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# Evaluation of structured lipids with behenic acid in the prevention of obesity

Débora Kono Taketa Moreira [a,*], Paola Souza Santos [b], Alessandra Gambero [b], Gabriela Alves Macedo [a,c]

[a] Department of Food Science, School of Food Engineering, State University of Campinas, Monteiro Lobato street, 80, 13083-862 Campinas, SP, Brazil
[b] Clinical Pharmacology and Gastroenterology Unit, Medical Science Faculty, São Franscisco University, Av. São Francisco de Assis, 218, 12916-900, Bragança Paulista, SP, Brazil
[c] Department of Food and Nutrition, School of Food Engineering, State University of Campinas, Monteiro Lobato St. 80, 13083-862 Campinas, SP, Brazil

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ABSTRACT

Obesity affects all social classes, making it necessary to develop effective products that aid weight loss or help prevent weight gain. The objective of this work was to study the anti-obesity effects of structured lipids (SL) obtained by enzymatic interesterification, based on olive oil, soy oil and fully hydrogenated crambe oil. Twenty-four C57Bl/6 mice were distributed into four experimental groups according to the diet consumed: Control Diet (CD), Structured Lipids Diet (SLD), High-fat Control Diet (HCD), High-fat Structured Lipids Diet (HSLD). The animals that were fed SLs presented a smaller weight gain, despite a larger intake of the diet. The lowest weight gain was reflected in reduced amounts of adipose tissue and lower liver weight. A significant increase in lipids excreted by the animals in the feces was observed, despite there being no sign of toxicity or presence of diarrhea. The animals that consumed the HSLD presented lower total and LDL-cholesterol, increased HDL-cholesterol and increased hepatic arachidonic acid and docosahexaenoic acid levels. In addition, they did not develop hepatic steatosis. The study therefore showed that SLs could play a major role in combating or preventing obesity and other resultant diseases, without producing side effects.



## 1. Introduction

According to the World Health Organization, obesity is one of the main public health problems in the world. It is predicted that by 2025, around 2.3 billion adults will be overweight and >700 million, obese. In Brazil, obesity is an increasing problem. In a public survey carried out in 2015 by the IBGE (Brazilian Institute of Geography and Statistics), around 82 million people (almost 60%) presented a BMI of 25 or more (overweight or obese), with females being more inclined to be overweight (58.2%) than males (55.6%). The IBGE data highlight the urgency to consider adequate public policies regarding the prevention and treatment of the overweight or obese population (ABESO, 2015).

Obesity is simply the accumulation of body fat resulting from excess dietary energy intake and the low burning of calories associated with chronic and systematic low-grade inflammation. Obesity is associated with various diseases, such as type 2 diabetes mellitus, systemic hypertension, cardiovascular disease, dyslipidemia, hyperventilation syndrome, osteoarticular diseases and some types of cancers, which have destructive effects on quality of life (Bastos, Rogero, & Arêas, 2009; Eckersley, 2001; Jebb & Prentice, 1997).

Different diets have been proposed to treat obesity, from those with a low energetic content and nutritional equivalent, to those that propose restrictions to certain nutrients, changing the proportions of proteins, carbohydrates and fats (Klein et al., 2004). Despite the studies showing positive effects, the rise in obesity levels around the world indicates that it is necessary to develop more effective strategies to combat this problem. Thus, the utilization of natural compounds with the ability to block the absorption of gastrointestinal fats help to accelerate the process of weight loss or prevent the development of obesity in a non-aggressive way, since they are composed of lipids that are present in food.

The increase in knowledge on the effects of fatty acids related to the length of the chain, unsaturation and stereospecific distribution on the metabolism and health, has led to an increased interest in the use of oils and fats to reduce the risks of disease, as well as improving health. One type of modification to oils and fats that has been largely used in recent times, is the process of enzymatic interesterification, which produces structured lipids. Structured lipids are usually combinations of triglycerides, modified to present a particular composition in fatty acids or triglycerides, in order to obtain a desirable property, such as caloric reduced or an altered melting point (Berry, 2009; Kubow, 1996; Petrauskaite, De Greyt, Kellens, & Huyghebaert, 1998).

* Corresponding author at: Department of Food Science, School of Food Engineering, State University of Campinas, Monteiro Lobato St. 80, 13083-970 Campinas, São Paulo, Brazil.
*E-mail addresses:* deborakono@hotmail.com (D.K.T. Moreira), pa.s.santos@hotmail.com (P.S. Santos), alessandra.gambero@usf.edu.br (A. Gambero), macedoga@gmail.com (G.A. Macedo).

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The interesterification of combinations of solid fats and vegetable oils can form products with excellent technological and nutritional characteristics. Thus, the objective of this work was to study the anti-obesity effects of structured lipids formulated from olive oil, soy oil and fully hydrogenated crambe oil. The raw material choice was based in the excellent antioxidant, anti-inflammatory and cardioprotective actions of olive oil (Perez-Jimenez et al., 2005); in the presence of polyunsaturated acids in soy oil that improve the levels of HDL-cholesterol and lower LDL-cholesterol (Russo, 2009), as well as being a low cost raw material in Brazil; in the content of behenic acid in the fully hydrogenated crambe oil, which is a natural inhibitor of pancreatic lipases (Kojima et al., 2010).

## 2. Material and methods

### 2.1. Raw material

In this experiment, we used a structured lipid (SL) composed of soy oil (45% m/m), extra virgin olive oil (45% m/m) and fully hydrogenated crambe oil (10% m/m), obtained by enzymatic interesterification, by immobilized *Thermomyces lanuginosus* lipase (Lipozyme TL IM) from Novozymes A/S, at a proportion of 60 U/g per gram of the total substrate. The reaction was carried out in a Dubnoff shaking water bath at 60 °C for 8 h at 180 rpm. After the reaction, the lipase was removed and stored in a freezer at −20 °C, until used. The lipase activity unit (U) was defined as the quantity of lipase required to release 1 μmol of fatty acid in 1 min, per milligram of enzyme at 37 °C.

The physical-chemical characterization of SL was obtained by Moreira, Ract, Ribeiro, and Macedo (2017).

### 2.2. Experimental protocol

Five-week old male C57B/l6 mice were used. The mice were obtained from Multidisciplinary Center for Biological Research (CEMIB; State University of Campinas, Campinas, SP, Brazil). The mice had a two-week adaptation period before being randomly distributed into four experimental groups containing 6 animals each: Control Diet (CD) - mice fed with AIN-93M diet; Structured Lipid Diet (SLD) - mice fed with AIN-93M diet modified by substituting soy oil by the structured lipid (7% of the diet or 16% of total calories); High-fat Control Diet (HCD) - mice fed with a high-fat diet composed of soy oil and lard; and the High-fat Structured Lipid Diet (HSLD) - mice fed with the high-fat diet modified by substituting the lard and soy oil with the structured lipid (29.8% of the diet or 52.7% of the total calories). The project was approved by the Animal Ethics Committee at the São Francisco University (CEUA/USF), Bragança Paulista, São Paulo, Brazil. Protocol number: 001.002.2014.

The composition of the diets can be seen in Table 1. The animals were kept in individual cages on artificial cycles of 12 h:12 h light: dark at a controlled temperature. The animals, which had free access to water and diets, were weighed on a weekly basis and their food intake was monitored for 8 weeks. The food intake was monitored by subtracting the amount of food consumed from the volume offered to the animals.

### 2.3. Evaluation of energy expenditure by indirect calorimetry

To evaluate the indirect calorimetry, in the final week of the experiment, the animals were kept individually in cages attached to the Oxylet/Physiocage system (Panlab, Barcelona/Spain) at 22-23 °C, 44–45 % humidity, light/dark cycle 12/12 h, air flow of 0.5 L/min for 24 h of analysis, where $O_2$ (%) and $CO_2$ (%) were measured every 9 min. The volume of oxygen consumed ($VO_2$); volume of carbonic gas produced ($VCO_2$); respiratory coefficient (RQ), which is the relation between the $VCO_2/VO_2$, and total energetic expenditure (EE) were calculated using Metabolism Software (Panlab, Barcelona, Spain).

### 2.4. Basal blood glucose and insulin tolerance test (ITT)

After 6 h of fasting, the glucose homeostasis was evaluated by the basal blood glucose levels and the insulin tolerance test as previously mentioned by Acedo et al. (2015). The constant velocity of the reduction in glucose during the insulin tolerance test (kITT) was calculated by the GraphPad Instat program, to obtain the angular coefficient of the curve and the glycemic values adjusted in a linear model (Bonora, Manicardi, Zavaroni, Coscelli, & Butturini, 1987).

### 2.5. Necropsy of animals and material collection

After 6 h of fasting, the animals were anesthetized with a 1:1 v/v mixture of 100 mg/mL ketamine and 2% xylazine at a volume of 0.3 μL for each 100 g of body weight, in order to achieve a deep anesthesia. Peripheral blood was collected by cardiac puncture. The stores of white adipose tissue (epididymal, subcutaneous, perirenal and mesenteric), liver, intestine and the gastrocnemius muscle were removed, weighed and the values given in grams. Biopsies of the tissue that was collected and frozen were adequately stored at −80 °C for later use.

### 2.6. Histopathological evaluation of the liver

Hydrated, 2.0 μm sections of paraformaldehyde-fixed, paraffin-embedded liver specimens were stained using the haematoxylin–eosin method for evaluation of liver histology. The percentage of steatotic cells (macrovesicular and microvesicular) was determined and graded as described by Turlin et al. (2001): 0, absent; 1, <25%; 2, 26–50%; 3, 51–75% or 4, >75% of the parenchyma.

### 2.7. Triglycerides and total cholesterol

Triglycerides and total cholesterol of the serum and lipid fractions of the liver and epididymal adipose tissue were measured using commercial kits (LaborLab® Photocolorimetric kits, MG, Brazil). The lipid fractions of hepatic and epididymal adipose tissues were extracted with isopropanol (100 mg/mL), homogenized in ultra-turrax and separated by centrifugation at 300 rpm/10 min. HDL-cholesterol was also measured in serum using commercial kit (LaborLab®). The LDL-cholesterol and the VLDL-cholesterol were indirectly determined, as given by Friedewald, Levy, and Fredrickson (1972).

### 2.8. Extraction and evaluation of total lipids from the hepatic tissue and feces

When evaluating the indirect calorimetry, the animal feces were collected, weighed and stored at −80 °C. A lipid fraction of feces and of the liver was extracted using the Folch, Lees, and Sloane (1957) and the solvent was evaporated with nitrogen gas. The total lipids were obtained *via* gravimetric analysis.

### 2.9. Evaluation of the liver and feces lipid profile

The analyses of the composition of fatty acids were carried out in gas chromatography, after sterilization, carried out according to Hartman and Lago (1973). The fatty acid methyl esters were separated according to the AOCS Ce 1f-96 (2009) method in a DB-23 Agilent capillary column (50% Cyanopropyl-methylpolysiloxane), with the dimensions: 60 m length × 0,25 mm internal diameter × 0,25 mm thickness. The operation conditions of the chromatography were: column flow = 1.0 mL/min; linear velocity = 24 cm/s; detector temperature = 280 °C; injector temperature = 250 °C; oven temperature = 110–215 °C to 5 °C/min, 215 °C for 24 min; carrier gas - helium; injection volume = 1.0 μL; injection split, ratio 1:50. The qualitative composition was determined by comparing the peak retention times with the respective standards of fatty acids.