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Structured Nanoemulsion Lipid as Alternative to Obesity Control: An *In Vivo* Study

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ABSTRACT

Due to the global obesity epidemic, creating low-calorie foods for the industry is critical. Behenic acid, by partially inhibiting pancreatic lipase and decreasing fat absorption, is a promising option when included in structured lipids. This study evaluated the impact of chronic consumption and the anti-obesity potential of lipids structured with behenic acid in mice, comparing traditional and nanoemulsified forms. Diets with behenic acid promoted an excellent lipid metabolic impact, with good absorption of essential fatty acids in the liver and demonstrated excretion of this fatty acid in feces, suggesting its low absorption. The nanoemulsinated version stood out for presenting 50% less weight gain than diets with lard, improved insulin resistance, reduction in adipose tissue and absence of hepatic steatosis. The literature on the behavior of nanoemulsions in the digestive process is limited. Therefore, this study presents significant results for the development of low-calorie products.

Keywords

Behenic acid, in vivo assay, Interesterification, Obesity, Structured lipids.

Abbreviations

SL: Structured Lipids, O/W: Oil-in-water, NeSL: nanoemulsions of SL, ITT: Insulin tolerance test, PTP1B: Protein tyrosine phosphatase 1B, PUFAs: polyunsaturated fatty acids, FFAs: free fatty acids, NAFLD: non-fatty liver disease alcohol, FAs: fatty acids, AA: arachidonic acid, DHA: docosahexaenoic acid.

Introduction

Obesity, as results from the excessive accumulation of body fat, represents a serious risk to global health [1]. In Brazil, more than 104 million people are considered overweight and 41.7 million are obese, significantly affecting public health and generating high costs for Health System [2]. Obesity, influenced by genetic and behavioral factors, occurs due to the imbalance between energy expenditure resulting from low physical activity and basal metabolism and excessive calorie intake, generally coming from foods rich in lipids [3], and can result in several chronic and inflammatory health problems.

The food industry around the world is search for healthier lipids, which have technically and economically viable fat bases. Recently, we observed a ban on the addition of trans fatty acids in processed foods, a process that was faced by the food industries. One approach included replacing trans fatty acids with saturated ones, but from a health point of view, their consumption is also associated with the establishment of cardiovascular diseases [4], despite their technological functionality. The transition to a different lipid matrix represents a complex challenge for industries [5]. An ideal alternative to make foods healthier would be to replace trans and saturated fatty acids with unsaturated fatty acids. However, unsaturated fatty acids are liquid at room temperature, which makes food processing challenging. A possible solution is to incorporate structured lipids, produced with nutritious lipid matrices, using the interesterification reactions to food lipid matrix

[6]. This approach is based on the rearranges of triacylglycerols, generating unique lipid compositions with desirable characteristics, such as for example, reduced caloric value and different melting points [7]. The association of different vegetable oils and solid fats makes it possible to obtain lipids with technological potential and nutritional properties. In this context, previous studies showed that a structured lipid containing behenic acid as a saturated fatty acid was able of inhibiting the establishment of obesity in mice fed in a high-fat diet [8,9]. It was also possible to promote metabolic improvement and aid in weight loss in mice that were already obese with the structured lipid containing behenic acid.

The production of a nanoemulsion using a structured lipid represents a unique and original approach, providing potential stability to the product. This would facilitate the lipid application, being more stable incorporated, and increase the bioavailability of some fatty acids in the formulation [10]. This approach is relevant, considering that positive results observed in previous studies can be attributed to the low absorbed fraction of the lipid [8,9]. Furthermore, the nanoemulsion could increases the interaction of the lipid with metabolic cells, but there is no significant data about the absorption of nanostructured lipids since this date [11]. The objective of this study was to evaluate *in vivo* antiobesity effects of nanoemulsified structured lipids rich in behenic acid produced by enzymatic synthesis and expand our understanding of the potential these fats may have as future sources of healthier foods.

Materials and Methods

Synthesis of structured lipid rich in behenic acid and nanoemulsion production

The synthesis of structured lipid (SL) rich in behenic acid was performed by enzymatic interesterification of a blend of olive oil, soybean oil, and fully hydrogenated crambe oil in a proportion of 3:3:1 (w/w) as described before [12]. The oil-in-water (O/W) nanoemulsions of SL (NeSL) was prepared using 30% lipid phase and aqueous phase containing 3% of the surfactant Tween 80 as described before [13].

Animals

Five-week-old C57Bl6 mice were purchased from the Multi-institutional Center for Bioterism of the Universidade Estadual de Campinas (CEMIB, Unicamp, Brazil). The animals were housed in cages and maintained with artificial cycles of 12 h light and dark, and 22°C. The Committee on Ethics in Animal Use of State University of Campinas approved the experimental protocols (Protocol number: 5457-1/2019).

Diet composition and experimental groups

Thirty mice were randomly distributed in six groups (n=5) and they were fed for 13 weeks with different diets formulated as described in Table S1. Control diet group (CD) received AIN-93 (3948 kCal/Kg), the control high-fat diet group (HFD) received standard high-fat diet formulated with lard, soybean-olive group (SO) received high-fat diet formulated with the same amount of soybean and olive oil, soybean-olive-crambe group (SOhC) received high-fat diet formulated with a blend of soybean, olive and hydrogenated crambe oil, structure lipid group (SL) received high-fat diet formulated with structured lipid as describe before and nanoemulsion SL group (NeSL) received a high-fat diet formulated with nanoemulsion of structured lipid (Figure 1). All high-fat diets had 45% of calories from lipid sources (4787 Kcal/kg). The animals were provided water and diet ad libitum and the food intake was monitored by subtracting the amount of food consumed from the volume offered to the animals. The body weight was assessed weekly.

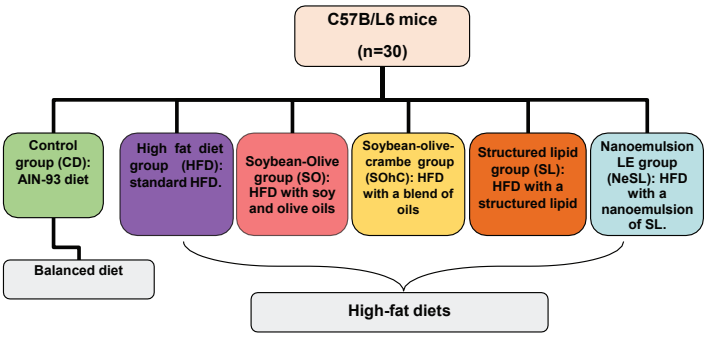

Figure 1: Experimental groups and diets.

Glucose Homeostasis Evaluation

In the last week of the protocol, mice fasted for 6 h were assessed for blood glucose by using a drop of blood from tails and a glucometer (Accutrend Plus, Roche Diagnostics, Mannheim, Germany). Insulin tolerance test was performed as previously described [12]. Results were expressed as kITT.

Necropsy and sample collection

Mice were fasted for 6 h and anesthetized with xylazine/ketamine overdose (0.1 mL per 30 g body weight of 1:1 v/v of 2% xylazine and 10% ketamine). Blood was collected via cardiac puncture. Adipose tissue depots (epididymal, perirenal, mesenteric, subcutaneous, and brown interscapular), liver and gastrocnemius muscles were carefully dissected, weighed, and expressed as a percentage of body weight (b.w.). Liver and adipose tissue samples were collected and stored at −80°C for further analyses. Liver samples were also stored in buffered paraformaldehyde for histologic analysis. Feces were collected directly from animal cages and stored at −80°C for lipid analyses.

Hepatic histologic analyses

Hydrated 2.0 µm sections of paraffin-embedded liver specimens were stained using the hematoxylin–eosin for histological evaluation. A blinded evaluation was performed for steatosis assessment using a 5-grade scale (0, absent or present in <5% of hepatocytes; 1+, ≥5% and <25%; 2+, ≥25% and <50%; 3+, ≥50% and <75%; and 4+, ≥75%), lobular inflammation (0-1), portal inflammation (0-1) and fibrosis (0-1).

Lipid extraction and characterization

The lipid fraction of the liver, adipose tissue, and feces was extracted using the Folch method and quantified by gravimetry [15]. The analyses of the composition of fatty acids were carried out in gas chromatography. 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). The qualitative composition was determined by comparing the peak retention times with the respective standards of fatty acids as previously described [12].

Serum Analysis

Triglycerides and total cholesterol were measured in the serum and in the lipid fractions of the liver and epididymal adipose tissue, using kits (Triglycerides GOD-PAP Liquid Stable; Cholesterol COD-PAP Liquid Stable, HDL Cholesterol Direct and LDL Cholesterol, from Laborlab®, MG, Brazil). Serum TNF-α measurement were performed by using EIA kit (BD OptEIA ™, USA).

Data Analysis

All data were expressed as the means ± standard error mean (SEM). Differences among groups were detected by performing an analysis of variance followed by the Tukey multiple comparison test. Values of $p < 0.05$ were considered significant.

Results

Food consumption and body composition

To evaluate the anti-obesity effects of nanoemulsified structured lipids in vivo a long-term dietary intervention of 13 weeks in mice was performed. We extended our protocol by comparing the results of the nanoemulsified structured lipid with a "classic" experimental high-fat diet, with a mixture of soybean and olive oil, with a blend of soybean, olive and crambe oil (used in structuring the lipid) and with the structured lipid. The results obtained regarding percentage of weight gain and average weekly diet consumption by the animals are presented in Figure 2. As we can observe, weight gain in HFD with oils was lower than classical HFD with lard. The addition of crambe oil was a point of weight gain reduction, as well as the nanoemulsification of structured lipid.

The final body composition demonstrated that mice fed with nanoemulfied structure lipid has lower visceral, brown, and subcutaneous adipose tissue amounts than mice fed with others

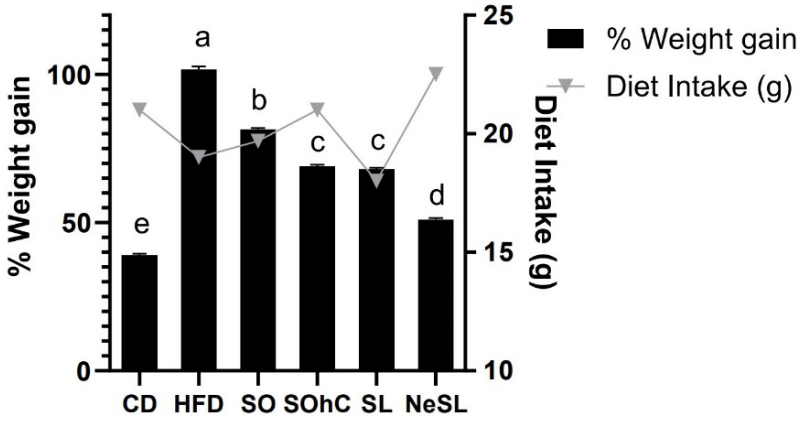

Figure 2: The percentage from initial body weight and diet ingestion of mice that consumed the control diet (CD), high-fat diet with soybean and olive oil (SO), high-fat diet with lard (HFD), high-fat diet with soybean, olive, and fully hydrogenated crambe oil (SOhC), high-fat diet with structured lipid (SL) and high-fat diet with nanoemulsified structured lipid (NeSL). Data are presented as means ± SEM of 5 mice. Statistical analysis was performed using Tukey multiple comparison test. For all columns with the same letter, the difference between the means is not statistically significant.

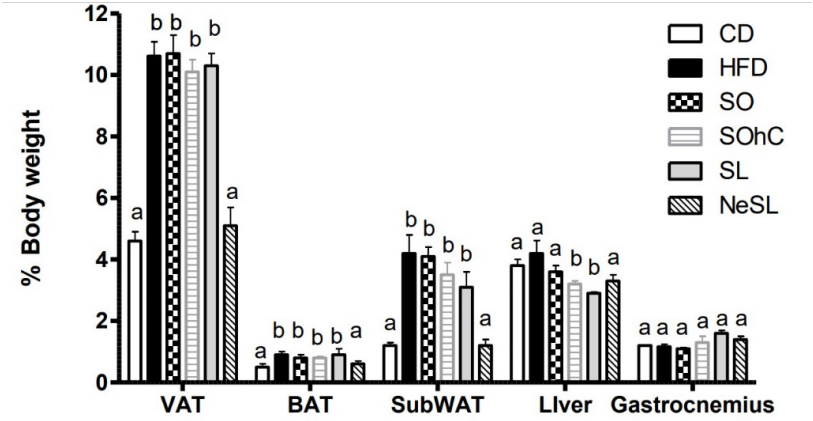

Figure 3: Tissue weight as percentage of total body weight of mice that consumed the control diet (CD), high-fat diet with lard (HFD), high-fat diet with soybean and olive oil (SO), high-fat diet with soybean, olive, and fully hydrogenated crambe oil (SOhC), high-fat diet with structured lipid (SL) and high-fat diet with nanoemulsified structured lipid (NeSL). Visceral adipose tissue (VAT) is the sum of epididymal, perirenal, mesenteric adipose tissue depots. Brown adipose tissue (BAT) and subcutaneous white adipose tissue (SubWAT) are shown separately. Data are presented as means ± SEM of 5 mice. Statistical analysis was performed using Tukey multiple comparison test. For all parameters with the same letter, the difference between the means is not statistically significant.

HFD (Figure 3). However, liver weigh was reduced only in HFD with crambe oil (SOhC and SL) in blend or in structured lipids.

Serum metabolic parameters

The results obtained by analyzing the animals' basal blood glucose are shown in Figure 4. Although the HFD group had a higher basal glycemia, of 203.5 mg/dL, there was no statistically significant difference compared to the other groups, possibly because more experimental time was needed for more pronounced effects on glycemia to occur. The same occurred with the NeSL sample as evaluated, in which the animals in this group had a fasting blood glucose level of 147.75 mg/dL, the lowest among all groups, including in relation to the control group that consumed a balanced and normocaloric diet. Which presented a blood glucose level of 150.00 mg/dL.

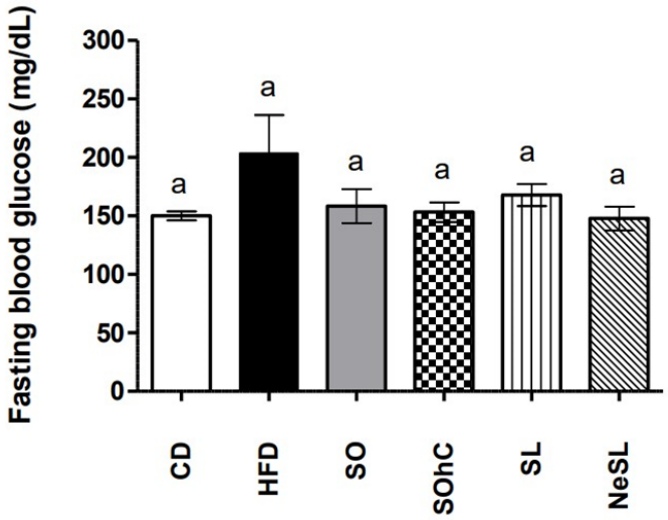

Figure 4: Fasting blood glucose of mice that consumed the control diet (CD), high-fat diet with lard (HFD), high-fat diet with soybean and olive oil (SO), high-fat diet with soybean, olive, and fully hydrogenated crambe oil (SOhC), high-fat diet with structured lipid (SL) and high-fat diet with nanoemulsified structured lipid (NeSL). Data are presented as means ± SEM of 5 mice. Statistical analysis was performed using Tukey multiple comparison test. For all columns with the same letter, the difference between the means is not statistically significant.

To evaluate peripheral insulin sensitivity, an insulin tolerance test (ITT of 30') was carried out in animals that had previously fasted for 6 hours. The results of this analysis are shown in Figure 5. Regarding the insulin tolerance test, kITT, a lower result is directly proportional to greater insulin resistance, that is, the higher the kITT, the more favorable to health. In this case, the groups that presented the best results were NeSL, animals that consumed the nanoemulsified structured lipid and the control group, which ingested a balanced and normocaloric diet, both of which did not present statistically significant differences between them. The group fed with "classical" HFD formulated with lard exhibited the lowest value of the insulin decay constant during the ITT, revealing itself as the group with a negative result, which showed an increase in insulin resistance.

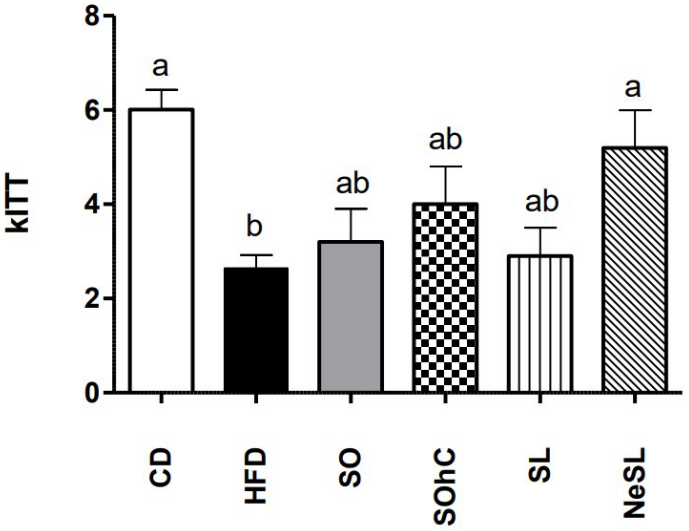

Figure 5: Insulin tolerance test of mice that consumed the control diet (CD), high-fat diet with lard (HFD), high-fat diet with soybean and olive oil (SO), high-fat diet with soybean, olive, and fully hydrogenated crambe oil (SOhC), high-fat diet with structured lipid (SL) and high-fat diet with nanoemulsified structured lipid (NeSL). Data are presented as means ± SEM of 5 mice. Statistical analysis was performed using Tukey multiple comparison test. For all columns with the same letter, the difference between the means is not statistically significant.

In the present study, the serum triglycerides of mice that consumed diets containing behenic acid or only soybean and olive oil were significantly lower than those of the group fed the lard-based HFD (Table 1). No statistical differences were found regarding total cholesterol levels between groups, and HFD with vegetable oils resulted in similar HDL-c blood levels, whereas LDL-c level was higher in the group fed the lard-based HFD and showed the lowest level in the group fed with nanoemulsified structured lipid (Table 1).

A serum inflammatory marker analysed, TNF-α, was higher only in HFD group with other groups presenting amounts of this cytokine similar to control group (Table 1).

Liver, Adipose Tissue and Feces Analysis

The histological analysis of liver samples by a blinded pathologist indicated the presence of severe steatosis associated with mild lymphoplasmacytic inflammation in HFD and SO groups, moderate steatosis associated with mild lymphoplasmacytic inflammation in SL group, mild steatosis associated with mild lymphoplasmacytic inflammation in SOhC group, and absence of steatosis in CD and NeSL group. A demonstrative slide could be observed in Figure 6.

The quantifications of total cholesterol and triglycerides in liver lipid extraction were higher in the groups HFD and SO (Table 2). No behenic acid was found in the liver tissue of the animals in any of the diets provided, indicating that it was not stored in the liver as we can observed in the qualitative lipid analysis performed in liver lipid extract (Table 3).

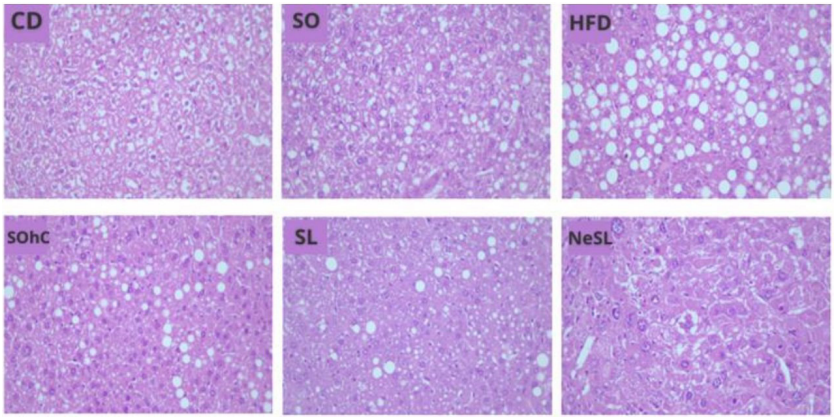

Figure 6: Liver histology of mice that consumed the control diet (CD), high-fat diet with lard (HFD), high-fat diet with soybean and olive oil (SO), high-fat diet with soybean, olive, and fully hydrogenated crambe oil (SOhC), high-fat diet with structured lipid (SL) and high-fat diet with nanoemulsified structured lipid (NeSL). Hematoxylin & Eosin stain (40x).

Table 1: Content of total cholesterol and triglycerides, cholesterol fractions (HDL-c, LDL-c) and TNF-α in the blood of mice fed with control diet (CD), high-fat diet with lard (HFD), high-fat diet with soybean and olive oil (SO), high-fat diet with soybean, olive, and fully hydrogenated crambe oil (SOhC), high-fat diet with structured lipid (SL) and high-fat diet with nanoemulsified structured lipid (NeSL).

	CD	HFD	SO	SOhC	SL	NeSL
Triglycerides (mg/dL)	116.8±24.6[a]	181.8±32.8[b]	68.6±15.6[a]	121.4±26.4[a]	114.6±9.0[a]	100.3±14.8[a]
Total Cholesterol (mg/dL)	132.6±9.6[a]	156.9±10.6[a]	160.7±21.9[a]	143.2±9.2[a]	131.3±7.0[a]	122.5±7.6[a]
HDL-c (mg/dL)	90.7±1.5[a]	123.7±3.5[b]	101.2±0.8[c]	103.7±0.9[c]	99.7±1.0[c]	103.2±2.5[c]
LDL-c (mg/dL)	37.2±0.9[a]	48.8±1.6[b]	46.5±1.9[b]	42.6±3.1[ab]	42.7±3.1[ab]	41.7±2.6[ab]
TNF- α* (pg/mL)	20.0±1.4a	61.0±22.6[b]	19.0±1.1[a]	35.3±11.0[a]	34.5±2.2[b]	20.4±5.8[a]

Data are presented as means ± SEM of 5 mice. Statistical analysis was performed using Tukey multiple comparison test. For all columns with the same letter, the difference between the means is not statistically significant.

Table 2: Content of total cholesterol and triglycerides in liver of mice fed with control diet (CD), high-fat diet with lard (HFD), high-fat diet with soybean and olive oil (SO), high-fat diet with soybean, olive, and fully hydrogenated crambe oil (SOhC), high-fat diet with structured lipid (SL) and high-fat diet with nanoemulsified structured lipid (NeSL).

	CD	HFD	SO	SOhC	SL	NeSL
Triglycerides (mg/dL)	68.0±8.0[a]	114.9±21.2[b]	196.9±15.1[c]	97.9±14.5[a]	94.8±9.1[a]	54.6±7.2[a]
Total cholesterol (mg/dL)	28.5±2.5 [a]	37.6±3.2 [b]	36.9±3.8 [b]	26.0±10.2 [a]	25.0±0.9 [a]	33.7±2.0[a]

Table 3: Qualitative profile of fatty acids in the liver tissue of animals fed the control diet (CD), high-fat diet with lard (HFD), high-fat diet with soybean and olive oil (SO), high-fat diet with soybean, olive, and fully hydrogenated crambe oil (SOhC), high-fat diet with structured lipid (SL) and high-fat diet with nanoemulsified structured lipid (NeSL). (SOhC), high-fat diet with structured lipid (SL) and high-fat diet with nanoemulsified structured lipid (NeSL).

Fatty acids (%)		CD	HFD	SO	SOhC	SL	NeSL
Lauric Acid	C12:0	0.71±0.2	0.08±0.0	0.11±0.2	0.11±0.0	0.37±0.1	0.53±0.1
Myristic Acid	C14:0	0.90±0.7	0.52 ±0.0	0.53±0.1	0.50±0.1	0.43±0.8	0.53±0.6
Palmitic Acid	C16:0	24.64±1.5	26.21±2. 5	19.98±1. 0	25.64±2. 2	20.72±0.2	22.23±0. 8
Palmitoleic Acid	C16:1	4.43±0.7	4.15±1.0	3.95±0.2	1.5±1.5	2.14±0.5	1.24±0.0
Heptadecanoic Acid	C17:0	0.24±0.2	0.21±0.2	0.28±0.0	0.3±0.0	0.23±0.0	0.44±0.2
Stearic acid	C18:0	10.10±2.5	3.81±0.4	3.78±2.8	12.70±3. 0	7.63±1.0	12.59±0. 5
Oleic acid	C18:1	26.10±0.5	41.08±1. 5	36.33±0. 9	20.70±4. 5	20.50±2.1	14.84±2. 5
Linoleic acid	C18:2	14.82±0.5	16.57±2. 5	24.73±0. 5	26.10±1. 0	30.90±0.8	24.97±2. 8
Linolenic Acid	C18:3	0.40±0.0	0.43±0.0	1.39±0.0	0.0±0.0	2.05±0.2	1.04±0.1
Arachidic Acid	C20:0	0.31±0.2	0.16±0.1	0.20±0.1	0.12±0.1	0.14±0.0	0.40±0.0
Gadoleic Acid	C20:1	0.68±0.1	0.69±0.1	0.67±0.0	0.69±0.2	0.48±0.1	0.66±0.0
Arachidonic Acid	C20:4	8.95±0.8	3.22±0.2	3.93±1.5	7.88±2.8	6.26±0.5	10.70±0. 1
Docosapentaen oic Acid	C22:5	0.0±0.0	0.40±0.0	0.46±0.4	0.80±1.5	0.92±0.5	0.76±0.8
Docosahexaeno ic Acid	C22:6	6.40±1.0	1.99±0.0	3.18±0.2	2.58±0.5	6.58±1.3	9.03±0.5
Saturated (%)		36.90±4. 5	31.10±2. 6	25.12±0. 8	39.57±2. 2	29.84±1.0	36.74±0. 5
Unsaturated (%)		62.33±4. 5	68.80±2. 6	74.89±0. 8	60.25±2. 2	70.16±1.0	63.26±0. 5

Analysis of visceral adipose tissue revealed that the amount of triglycerides and total cholesterol of control group and HFD with nanoemulsified structured lipid was similar (Table 4). In visceral adipose tissue, we could observed behenic acid in SL and NeSL group, indicating that occur some intestinal absorption and adipose tissue deposition when behenic acid is incorporated in TAGs by esterification (Table 5).

The presence of behenic acid in feces was observed in all groups that were fed with HFD containing fully hydrogenated crambe oil (Table 6).

Discussion

The evaluation of antiobesity effect of an structured lipid containing behenic acid was already reported by our group, attesting the low toxicity and good performance of these lipids in

Table 4: Content of total cholesterol and triglycerides in visceral adipose tissue of mice fed with control diet (CD), high-fat diet with lard (HFD), high-fat diet with soybean and olive oil (SO), high-fat diet with soybean, olive, and fully hydrogenated crambe oil (SOhC), high-fat diet with structured lipid (SL) and high-fat diet with nanoemulsified structured lipid (NeSL).

	CD	HFD	SO	SOhC	SL	NeSL
Triglycerides (mg/dL)	108.2±21.9[a]	202.1±26.1[b]	273.2±24.5[c]	226.8±32.2 [b]	230.9±14.6 [b]	109.3±10.1[a]
Total cholesterol (mg/dL)	18.3±7.5[a]	35.5±3.1[b]	36.6±10.1[b]	43.9±1.0[b]	41.1±3.5[b]	21.4±2.5[a]

Table 5: Qualitative profile of fatty acids in the adipose tissue of animals fed the control diet (CD), high-fat diet with lard (HFD), high-fat diet with soybean and olive oil (SO), high-fat diet with soybean, olive, and fully hydrogenated crambe oil (SOhC), high-fat diet with structured lipid (SL) and high-fat diet with nanoemulsified structured lipid (NeSL).

Fatty acids (%)		CD	HFD	SO	SOhC	SL	NeSL
Lauric acid	C12:0	0.11±0.1	0.06±0.1	0.02±0.0	0.10±0.2	0.08±0.2	0.08±0.1
Myristic acid	C14:0	1.02±0.1	0.73±0.0	0.72±0.6	0.61±0.0	0.66±0.7	0.67±0.2
Palmitic acid	C16:0	16.51±2.2	18.72±0.5	15.90±0.5	14.59±0. 8	16.81±1.5	14.51±1.0
Palmitoleic acid	C16:1	8.72±0.1	3.83±0.8	8.71±1.5	4.09±2.2	3.60±0.2	4.04±0.2
Heptadecanoic acid	C17:0	0.17±0.0	0.29±0.1	0.16±0.0	0.15±1.5	0.19±0.9	0.16±0.2
Stearic acid	C18:0	1.55±1.0	4.79±0.5	3.05±0.2	3.23±2.2	4.91±3.0	3.86±0.0
Oleic acid	C18:1	42.67±0.5	50.02±1.5	42.59±0.8	43.04±3. 0	33.66±0.8	36.60±0.5
Linoleic acid	C18:2	24.68±0.8	19.10±1.3	25.97±0.0	30.54±0. 1	35.54±0.2	35.32±2.5
Linolenic acid	C18:3	2.23±0.0	0.62±0.1	0.92±0.2	1.91±0.0	2.37±0.2	2.04±0.1
Arachidic acid	C20:0	0.08±0.1	0.09±0.2	0.06±0.5	0.08±0.2	0.18±0.0	0.21±0.0
Gadoleic acid	C20:1	0.67±0.1	0.73±0.2	0.91±0.5	0.43±0.1	0.45±0.1	0.61±0.1
Arachidonic acid	C20:4	0.31±0.0	0.25±0.2	0.32±2.8	0.29±2.2	0.30±0.5	0.35±0.1
Behenic acid	C22:0	0.03±0.1	0.02±0.0	0.01±0.2	0.07±0.5	0.29±0.0	0.35±0.0
Docosapentaen oic acid	C22:5	0.04±0.1	0.04±0.4	0.04±0.8	0.09±0.0	0.08±0.1	0.09±0.2
Docosahexaenoi c acid	C22:6	0.16±0.1	0.10±0.0	0.10±0.1	0.15±0.2	0.17±0.5	0.18±0.0
Saturated (%)		19.71±0.5	24.81±0.8	20.08±2.5	18.97±1. 5	23.27±0.2	19.97±0.5
Unsaturated (%)		80.22±0.5	75.05±0.2	79.92±2.5	80.79±1. 5	76.38±0.2	79.59±0.5

Table 6: Qualitative profile of fatty acids in the feces of animals fed the control diet (CD), high-fat diet with lard (HFD), high-fat diet with soybean and olive oil (SO), high-fat diet with soybean, olive, and fully hydrogenated crambe oil (SOhC), high-fat diet with structured lipid (SL) and high-fat diet with nanoemulsified structured lipid (NeSL).

Fatty acids (%)	CD	HFD	SO	SOhC	SL	NeSL	
Lauric Acid	C12:0	0.0±0.0	0.30±0.2	1.36±0.1	0.24±0.0	0.24±0.1	0.42±0.0
Myristic Acid	C14:0	1.96±0.0	0.61±0.0	1.55±0.4	0.38±2.69	0.37±0.1	0.38±0.1
Palmitic Acid	C16:0	31.33±0.5	22.02±0.8	30.96±0.3	21.54±1.7	22.65±0.9	18.23±0.5
Heptadecanoic Acid	C17:0	0.0±0.0	0.62±0.0	0.0±0.0	0.14 ±0.1	0.33±0.4	0.37±0.0
Stearic acid	C18:0	26.02±1.5	42.10±0.5	24.26±0.32	31.12±0.6	29.60±2. 5	27.84±1. 8
Oleic acid	C18:1	13.76±0.8	15.08±0.4	17.33±0.4	11.75±0.2	6.04±1.2	6.07±0.7
Linoleic acid	C18:2	4.92±2.5	4.00±0.5	6.71±0.1	3.20±0.1	3.99±0.8	1.20±0.2
Arachidic Acid	C20:0	5.26±0.5	2.19±0.1	4.17±0.0	3.97±0.1	4.51±0.4	5.93±0.5
Gadoleic Acid	C20:1	2.90±0.0	1.03±0.0	0.0±0.0	0.70±0.1	0.50±0.0	0.38±0.2
Arachidonic Acid	C20:4	0.0±0.0	0.32±0.0	0.0±0.0	0.38±0.0	0.0±0.2	0.21±0.0
Behenic Acid	C22:0	5.90±0.2	8.64±0.6	5.59±0.0	23.88±2.9	27.91±0. 5	35.80±1. 0
Lignoceric Acid	C24:0	0.0±0.0	0.83±0.2	3.33±0.8	1.41±0.1	2.30±0.1	3.10±0.9
Saturated (%)		70.48±1.5	77.31±2.8	71.26±0.64	82.70±0.63	87.93±0. 5	92.07±1.0
Unsaturated (%)		21.59±1.5	20.69±2.8	24.04±0.64	16.04±0.63	10.53±0. 5	7.86±1.0

mice [8,9]. The effect and toxicity of nanoemulsified structured lipid containing behenic was evaluated in animal cells model [13]. There are several studies reporting that the ingestion of structured lipids with different compositions can help reduce body fat in rodents. Mice fed with high-fat diet formulated with structured lipid rich in behenic acid showed less body mass gain compared to the group fed a diet rich with conventional lipids [8]. Male C57BL/6J mice fed for 12 weeks with a structured lipid diet with 10 to 30% medium-chain fatty acids and these animals showed a significant difference in weight gain when compared to the high-fat control group [16]. Another study evaluated a new structured lipid prepared from soybean oil and coconut oil that resulted in reduced body mass gains than C57BL/6J mice fed a high-fat diet and control diet [17]. This is because in the absence of pancreatic lipase activity, more TAGs remain intact and are not absorbed. An in vitro simulation, found that the sample with nanoemulsified structured lipid containing behenic acid exhibited a partial inhibition of 20% in the activity of pancreatic lipase [9]. The sample preserved 11.31% of TAG after digestion, demonstrating its effectiveness as a partial inhibitor of pancreatic lipase activity.

Another *in vitro* assay using cellular models of adipose tissue revealed that lipid accumulation was reduced by 42% when using the same nanoemulsion as in the present study [13]. This study evaluated the effects of consuming a high-fat diet made with nanoemulsified structured lipids versus other high-fat diets using mice as a model. The mice group that consumed the experimental high-fat diet with lard (HFD) recorded the highest weight gain, followed by the control group that did not contain behenic acid in the diet (SO), which presented the second highest weight gain. Among the high-fat diets with behenic acid studied, the final body weight of mice fed with the HFD with nanoemulsified structured lipid (NeSL) was the closest to the balanced diet. Despite being rich in lipids and slightly higher feed consumption by the animals, this diet resulted in less body mass gain.

The diet that promoted the greatest excretion of total lipids in feces was NeSL, demonstrating a result four times better compared to lipid without behenic acid (SO). The interesterified sample (SL) showed almost 2.5 times more lipid excretion through feces than the non-interesterified blend sample, with the same composition. The NeSL group had a greater release of behenic in feces, representing 35.8%. Subsequently, DHLE promoted 27.51% fecal behenic acid release, indicating that behenic acid was not fully absorbed by the animals' bodies during the experiment. Having large amounts of lipids in feces infers that this fat was partially absorbed and mostly excreted, in a greater proportion for behenic diets. It is worth noting that the mice did not present diarrhea and none of the groups, which consumed the lipids in the quantities tested, manifested any adverse reaction.

Considering the weight gain control observed in the NeSL group, the better performance in the insulin tolerance test in the same animals may be related to it. In contrast, the HFD group showed the lowest insulin decay constant value associated to a higher weight gain indicating increased insulin resistance. Studies carried out with behenic acid have inversely associated it with the onset of type 2 diabetes and no significant correlation was found, in contrast to what occurred with other fatty acids [18,19]. Behenic acid appears to be effective in inhibiting the activity of protein tyrosine phosphatase 1B (PTP1B), a relevant negative regulator of the insulin signal transduction pathway, which has proven to be a new therapeutic approach for the treatment of type 2 diabetes [20-22].

The serum triglycerides of mice that consumed diets containing behenic acid were significantly lower than those of the DHB group. In a 60-day chronic study with rats and rabbits fed a structured lipid diet containing sunflower oil or soybean oil and ethyl behenate, there was a significant decrease in serum triglyceride levels [23]. In another study, rats that ingested a diet containing behenic acid showed a reduction in plasma and liver triglyceride levels, as well as less visceral fat deposition [24]. Both authors associated these effects with the reduction of intestinal absorption of dietary fat by behenic acid, which occurs partially due to the inhibition of the action of pancreatic lipase. Blood levels of total cholesterol were lower in groups where behenic was present, a result already seen in previous studies [8]. Similarly, a study with C57BL/6 mice fed diets containing 10 or 15% structured lipids had significantly lower serum total cholesterol concentrations compared to those fed lard [17].

The fatty acid composition is recognized as the gold standard to represent the fatty acids present in the diet. Studies have already proven that the amount of PUFAs (polyunsaturated fatty acids) in food has a good correlation with the composition of fatty acids in adipose tissue and in the lipid fractions of the blood [25]. A greater cardiometabolic risk is related to the location of fat accumulation, especially when accumulated in visceral adipose tissue and in ectopic deposits, such as the liver. When histologically analyzing the liver fragments of the animals, a marked disparity was noted in the hepatocytes of the HFD group. These hepatocytes showed evident steatosis, both on a macro and micro-droplet scale. The NeSL diet, despite being high in fat, was able to inhibit the accumulation of lipids in hepatocytes. Insulin resistance induced by increased "de novo" hepatic lipogenesis may result in the aggregation of free fatty acids (FFAs) from adipose tissue, contributing to the accumulation of hepatic triglycerides and triggering simple steatosis, the initial stage of non-fatty liver disease alcohol (NAFLD) [26]. The fecal lipid profile revealed a reduction in the amounts of fatty acids (FAs) present in feces as the amount of SLs in the diet increased, suggesting that this structured lipid facilitates the digestion and absorption of unsaturated fatty acids in the intestine. Our results indicate that a high concentration of saturated FAs in feces is related to a lower accumulation of body fat. In the composition of fatty acids in the liver, the NeSL group presented a significantly higher amount of arachidonic acid (AA) and docosahexaenoic acid (DHA). Docosahexaenoic acid is derived from the omega-3 and omega-6 series of fatty acids. This indicates that the structured nanoemulsified lipid can facilitate the transformation of essential fatty acids in the liver, thus reducing the accumulation of liver fat and providing essential fatty acids for

maintaining the individual's health. In the case of DHA, DHLE stood out with higher levels than the DHMS group. The higher content of AA found in the liver of animals that consumed the behenic diet is the result of the high content of linoleic acid in the lipid matrices used to prepare the lipids, as this originates AA through biotransformation. These results can be associated with the unsaturated fatty acids present in the composition of NeSL, such as oleic acid and linoleic acid. The linoleic acid present in the diet can be transformed into arachidonic acid and several other fatty acids by the liver [27].

Considering the results obtained, it can be assumed that the structured lipid did not be deleterious the lipid metabolism of the liver, but rather increased the levels of essential fatty acids, in addition to showing anti-inflammatory action [28], as observed with TNF-α serum levels. Our results agree with another study who states that interesterified structured lipids with long-chain fatty acids are capable of improving the inflammatory state and lipid metabolism in obese rats induced by a high-fat diet [29]. An *in vitro* study found that our NeSL was able to reduce the levels of TNF-α and IL-6 by macrophages, possibly due to the combination of fatty acids, especially behenic acid and also due to the presence of minority compounds in the sample [13].

The significant results of our study can be justified by the action of the emulsifier, which, by forming a semi-impermeable layer around the droplets, can restrict the total access of lipase to the surfaces of nanoemulsified lipid particles, delaying lipid digestion [30]. Another hypothesis is that a steric hindrance of the enzyme-substrate complex occurs when the lipid is reduced in size in the form of nanoemulsion. Some long-chain fatty acids may remain at the oil-water interface, limiting lipase access to particle surfaces during digestion [31]. There is concern regarding the possible toxicity of nanoparticles used in food. However, the nanoemulsions examined in this research demonstrated a lack of toxicity, as evidenced in cellular cytotoxicity tests conducted previously [13]. It is important to highlight that the food-grade nanoemulsion will be combined with other ingredients in the formulation of a product, and its replacement with a conventional lipid will be partial. Therefore, the amount needed would be even smaller than that used in dietary formulations, not being enough to cause harm.

In summary, when evaluating anti-obesity effects in C57BL/6 mice, behenic acid diets demonstrated promising results, including less weight gain, reduced lipid accumulation in the liver, reduced impact on serum triglycerides and insulin resistance associated with control of inflammation. Furthermore, the absorption of essential fatty acids by the body was promoted. The diet demonstrated effectiveness in eliminating lipids in feces, highlighting that the interesterification process not only leads to technological improvements, but also to metabolism, without causing diarrhea or adverse reactions. The results have relevant implications, as nanoemulsions can be formulated for use as ingredients in functional foods, serving as a fat substitute and contributing to the development of foods that slow down the digestion of lipids.

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References

1. Tutor AW, Lavie CJ, Kachur S, et al. Updates on obesity and the obesity paradox in cardiovascular diseases. Prog Cardiovasc Dis. 2023; 78: 2-10.
2. BRASIL, Ministério da Saúde. O impacto da obesidade, 2022.
3. Khanna D, Khanna S, Khanna P, et al. Obesity: A Chronic Low-Grade Inflammation and Its Markers. Cureus. 2022; 14: e22711.
4. Maki KC, Dicklin MR, Kirkpatrick CF. Saturated fats and cardiovascular health: Current evidence and controversies. J Clin Lipidol. 2021; 15: 765-772.
5. Hughes NE, Marangoni AG, Wright AJ, et al. Potential food applications of edible oil organogels. Trends Food Sci Tech. 2009; 20: 470-480.
6. ZUIN JC, Gandra RLDP, Ribeiro APB, et al. Comparing chemical and enzymatic synthesis of rich behenic lipids products: technological and nutritional potential. Food Sci Technol. 2022; 42: e105821.
7. Wang YD, Zhang T, Liu RJ, et al. Reviews of medium and long-chain triglyceride with respect to nutritional benefits and digestion and absorption behavior. Food Res Int. 2022; 155: 111058.
8. Moreira DK, Santos PS, Gambero A, et al. Evaluation of structured lipids with behenic acid in the prevention of obesity. Food Res Int. 2017; 95: 52-58.
9. Da Silva RM, Zarricueta ML, Moreira DKT, et al. Structured Lipid Containing Behenic Acid Versus Orlistat for Weight Loss: An Experimental Study in Mice. Pharmanutrition. 2020; 14: 100213.
10. Dhiman N, Awasthi R, Sharma B, et al. Lipid Nanoparticles as Carriers for Bioactive Delivery. Front Chem. 2021; 9: 580118.
11. He X, Deng H, Hwang HM. The current application of nanotechnology in food and agriculture. J Food Drug Anal. 2019; 27: 1-21.
12. Moreira DKT, Gandra RLD, Zuin JC, et al. Synthesis and characterization of structured lipid rich in behenic acid by enzymatic interesterification. Food Bioprod Process. 2020; 122: 303-310.
13. Gandra RLD, Zuin JC, Moreira DKT, et al. Production and characterization of nanoemulsion with low-calorie structured lipids and its potential to modulate biomarkers associated with obesity and comorbidities. Food Res Int. 2021; 150: 110782.
14. Pinto LdeF, Compri CM, Fornari JV, et al. The immunosuppressant drug, thalidomide, improves hepatic alterations induced by a high-fat diet in mice. Liver Int. 2010; 30: 603-610.

15. Folch J, Lees M, Sloane Stanley GH. A simple method for the isolation and purification of total lipides from animal tissues. J Biol Chem. 1957; 226: 497-509.

16. Zhou H, Urso CJ, Jadeja V. Saturated Fatty Acids in Obesity-Associated Inflammation. J Inflamm Res. 2020; 13: 1-14.

17. Ji SY, Xu FR, Zhang N, et al. Dietary a novel structured lipid synthesized by soybean oil and coconut oil alter fatty acid metabolism in C57BL/6J mice. Food Biosci. 2021; 44: 101396.

18. Forouhi NG, Koulman A, Sharp S, et al. Differences in the prospective association between individual plasma phospholipid saturated fatty acids and incident type 2 diabetes: the EPIC-InterAct case-cohort study. Lancet Diabetes Endocrinol. 2014; 2: 810-818.

19. Ardisson Korat AV, Malik VS, Furtado JD, et al. Circulating Very-Long-Chain SFA Concentrations Are Inversely Associated with Incident Type 2 Diabetes in US Men and Women. J Nutr. 2020; 150: 340-349.

20. Lai KZH, Semnani-Azad Z, Boucher BA, et al. Association of Serum Very-Long-Chain Saturated Fatty Acids With Changes in Insulin Sensitivity and β-Cell Function: The Prospective Metabolism and Islet Cell Evaluation (PROMISE) Cohort. Diabetes. 2023; 72: 1664-1670.

21. Shibata E, Kanno T, Tsuchiya A, et al. Free fatty acids inhibit protein tyrosine phosphatase 1B and activate Akt. Cell Physiol Biochem. 2013; 32: 871-879.

22. Tamrakar AK, Maurya CK, Rai AK. PTP1B inhibitors for type 2 diabetes treatment: a patent review (2011 - 2014). Expert Opin Ther Pat. 2014; 24: 1101-1115.

23. Kanjilal S, Kaki SS, Rao BV, et al. Hypocholesterolemic effects of low calorie structured lipids on rats and rabbits fed on normal and atherogenic diet. Food Chem. 2013; 136: 259-265.

24. Kojima M, Tachibana N, Yamahira T, et al. Structured triacylglycerol containing behenic and oleic acids suppresses triacylglycerol absorption and prevents obesity in rats. Lipids Health Dis. 2010; 9: 77.

25. HodsonL, Skeaff C, Fielding BA. Fatty acid composition of adipose tissue and blood in humans and its use as a biomarker of dietary intake. Progress in lipid research. 2008; 47: 348-380.

26. Buzzetti E, Pinzani M, Tsochatzis EA. The multiple-hit pathogenesis of non-alcoholic fatty liver disease (NAFLD). Metabolism. 2016; 65: 1038-1048.

27. Monnard CR, Dulloo AG. Polyunsaturated fatty acids as modulators of fat mass and lean mass in human body composition regulation and cardiometabolic health. Obesity Reviews. 2021; 22: e13197.

28. Spite M, Claria J, Serhan CN. Resolvins, specialized proresolving lipid mediators, and their potential roles in metabolic diseases. Cell Metab. 2014; 19: 21-36.

29. Du YX, Chen SN, Zhu HL, et al. Consumption of Interesterified Medium- and Long-Chain Triacylglycerols Improves Lipid Metabolism and Reduces Inflammation in High-Fat Diet-Induced Obese Rats. J Agric Food Chem. 2020; 68: 8255-8262.

30. Li Y, Hu M, McClements DJ. Factors affecting lipase digestibility of emulsified lipids using an digestion model: Proposal for a standardised pH-stat method. Food Chemistry. 2011; 126: 498-505.

31. McClements DJ, Li Y. Structured emulsion-based delivery systems: Controlling the digestion and release of lipophilic food components. Adv Colloid Interfac. 2010; 159: 213-228.