects on growth and food intake. However, the structured lipids beneficially lowered serum and liver lipids, particularly cholesterol, LDL cholesterol, triglycerides and also maintains the essential fatty acid status in serum and liver. The lipid deposition observed in the arteries of rabbits fed on atherogenic diets was significantly reduced when structured lipids were included in the diet. These observations coincided with reduced levels of serum cholesterol particularly LDL cholesterol observed in experimental groups. Therefore the structured lipids, designed to have low calorific value also beneficially lower serum lipids and lipid deposition in animals fed on atherogenic diets.

1. Introduction

Structured lipids (SLs) are defined as triglycerides (TAG), restructured or modified to change the fatty acid composition and/or their positional distribution in the glycerol molecule by chemical or enzymatic process (Akoh, 1996; Haumann, 1997). The SL combines the unique characteristics of component fatty acids such as melting behaviour, digestion, absorption and metabolism which enhance their utility in foods, nutrition and therapeutics (Osborn & Akoh, 2002). With increasing consumer awareness of the risks associated with high fat intake and excess calories provided by them, a market for reduced calorie fats and fat replacers has opened up in recent times. Carbohydrate and protein based fat replacers are currently available, but they have limitations for their use at high temperatures (Hassel, 1993). Therefore, lipid based fat substitutes are the viable option for use in cooking and deep fat frying applications and for mimicking all attributes of a natural fat. Several such low calorie SLs are reported in the literature and notable among them are Caprenin, Salatrim, MCT, Captex etc., (Akoh, Long, Flatt, Rose, & Martin, 1998; Mela, 1996; Osborn & Akoh, 2002; Wardlaw et al., 1995). Developed as a reduced-calorie fat with 5 kcal/g, Caprenin is a SL containing behenic acid (C22:0) in random position obtained from hydrogenated rapeseed oil and two medium chain fatty acids – caprylic and capric acids. Studies using rats have shown that absorption of this SL is significantly lowered and its excretion in the faecal matter was higher than that observed with native oils (Peters, Holcombe, Hiller, & Webb, 1991; Webb & Sanders, 1991). Salatrim, on the other hand is a SL containing a mixture of long-chain (stearic acid) and short chain (acetic, propionic or butyric acids) aliphatic acids randomly distributed among the three positions of glycerol molecule (Smith, Finley, & Leveille, 1994). Extensive feeding studies in animals revealed that Salatrim does not change the intestinal microflora and secondary bile acids and does not show mutagencity or other toxicological effects (Scheinbach et al., 1994). However, both Caprenin and Salatrim are devoid of essential fatty acids (EFA). Olestra, another SL is a mixture of hexa-, hepta- and octanoic esters of sucrose (Peters, Lawson, Middleton, & Triebwasser, 1997). The physical properties of sucrose polyesters are similar to normal triacylglycerol. Olestra is adaptable to most applications where fats and oils are used. However, the major drawback of olestra is 'anal leakage', the result of a non-digestible fat passing through the digestive system.

Reduced calorie SLs are designed by taking advantage of either lower caloric density of short chain fatty acids or limited absorption

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of long chain saturated fatty acids (Haumann, 1997; Wardlaw et al., 1995). The majority of reduced calorie fats and fat substitutes available today are devoid of essential fatty acids and contain unusual fatty acids, not encountered in edible oils and fats. A nutritionally improved SL (SL1) was developed in our laboratory by enzymatic interesterification of sunflower oil with ethyl behenate and its calorific value was found to be 5.36 kcal/g (Kanjilal, Prasad, Kaimal, Ghafoorunissa, & Rao, 1999; Kaimal, Kanjilal, & Prasad, 2003). In continuation, another SL (SL2) was prepared in the present study by enzymatic interesterification of soybean oil with ethyl behenate. Both these structured lipids contain 28–29% behenic acid and 38–40% linoleic acid. In addition, SL2 contains about 4.8% linolenic acid. The objective of this investigation was to study the long term feeding of these two SLs (SL1 and SL2) on some of the risk factors associated with cardiovascular disease, especially their influence on blood cholesterol levels. Effect of SL on cholesterol levels were studied in both rats and rabbits given normal or atherogenic diets. Effect of the two SLs on lipids accumulated in the arteries of rabbits fed on atherogenic diets was also studied.

2. Materials and methods

2.1. Materials

Refined sunflower (SO) and soybean oil (SBO) of popular brands were purchased from the local market. The lipase, lipozyme TL IM was purchased from Novozymes A/S, (Bagsvaerd, Denmark). Ethyl behenate was a generous gift of M/s VVF Ltd., (Mumbai, India). All the solvents needed for the preparations as well as analysis were of AR grade and purchased from Ranbaxy Fine Chemicals Ltd., Gurgaon, India. Chemicals needed for the estimation of cholesterol, phospholipids and triglycerides were obtained from Sisco Research Laboratory Pvt. Ltd., Mumbai, India and Qualigens, India. The standard mixture of fatty acid methyl esters was purchased from Sigma (FAME, Mix C4-C24; Supelco, Cat. No. 18919). All the ingredients needed for preparation of AIN-93 atherogenic diet were purchased from the Himedia, Mumbai, India. All the feeding experiments were conducted on inbred Wistar male rats and New Zealand male White rabbits obtained from animal house of Central Food Technological Research Institute (CFTRI), Mysore, India.

2.2. Preparation of structured lipids

Structured Lipids (SL) were prepared according to procedure reported in the literature, with minor modifications (Kanjilal et al., 1999). Briefly, SO (1.6 kg) and ethyl behenate (1.2 kg) were interesterified using 4% (by weight of substrates) *Lipozyme TL IM* for 6 h for the preparation of SL1. For SL2, SBO (1.6 kg) was interesterified with ethyl behenate (1.1 kg). After 6 h, the enzyme was filtered and the crude product was subjected to short path distilation (UIC GmbH, Germany) to distil out the unreacted fatty acid ethyl esters. The optimised distilation conditions for efficient separation of ethyl esters of fatty acids from structured lipids were: temperature 200 °C, pressure 0.1 mbar, feeding temperature 65 °C, feed rate of 10 ml/min at 430 rpm speed. The isolated yields of structured lipids were in the range of 95–97%.

2.3. Estimation of calorific value

The calorific value of the SLs were determined according to the literature reported protocol (Kanjilal et al., 1999; Finley et al., 1994). The calorific values of SL1 and SL2 are found to be 5.36 and 5.2 kcal/g respectively.

2.4. Animal experiments

2.4.1. Growth study on ad libitum diet (normal diet)

In this experiment, 18 weanling Wistar male rats weighing 39–40 g were divided randomly into three groups. One group of rats was fed ad libitum AIN-93 supplemented with 10% SO (control group), and other two groups were fed ad libitum AIN-93 diet (Reaves, Nielsen, & Fohey, 1993) containing 10% of SL1 or SL2 (experimental group). The feeding experiment was conducted over a period of 60 days. 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 700*g* for 20 min at 4 °C. The liver, heart and kidney were removed, rinsed with ice cold saline, blotted, weighed, and stored at −20 °C until analysed. The experimental protocol was approved by the institutional animal ethics committee

2.4.2. Growth study on atherogenic diet given to rats

Male Wistar rats [OUBT-Wistar, IND-cft (2c)] weighing 45 g ± 2 g were grouped (five rats in each group) by random distribution and housed in individual cages, under a 12 h light/dark cycle, and in an approved animal house facility at the Central Food Technological Research Institute in Mysore, India. Animals were given a fresh AIN-93 atherogenic diet daily, and left over food was 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. Each group of rats was fed for a total of 60 days. 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 700*g* for 20 min at 4 °C. The liver, heart and kidney were removed, rinsed with ice cold saline, blotted, weighed, and stored at −20 °C until analysed. The experimental protocol was approved by the institutional animal ethics committee.

2.4.3. Growth study on atherogenic diet given to New Zealand white rabbits

New Zealand male white rabbits weighing 1.5 kg (five rabbits 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 Research Institute in Mysore, India. Animals were given a fresh diet daily, and leftover food was 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. Each group of rabbits was fed for a total of 45 days. After 45 days of feeding, rabbits were fasted overnight and sacrificed under diethyl ether anaesthesia. Blood was drawn by cardiac puncture, and serum was separated by centrifuging at 700*g* for 20 min at 4 °C. The liver, heart and kidney were removed, rinsed with ice cold saline, blotted, weighed, and stored at –20 °C until analysed. The experimental protocol was approved by the institutional animal ethics committee.

2.5. Analytical procedures

2.5.1. Physico-chemical characterisation

The acid value, peroxide value, iodine value, saponification value and unsaponifiable matters of the structured lipids, SL1 and SL2 were assayed following AOCS methods (Firestone, 2004a,b,c,d,e). The composition of fatty acids of SO, SBO, SL1 and SL2 were determined by GC. GC was performed on HP 6850 Series gas chromatograph equipped with a FID detector and the capillary column DB-225 (30 m × 0.25 mm × 0.5 μm). The oven temperature was programmed for 2 min at 160 °C, raised at 6 °C min^{-1} to 180 °C, hold for 2 min at 180 °C, raised again at 4 °C min^{-1} to 230 °C and finally hold for 10 min at 230 °C. The carrier gas, N_2