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ORIGINAL ARTICLE

Postprandial effects on plasma lipids and satiety hormones from intake of liposomes made from fractionated oat oil: two randomized crossover studies

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Abstract

Background: The composition and surface structure of dietary lipids influence their intestinal degradation. Intake of liposomes made of fractionated oat oil (LOO) is suggested to affect the digestion process and postprandial lipemia and also induce satiety.
Objective: In the present study, the metabolic effects on plasma lipids and gut hormones related to satiety were investigated in healthy individuals after intake of LOO, with dairy lipids as placebo.
Design: Two blinded randomized studies with crossover design were performed. In the first study, 19 subjects consumed 35 g lipids from LOO or yoghurt in a breakfast meal. In a follow-up study, 15 women consumed 14 or 1.8 g lipids from LOO mixed in yoghurt. Blood samples were analyzed for plasma lipids, insulin, glucose, and intestinal hormones CCK, PYY, GLP-1, and GLP-2 before and four times after the meal. Subjective analysis of satiety was measured using a visual analog scale questionnaire. Participants recorded their food intake during the rest of the day.
Results: Intake of 35 and 14 g lipids from LOO significantly increased plasma concentrations of CCK, GLP-1, GLP-2, and PYY postprandially. This coincided with a prolonged elevation of triglycerides and large cholesterol-containing particles. Non-esterified fatty acids decreased after intake of 14 and 1.8 g lipids from LOO. The subjective sensation of satiety in women was increased 7 h after intake of 35 g lipids from LOO without any difference in food intake. Our results indicate that intake of 14 g lipids from LOO at breakfast substantially reduced energy intake during the rest of the day.
Conclusions: This study suggests that intake of LOO prolong lipid digestion, affect postprandial plasma lipids and have an effect on satiety. The effect of LOO on GLP-2 indicates that intake of LOO also improve gut health.

Keywords: *polar lipids*; *liposomes*; *postprandial lipemia*; *intestinal hormones*; *satiety*; *gut health*

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Food structure and composition influence the digestion rate and nutrient uptake. The presence of fat in the distal small intestine stimulates release of gastric hormones, slows down gastric emptying and pancreatic secretion, and induces satiety (1–3). Dietary fat is normally emulsified by the action of bile salts and polar lipids, and the core lipids of micelles and emulsion particles are accessible to the battery of pancreatic lipolytic enzymes. Polar lipids in general are very surface-active compounds (4).

Changes in the surface structure of dietary lipids, caused by changes in the composition of the polar lipids around the core lipids, may affect the rate by which the core lipids are being hydrolyzed (5). Consequently, hormone-induced satiety and postprandial plasma lipid output can be affected by modification of the polar lipids around the core lipids.

The gut hormones cholecystokinin (CCK), glucagon-like peptide 1 (GLP-1), and peptide YY (PYY) are released after a meal and influence the appetite center in hypothalamus. CCK induces satiety in a dose-dependent manner (6). GLP-1 is released as response to intake of macronutrients and reduces gastric emptying (7). Dietary fat was reported to be the most powerful stimulant of PYY secretion when investigating single nutrients (8). PYY is also a key mediator of ileal brake (9).

The glucagon-like peptide 2 (GLP-2) is not a satiety hormone, but it is co-expressed with GLP-1, and acts locally as a growth factor for the intestinal cells. Factors stimulating the increase in postprandial GLP-2 are not fully identified, but undigested or partly digested lipids in the terminal ileum are suggested factors (10, 11).

Polar lipids, comprising glycerophospholipids, sphingolipids, and in plants also galactolipids, are major lipid constituents in cell membranes. They represent a minor component in our diet with an average intake of 3–5 g/day (12).

Oats contain more lipids than other cereals, and extracted oat oil has a high concentration of polar lipids, about 15 wt%. The main component of polar lipids in oats is the galactolipid digalactosyldiacylglycerol (DGDG), and 50% of the DGDG in oats contain a hydroxyl fatty acid, 15(R)-hydroxy linoleic acid, mainly in *sn*-2 position on the glycerol. The hydroxyl group is completely esterified with another fatty acid. This acyl-digalactosyl diacylglycerol is a natural estolide, which is unique for oats (13–15).

Galactolipids are mainly hydrolyzed by the pancreatic lipase related protein 2 (PLRP2), an enzyme from the pancreatic lipase family which has been shown to be a galactolipase (16, 17). When surface lipids contain not only phospholipids but also galactolipids, the lipolysis of the core triglycerides (TGs) can be affected (4). However, the digestion of oat estolides is unknown.

We propose that after ingestion of galactolipid-rich oils dispersed into very small liposomes, the amount of lipids in distal parts of the small intestine will increase. The mechanism for this may be that the very small liposomes may cause hindrance of the lipolytic enzymes to hydrolyze the lipids; that is, a film rich in galactolipids will cover the active sites of the lipases which may hamper the rate of lipid hydrolysis. Another suggested mechanism is that particles with a galactolipid-rich surface will be hydrolyzed at a slower rate and leave partly undigested and unabsorbed lipid products in distal part of the intestine. An increasing amount of lipids in the ileum will thereby stimulate hormonal signals of satiety and affect postprandial lipemia at a relative low energy intake.

In order to investigate the hypothesis that galactolipid-rich small particles will affect gastrointestinal satiety hormones and postprandial lipemia, we have developed processes to produce very small, stable, and uniform liposomes from fractionated oat oil (LOO) (18, 19). Different concentrations of LOO were given to healthy volunteers in a breakfast meal and we investigated: plasma levels of gastrointestinal hormones, metabolic parameters, perceived satiety, and total energy intake during the experimental day. We used milk fat as dairy yoghurt as control for two reasons: because it represents a commonly used food item in a Nordic breakfast, and because in the few numbers of studies similar to this one (20, 21), dairy products were used as control.

Methods

Production of LOO

The oat oil fraction used in these experiments was high in polar lipids and obtained by controlling the concentration of ethanol, water, and sugar in different fractionation steps after ethanol extraction of oats. The lipid class composition of the fractionated oat oil was determined by HPLC (22). The main lipid classes are presented in Table 1.

Very small, stable, and uniform liposomes were formed almost spontaneously during dilution of the fractionated oat oil in a carefully controlled composition of ethanol–water mixture. The liposomes formed were diluted further in water and finally the ethanol was evaporated (19). The final liposome preparation contained 10 wt% lipids and 90 wt% water. The volumetric average diameter of the liposomes was 100 nm, measured by dynamic light scattering (Zetasizer Nanoseries, Malvern Instruments).

The stability of the liposomes was investigated by dilution 1:1 in: water; citric acid buffers pH 4, pH 3; citric acid 1 M. It was not possible to detect any changes in structure after 4 months in water. The stability decreased at decreasing pH. Using 1 M citric acid, it lasted 4 days before any changes could be seen. Thus, it is very reasonable that these liposomes were sufficiently stable to survive the low pH in the stomach and that they could enter the duodenum intact.

Human studies

In the first part, 19 healthy men and women (10 women, 9 men) of mean age 42, BMI 25, were recruited through announcements on the local intranet and advertisements. On two occasions 10–14 days apart, they were randomly assigned either a test- or placebo breakfast. In the second part of the study, we enrolled 15 women of mean age 34 and BMI 24. On three occasions, the 15 women received two doses of LOO in test breakfast or yoghurt in the placebo breakfast.

Table 1. Lipid class composition of LOO and milk fat in placebo yoghurt

	LOO[a] wt% of lipids	Milk fat[b] wt% of lipids
Triglycerides	40.9	95.8
Galactolipids	31.4	0.0
Phospholipids	20.0	1.1
Sterols	6.6	0.5
Others	1.3	2.6

[a]Measured by HPLC.
[b]From literature (23).

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Exclusion criteria were abnormal blood glucose levels in the fasting state, and hyperlipidaemia in addition to signs of liver or kidney disease or inflammatory activity as indicated by raised C-reactive protein, liver tests (bilirubin, alkaline phosphatase, gamma-glutamyl transpeptidase, and aminotransferases), and creatinine levels. All participants gave their written informed consent prior to the study. The studies were approved by the Regional Human Ethics Committee of Lund-Malmö, Sweden (Dnr 2010/18, 2011/55).

Participants conducted food registrations in the form of diaries over 4 days before the experiments and also of the remainder of the day of each trial. The intake was analyzed by the use of Dietist XP software with the Swedish National Food Administration database (2008 03 06) (Kost och Näringsdata, Sweden). Dietary intakes were related to recommended Swedish dietary guidelines (24).

Trial setup and analysis

The breakfast meals were composed of one slice of bread (37 g), one slice of smoked ham with 3% fat (14 g), blackberry jam (40 g), coffee or tea without milk or sugar and free access to water. The blackberry jam was mixed into the placebo and test meal before serving in order to mask any difference in taste and appearance. Both test- and placebo yoghurt contained 35 g of lipids at a concentration of 10 wt%. The placebo yoghurt was plain non-flavored dairy yoghurt with 10% fat. The test yoghurt contained three different amounts of LOO containing 35 g lipids in the first study, and 14 and 1.8 g lipids in the follow-up study. The remaining lipids up to 35 g were from milk; the total fat concentration was 10%. The fatty acid composition of the two lipid sources is presented in Table 2 and the lipid classes are presented in Table 1. A fat-free milk powder was added to the LOO in order to balance intake of protein, calcium, and carbohydrate in the meal (Table 3).

Table 2. Fatty acid composition of LOO and milk fat in placebo yoghurt

	LOO[a] wt% of FA	Milk fat[b] wt% of FA
C 4:0–12:0	0.0	15.9
C 14:0	0.2	11.3
C 16:0	15.0	30.3
C 18:0	1.1	11.5
C 18:1	37.4	21.6
C 18:2	31.1	1.5
C 18:3	1.1	0.6
C 18:2 15-OH	12.4	0.0
Others	1.8	7.3

[a]Derived from our lipid class analysis by HPLC in combination with FA analysis from literature (25).
[b]From literature (23).

Table 3. The composition of the whole meals from the first part of the study including either dairy yoghurt or LOO with milk powder

Total meal	Placebo/yoghurt	LOO and milk powder
Weight (g)	521	604
Energy (KJ)	2,892	3,056
Energy (Kcal)	690	730
Protein (g)	19.3	18.9
Carbohydrates (g)	65.4	65.7
Total fat (g)	38.7	43.4
Saturated fat (g)	24	9.1
Monounsaturated fat (g)	8.6	13.6
Polyunsaturated fat (g)	0.7	17.9
Cholesterol (mg)	115.1	9.8
Plant sterols (mg)	0	900
Fiber (g)	3.4	4.6
Calcium (mg)	407	443

Participants arrived at 7.00 am. Blood samples from the fore arm vein were taken before breakfast, when the subjects had been fasting since 9.00 pm the night before. The succeeding blood samples were taken 1, 3, 5, and 7 h after breakfast. All blood samples were immediately centrifuged and plasma was kept at −70°C until analysis. The participants were not allowed to eat anything for the following 5 h after breakfast. After blood sampling at 5 h after breakfast, the participants were served *ad libitum* a selection of pre-weighed food items (bread, egg, tomato, red bell pepper, low-fat spread cheese, smoked ham, and a banana) and the amounts were registered. The final blood sample was taken 7 h after breakfast. Finally, the participants registered what they ate for the rest of that day.

The blood samples were analyzed for triglycerides, cholesterol, blood glucose, insulin, HDL and LDL cholesterol, liver tests, C-reactive protein, hemoglobin, white blood cell count, and platelet count. All analyses were done at the Department of Clinical Chemistry, University Hospital of Lund, Sweden using the accredited routine methods developed by Roche Diagnostics (MSDS – COBAS Integra® – General Chemistry). Two milliliter EDTA-plasma with the addition of a protease inhibitor, aprotinin (Trasylol®), were immediately frozen at −70°C for later analyses of non-esterified fatty acids (NEFAs) (NEFA-c kit, Wako Chemicals, USA) the gut hormones PYY, GLP-1, GLP-2, and CCK. Total PYY (3–36 and 1–36), total GLP-1 (7–36 and 9–36) and GLP-2 were analyzed by ELISA from Merck Millipore, Solna Sweden. CCK was measured by a RIA, which is entirely specific for the bioactive CCKs in plasma (26, 27).

Subjective measurements of satiety

Participants were asked to subjectively rate their feeling of fullness (satiety), hunger, and desire to eat on a 10-cm visual analog scale (VAS) at each time point of blood

sampling. For example, the level of satiety was expressed as a percentage on a 10-cm scale where 100% means totally satiety and 0 means no sensation of hunger at all.

They were also asked to report any gastrointestinal sensation/side effect and notable difference between each meal regarding satiety and hunger.

Statistics

Plasma values were normalized according to an averaged blank between placebo and test meal(s). Significance of differences, between the results obtained with the LOO and placebo yoghurt, was examined using Student's paired *t*-test or paired ANOVA multiple measures. Bonferroni post hoc test was carried out when significant differences between diets were found. The calculation of incremental area under curve (iAUC) was done using the trapezoid rule on the period 0–5 h. The Student's paired *t*-test was used to calculate if there were any difference between the LOO and placebo after specific time points. Linear regression analysis was performed on the correlation between caloric intake and plasma PYY concentrations. All statistical analyses and calculations of iAUC were performed with GraphPad Prism (version 5.00. GraphPad software, San Diego, CA, USA).

Results

Study with high intake of LOO

In the first study, 19 participants ingested 410 ml LOO containing 35 g lipids or placebo meal with an equal amount of dairy lipids (Tables 2 and 3). There were both men and women in both groups.

Analysis of blood samples

The $iAUC_{0-5\,h}$ for plasma TG was not significantly different after the LOO compared to placebo; however, after 7 h the TG response was significantly higher with the LOO meal (Fig. 1) ($n = 19$, $p = 0.016$).

The $iAUC_{0-5\ h}$ for plasma glucose was significantly decreased ($n = 19$, $p = 0.03$) after intake of LOO compared to placebo (Fig. 2), with a significant lower concentration of plasma glucose at all postprandial time points ($n = 19$, $p = 0.01$, 0.0004, 0.01, and 0.0027).

The concentration of NEFAs in plasma was slightly lower after LOO compared to after placebo at 1, 3, and 5 h (trends $p < 0.1$). The plasma concentrations of total-, HDL-, and LDL cholesterol did not change postprandial. The minor fluctuations of the plasma concentrations of cholesterol seen after placebo were not significantly different from concentrations after LOO. We calculated the concentration of large cholesterol-containing particles (LC particles) by subtracting HDL and LDL cholesterol from total cholesterol. We found that the concentration of LC particles was more evenly distributed over time after LOO and did not display any peak

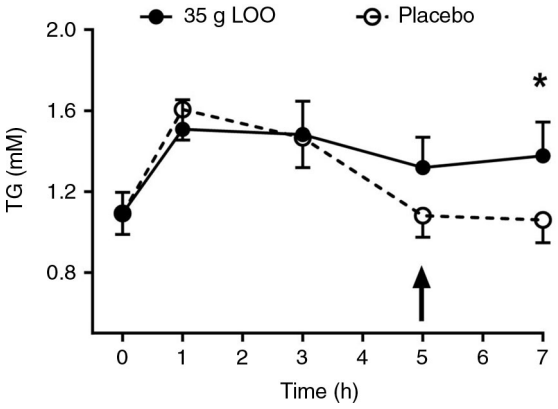
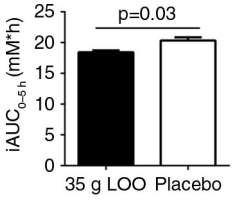


Fig. 1. The postprandial plasma concentrations of triglycerides after intake of LOO and placebo. The triglyceride concentrations after intake of breakfast with LOO containing 35 g lipids (●) or after a placebo yoghurt (○). The arrow at 5 h indicates intake of lunch. Data are presented as mM TG and are mean ± SEM, $n = 19$. *Values after 7 h were significantly different ($p < 0.05$).

concentration at 3 h as after placebo yoghurt (Fig. 3). This difference in pattern reflects a prolonged output of TG-rich lipoproteins and their remnants after a breakfast with LOO compared to the placebo yoghurt.

The concentrations of the hormones PYY, GLP-1, and CCK after intake of LOO with 35 g lipids were significantly



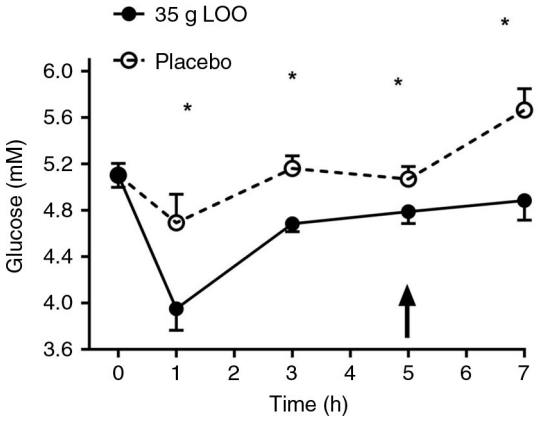


Fig. 2. The postprandial plasma concentration of glucose after intake of LOO and placebo. Plasma glucose after intake of a breakfast containing LOO with 35 g lipids (●) or after a placebo yoghurt (○). The difference in $iAUC_{0-5h}$ was significantly lower ($p = 0.03$) with LOO. The arrow at 5 h indicates intake of lunch. Data are presented as mM glucose and mean ± SEM, $n = 19$. *Values at all postprandial time points were significantly different ($p < 0.05$).

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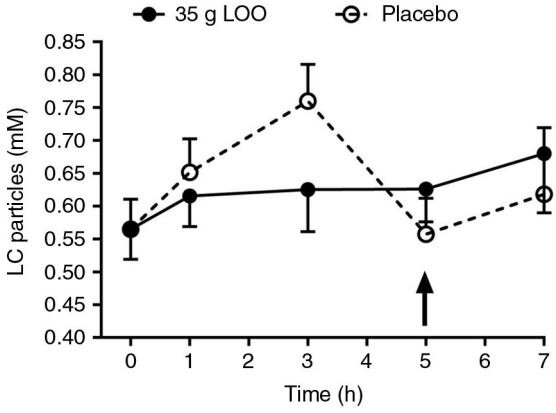

Fig. 3. The postprandial plasma concentration of large cholesterol-containing particles (LC) after intake of LOO and placebo. Plasma concentration of LC particles after intake of a breakfast containing LOO with 35 g lipids (●) or after a placebo yoghurt (○). LC particles are the values for total cholesterol with HDL and LDL subtracted. The arrow at 5 h indicates intake of lunch. Data are presented as mM cholesterol and mean ± SEM, n = 19.

increased compared to placebo, especially at the later time points 3, 5, and 7 h (Fig. 4a–c). The $iAUC_{0-5\ h}$ for GLP-1 and CCK was significantly increased after LOO compared to placebo ($p = 0.028$ and 0.048 respectively). CCK was significantly increased after 3 h with LOO ($p = 0.019$) and GLP-1 was significantly increased after 5 h ($p = 0.021$). PYY, GLP-1, and CCK were significantly increased with LOO after 7 h compared to placebo ($p = 0.004$, 0.014, and 0.012, respectively).

The levels of PYY, GLP-1, and CCK were significantly higher after 5 h with LOO (Figs. 4 and 7). It is reasonable that high levels of satiety hormones before lunch will lead to reduced energy intake during the rest of the day. Therefore, we investigated whether there was a correlation between the concentration of the satiety hormones just before lunch and the total energy intake on the experimental day. In Fig. 5, the results for PYY just before lunch ($PYY_{5\,h}$) are plotted against the total energy intake during the experimental day for all the participants with completed data (both 35 g LOO and placebo). By using these data, we derived an equation (Eq. 1). There was a negative trend between $PYY_{5\ h}$ and total energy during the rest of the day (Spearman r -0,247, CI-0.5255 to 0.08024, p = 0.0986). When we use Eq. 1 on the PYY results from the second study in this paper (14 g LOO vs. placebo), it indicates that an intake of LOO containing 14 g lipids at breakfast will reduce the total energy intake for the whole day with 7% compared to intake of the same amount of lipids from yoghurt at breakfast. GLP-1 showed a similar trend as PYY (data not shown).

$$\text{Energy Intake}_{\text{rest of day}} = 2036 - 17.72^{*}\text{PYY}_{5\text{th}} \quad (1)$$

On the VAS scale formula, women reported significantly higher satiety 7 h after intake of LOO compared to placebo, 70% satiety after LOO vs. 58% after placebo ($n = 10$, $p = 0.0056$). However, for men ($n = 9$) we did not find any significant differences between the meals (data not shown).

Study with lower intake of LOO

The follow-up study on 15 women investigated the effects from intake of two lower concentrations of LOO containing 14 and 1.8 g lipids. The meals were compensated with fat-free milk powder to balance the intake of protein, calcium and carbohydrate and plain non-flavored yoghurt to balance the total amount of fat to 35 g. The composition of the meals and study design were otherwise identical to the first study.

Analysis of blood samples

The iAUC for TG was not different after intake of LOO containing 14 and 1.8 g lipids compared to placebo and there were no postprandial differences in plasma glucose or insulin levels (data not shown). The $iAUC_{0-5\ h}$ for NEFAs was significantly lower after 14 g lipids from LOO compared to placebo ($p = 0.024$) (Fig. 6) and after 3 h the NEFA concentration was significantly lower with 14 g lipids from LOO compared to placebo ($p = 0.015$). There were no differences in plasma total, HDL or LDL cholesterol levels after the different LOO intake and placebo yoghurt (data not shown).

The $iAUC_{0-5\ h}$ for PYY and CCK were significantly higher with LOO containing 14 g lipids compared to 1.8 g and placebo (PYY $p = 0.008$ for 14 g vs. 1.8 g and $p = 0.009$ for 14 g vs. placebo, for CCK $p = 0.007$ for 14 g vs. 1.8 g and $p = 0.002$ for 14 g vs. placebo), whereas no significant difference in the $iAUC_{0-5\ h}$ for GLP-1 was observed (Fig. 7a–c). Both PYY and CCK were significantly increased with LOO containing 14 g lipids compared to placebo or to LOO containing 1.8 g lipids (PYY $p = 0.009$ and $p = 0.008$ respectively and CCK $p = 0.001$ and $p < 0.0001$, respectively). GLP-1 was higher ($p < 0.1$) or significantly higher after 1 and 5 h with 14 g of LOO compared to placebo (1 h $p = 0.043$, 5 h $p = 0.064$) and there was a trend towards a difference between LOO with 1.8 g lipids and placebo at 3 h ($p = 0.09$).

GLP-2 was only analyzed after a breakfast containing LOO with 14 g lipids and placebo yoghurt. The $iAUC_{0-5\ h}$ as well as the concentration of GLP-2 at all postprandial time points was significantly higher after LOO compared to placebo ($iAUC_{0-5\ h}$ $p = 0.002$, 1 h $p = 0.0001$, 3 h $p = 0.04$, 5 h $p = 0.017$, and 7 h $p = 0.029$) (Fig. 8).

Records of intake and side effects in both studies

The participants registered their food intake during the *ad libitum* served lunch and reminder of that day. Energy intake was 14% lower for women the rest of the day after breakfast with LOO containing 35 g lipids compared to placebo, but this difference was not statistically significant. The average energy intake at lunch was similar

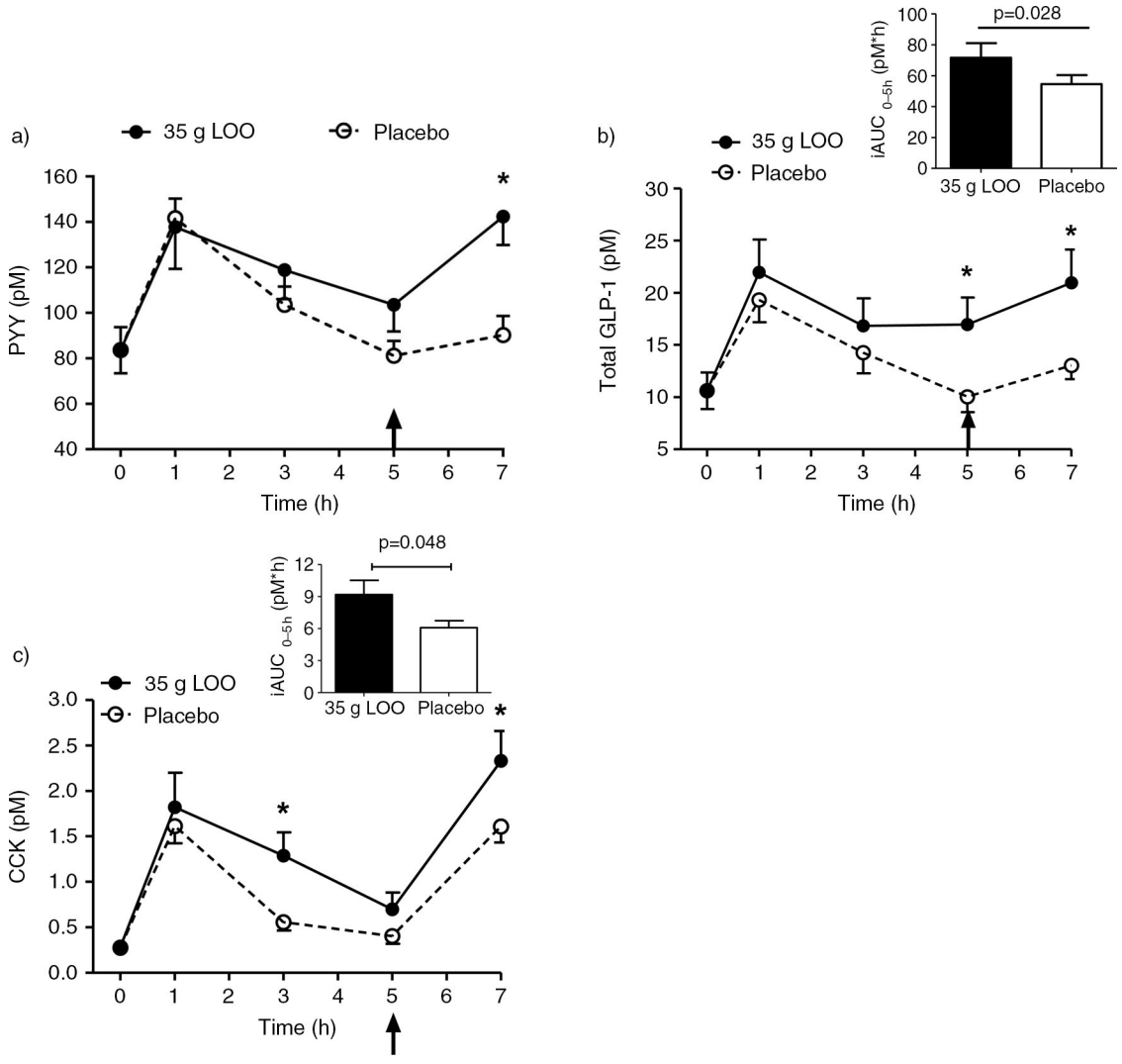

Fig. 4. The postprandial plasma concentration of PYY, GLP-1, and CCK after intake of LOO and placebo. Plasma concentrations of gastrointestinal hormones after intake of breakfast containing LOO with 35 g lipids (●) or after a placebo yoghurt (○). The arrow at 5 h indicates intake of lunch. Data are presented as pM and mean ± SEM, n = 12–19. (a) Shows PYY and there was a significant difference between LOO and placebo 7 h postprandially ($p = 0.004$). (b) Shows total GLP-1 and there was a significant difference between LOO and placebo 5 h postprandially ($p = 0.028$). (c) Shows CCK and there were significant differences between LOO and placebo after 3 and 5 h postprandially ($p = 0.02$ and $p = 0.012$, respectively). *Values were significantly different ($p < 0.05$).

around 424 kcal (441 after LOO containing 35 g lipids and 407 kcal after placebo) (range 275–725 in both groups) and not significantly different. The rest of the day was also not different between the three different meals and placebo in the follow-up study (data not shown).

The highest amount of LOO was not very palatable despite the addition of jam. However, LOO containing both 14 and 1.8 g of lipids could easily be mixed with milk powder and yoghurt and flavored; these meals were therefore not very different from placebo.

A few subjects reported an uncomfortable feeling in their gut the first hours after intake of breakfast. However, no difference between any of the test products and placebo could be distinguished.

Discussion

The present work aimed to answer the question whether intake of liposomes made of fractionated oat oil (LOO) could affect metabolic parameters related to satiety and postprandial plasma lipids in healthy non-obese subjects. The LOO was given in a breakfast meal after an overnight fast and compared to milk fat in dairy yoghurt.

The main finding was that intake of LOO significantly increased the appetite regulating hormones PYY, GLP-1, and CCK as well as the intestinal growth promoting hormone GLP-2, when the dose of LOO contained 14 and 35 g of lipids.

The proportions of TG, polar lipids, and sterols, as well as the proportion of saturated and unsaturated fatty acids

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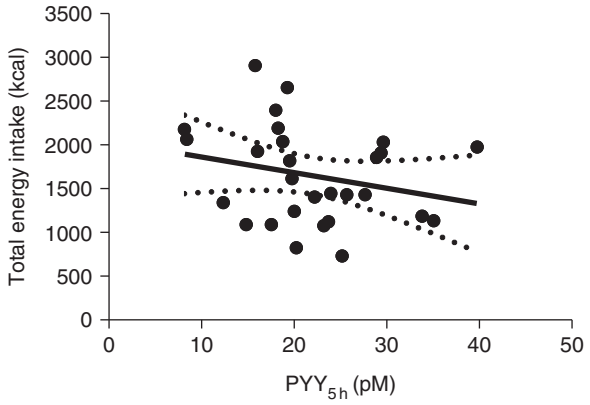

Fig. 5. The correlation between the total energy intake during the trial day vs. the plasma PYY concentration at 5 h after breakfast. Data are plotted as kcal vs. pM PYY. They represent subjects from both breakfast containing LOO with 35 g lipids and breakfast with placebo yoghurt (n = 28, 95% confidence intervals are shown as dotted lines, r2 = 0.059, p = 0.21 and the regression equation is Y = 2036 – 17.72*X).

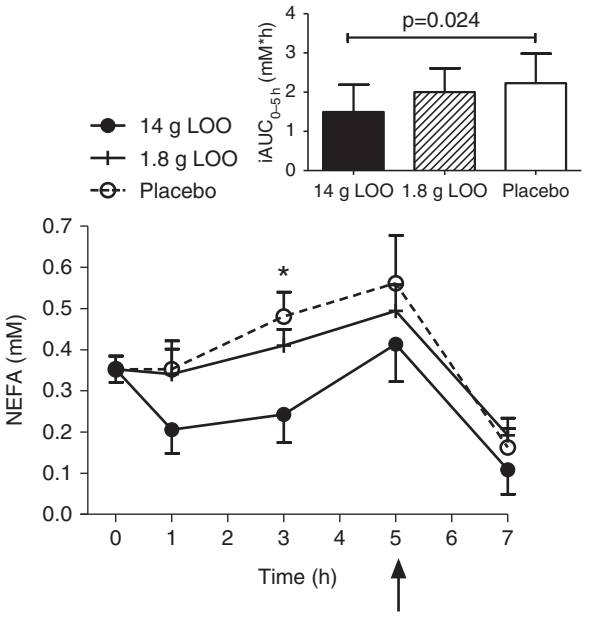

Fig. 6. The postprandial plasma concentration of non-esterified fatty acids (NEFAs) after intake of two doses of LOO and placebo. Plasma NEFA concentrations after intake of breakfast containing LOO with 14 g lipids (●), LOO with 1.8 g lipids (+), or after a placebo yoghurt (○). The arrow at 5 h indicates intake of lunch. Data are presented as mM free fatty acids and mean ± SEM, n = 15. The $iAUC_{0–5h}$ was significantly lower after 14 g lipids from LOO compared to placebo (p = 0.024). After 3 h, the NEFA concentration was significantly lower with 14 g lipids from LOO compared to placebo (p = 0.015). *Values after 3 h were significantly different (p < 0.05).

in the meals were most different with the highest dose of LOO, and we can see how this reflects the postprandial plasma lipids accordingly. With the highest dose of LOO, we found prolonged elevated postprandial concentrations of plasma TG accompanied with decreased plasma glucose concentration. The peak concentration of TG is significantly lower than with dairy yoghurt, which reflects both the lower concentration of TG in LOO and also the lower concentration of saturated fatty acids (Tables 1 and 2).

The concentration of NEFAs was lower after LOO than with milk fat. This can be indications of medium- and short chain fatty acids from the milk migrating as free fatty acids and also a smaller pool of TG-rich lipoproteins from LOO available for hydrolysis by lipoprotein lipase, but also indicate an effect of a hampered lipid digestion. A lowering of NEFAs is an important and desirable effect since elevated p-NEFA concentrations can increase the production of VLDLs and subsequently raise LDL-C levels (28).

The prolonged TG elevation after intake of 35 g LOO was accompanied with an increase in large cholesterol-containing particles without any peak and a delayed return to baseline. The nature of these cholesterol particles was not investigated, but remnant particles from chylomicron and VLDL are expected to dominate at the later time intervals (29).

In general we saw very little influence of LOO and placebo yoghurt on the plasma cholesterol levels. The acute intake of a meal containing 35 g fat or less has a low impact on postprandial cholesterol-rich particles, which has been shown in similar postprandial experiments (30). However, surface material from LOO and milk fat globule membrane might influence the formation of lipoprotein particles both in number and size and composition differently and thereby their degradation and uptake. In this case, the polar lipids from milk fat seem more accessible for the degrading enzymes and for the adsorbing surfaces than the polar lipids from LOO.

In healthy humans, the main glucose lowering effect following a meal is the inhibition of gastric emptying and increased insulin secretion both mediated by GLP-1. In the first study, intake of LOO with 35 g lipids might indicate a slower gastric emptying due to the hampered but prolonged lipemia. However, neither the postprandial glucose peak nor insulin appearing within the first hour was investigated.

The prolonged lipemia is in line with a significant increase of the intestinal hormones PYY, CCK, and GLP-1 and sensation of fullness at later time points.

We hypothesized to find a reversed correlation between postprandial gut hormone levels and total energy intake during the experimental day. The levels of PYY, GLP-1, and CCK were surprisingly high 5 h after consumption of LOO containing more than 14 g lipids (Figs. 4 and 7). The trend we found between PYY concentration and total energy intake during the rest of the day, Fig 5 and Eq. 1, indicates a reduction of energy intake during the

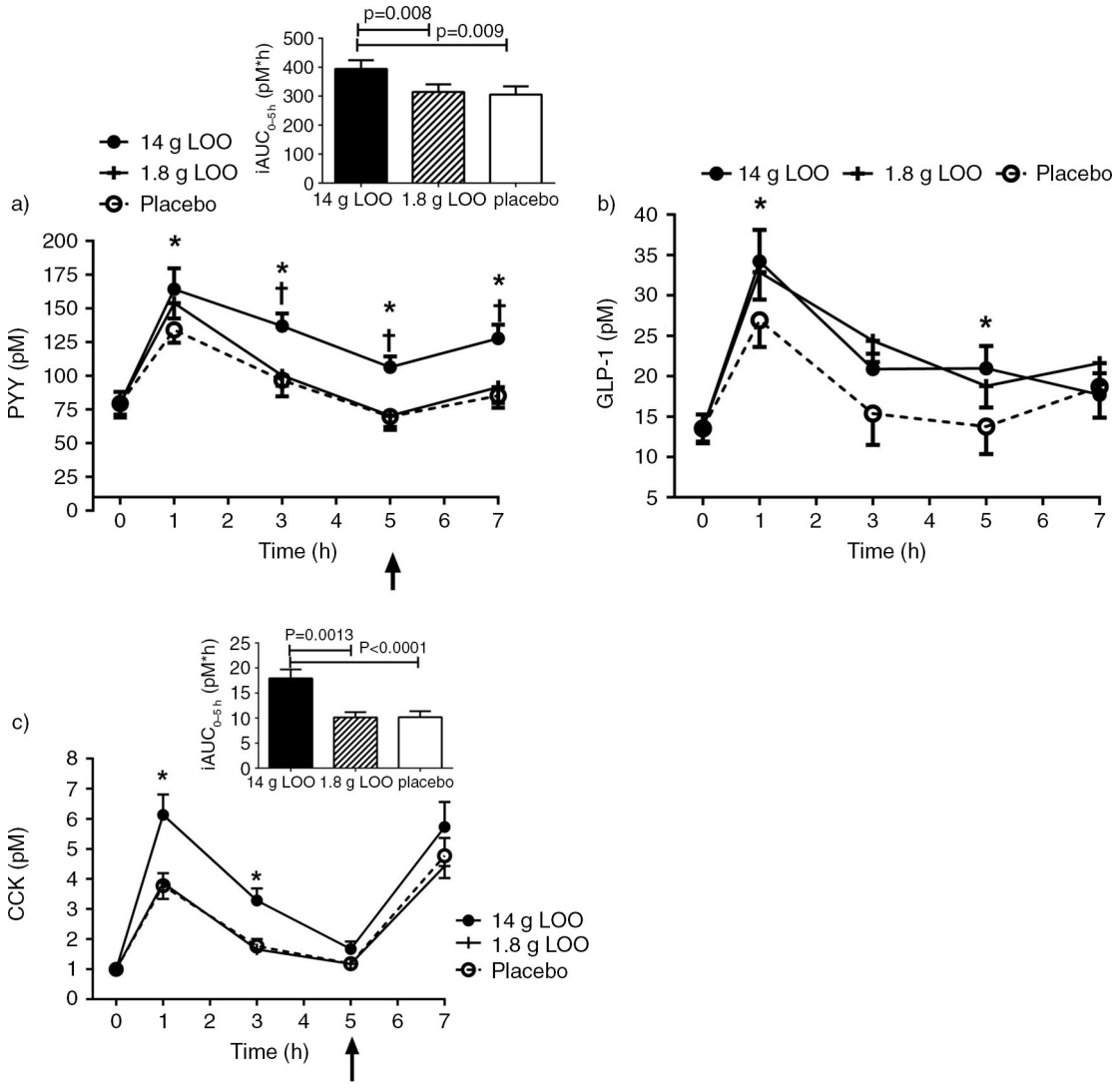

Fig. 7. The postprandial plasma concentration of PYY, GLP-1, and CCK after intake of two doses of LOO and placebo. Plasma concentrations of gastrointestinal hormones after intake of a breakfast containing LOO with 14 g lipids (●), LOO with 1.8 g lipids (+), or after a placebo yoghurt (○). The arrow at 5 h indicates intake of lunch. (a) Shows PYY and there were significant different iAUCs between LOO with 14 g lipids and placebo ($p = 0.009$) and between LOO with 14 g and 1.8 g lipids ($p = 0.008$). (b) Shows total GLP-1 and the difference between LOO with14 g lipids and placebo was significant at 1 and 5 h. ($p < 0.05$) and there was a trend towards a difference between LOO with 1.8 g lipids and placebo at 3 h ($p = 0.09$). (c) Shows CCK and there were significant differences in $iAUC_{0-5h}$ between LOO with 14 g lipids and placebo ($p < 0.0001$), and between LOO with 14 and 1.8 g lipids ($p = 0.0013$). Data are presented as pM and mean ± SEM, n = 12–15. *Values were significantly different ($p < 0.05$) between 14 g and placebo; †values were significantly different between 14 and 1.8 g LOO ($p < 0.05$).

rest of the day after a breakfast with LOO. Therefore we believe that we confirm other studies that show that higher plasma concentrations of PYY between meals are indicative of a reduction in energy intake in following meals (31, 32). However, more investigations are required to find out if lower doses of LOO can achieve similar effects and if these effects remain after a long period of time.

We found significant differences in satiety hormones (PYY, GLP-1, and CCK) but also for other parameters like glucose, NEFAs, and GLP-2 compared to control for the two highest LOO doses (35 and 14 g lipids). However, for the lowest dose of LOO (1.8 g lipids), we could not detect any significant differences from placebo in this study. The responses were rather similar for the two highest doses, indicating a stepwise response rather than a linear response. Therefore, it is of interest to perform further studies with doses of LOO in the interval between 1.8 and 14 g lipids.

Maintaining a normal body weight is getting more and more difficult around the world due to constant access to energy dense food and less physical activity.

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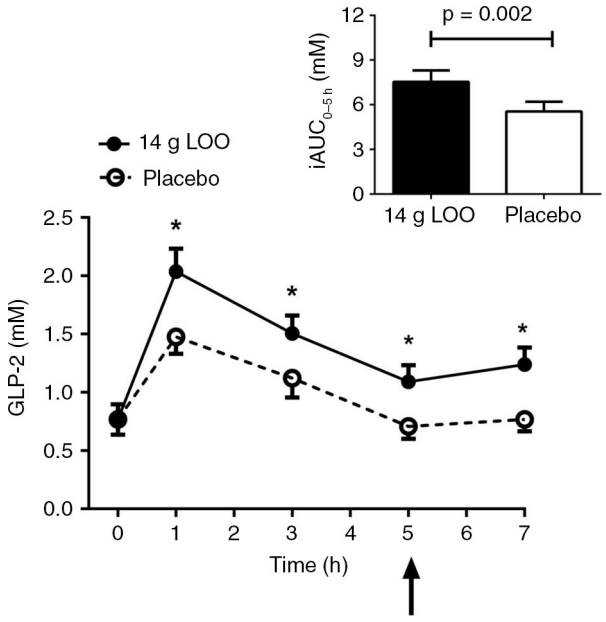

Fig. 8. The plasma concentration of GLP-2 after intake of LOO and placebo. Concentrations of GLP-2 after intake a breakfast containing LOO with 14 g lipids (●) or a placebo yoghurt (○). There was a significant difference in $iAUC_{0-5h}$ between LOO with 14 g lipids and placebo ($p = 0.002$). The arrow at 5 h indicates intake of lunch. Data are presented as mM and mean ± SEM, $n = 8$. *Values at all postprandial time points were significantly different ($p < 0.05$).

In order to reduce obesity and overweight and to regain health, energy intake must be cut down and regular physical activity must be performed. It is often difficult to treat obesity efficiently due to physiological and psychological aspects. The most efficient means available today are by performing surgical gastric bypass and guidance by professional nutritionists. Pharmacological drugs and intake of functional food can offer a significant additive effect to a weight loss regime.

There are several studies addressing the issue of increased and prolonged satiety as a means to better control food intake (33). To add food components like soluble fiber or protein to beverages or liquid meal to reduce consecutive food intake has had some success (34, 35). Also, recently concentrated thylakoid membranes from spinach leafs was suggested to promote appetite control and weight loss (36). Intake of different fatty acids PUFA may have effects on obesity but there is no consensus (37).

Earlier intervention studies with Fabuless/Olibra, an emulsion containing 40 wt% fractionated palm oil, 2.5 wt% fractionated oat oil and 57.5 wt% water, showed a significant dose-dependent decrease in energy intake 4 h and the following day after intake of Fabuless/Olibra in a dairy yoghurt (20, 38, 39). These early results have been regarded as inconclusive since positive findings regarding reduced energy intake could not be repeated (40, 41). The difficulties in obtaining conclusive results are believed to be derived from factors such as eating behavior, study design, and processing of the Fabuless-yoghurt mixture.

The lipids used in the present study do not contain palm oil, only fractionated oat oil. The concentration of polar lipids in LOO was considerably higher than in Fabuless (56 vs. 2.5 lipid %). A consequence of this is that the structure of the particles is different; the core of liposomes (LOO) is water, while the core of the particles in an emulsion (Fabuless) is oil (21, 42).

LOO can only be prepared when the concentration of polar lipids are above 50 lipid %. We have invented new procedures to prepare such liposomes (18, 19). The resulting LOOs are very stable and can pass the stomach without any substantial changes in the structure of the LOO caused by the low pH. The particle size of the used LOO was much smaller than milk fat globules, 100 vs. 1,000 nm. The positive postprandial effects are more obvious with LOO than with Fabuless, but the long-term effects from ingestion of LOO are not known.

We believe that due to the unique composition of the fractionated oat oil and the structure of the LOO used in this study, that LOO was digested differently than the dairy emulsion. The main factors that may influence the digestion of LOO compared to milk fat here is a physically changed surface structure affecting the hydrolysis of the lipids, or a different hydrolysis pattern of the galactolipids compared to the phospholipids by pancreatic lipases, and the influence of the size of the particles. The unique structure of the oat galactolipids, the estolides, may also contribute to the observed delayed digestion. The outcome of the delayed/hampered digestion of LOO is an increased exposure of the distal ileum to digested or partly digested LOO compared to milk fat.

Conclusions

We conclude that intake of LOO significantly affected postprandial TG, LC particles, glucose and the appetite regulating hormones CCK, PYY, GLP-1 as well as the sensation of satiety. Intake of LOO also significantly stimulated the growth promoting gut hormone GLP-2. These postprandial consentaneous results on healthy individuals argue for possible long-term effects from intake of LOO on satiety and food intake and possible also on gut health. A well-controlled long-term study aiming at satiety, weight control, and gut health on suitable subjects is therefore warranted.

Authors' contributions

LO designed the studies, analyzed the results and wrote the manuscript together with MH. MH formulated the research question and designed the liposomes. AR carried out the larger part of the study and analysis of

data together with LO. AR also contributed to the manuscript writing. JFR analyzed hormones and contributed to the manuscript writing.

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Conflict of interest and funding

LO, AR, and JFR declare that they have no competing interests. MH is one of the inventors to a patent about LOO and satiety. MH, LO, and AR have received reimbursement from Swedish Oat Fibre AB, the owner to a patent about LOO and satiety.

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