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Chloroplast membranes retard fat digestion and induce satiety

Effect of biological membranes on pancreatic lipase-colipase

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Running title: Membranes as lipase inhibitor and appetite suppressor

Key words: high-fat food intake, blood lipids, CCK, enterostatin, thylakoid, lipase, colipase, membrane proteins.

Abbreviations used: CCK, cholecystokinin, LHCII, light harvesting chlorophyll a/b protein complex II, MGDG and DGDG, monogalactosyldiacylglycerol and digalactosyldiacylglycerol respectively; NaTDC, sodium taurodeoxycholate.

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Synopsis

Human obesity is a global epidemic, which causes a rapidly increased frequency of diabetes and cardiovascular disease. One reason for obesity is the ready availability of refined food products with high caloric density, an evolutionarily new event, which makes over consumption of food inevitable.

Fat is a food product with high caloric density. The mechanism for regulation of fat intake has therefore been studied to a great extent. Such studies have shown that as long as fat stays in the intestine, satiety is promoted. This occurs through the fat released peptide hormones, the best known being cholecystokinin, CCK, which is released by fatty acids. Hence, retarded fat digestion with prolonged time for delivery of fatty acids promote satiety. Pancreatic lipase, together with its protein cofactor, colipase, is the main enzymatic system responsible for intestinal fat digestion. We found that biological membranes, isolated from plants, animals or bacteria, inhibit the lipase/colipase-catalysed hydrolysis of triglycerides even in the presence of bile salt. We propose that the inhibition is due to binding of lipase/colipase to the membranes and adsorption of the membranes to the aqueous/triglyceride interface, thereby hindering lipase/colipase from acting on its lipid substrate. We also found that chloroplast membranes (thylakoids), when added to refined food, suppressed food intake in rats, lowered blood lipids and raised the satiety hormones, CCK and enterostatin. Consequently, the mechanism for satiety seems to be retardation of fat digestion allowing the fat products to stay longer in the intestine.

INTRODUCTION

Human obesity is a global epidemic that increases the occurrence of type 2 diabetes and cardiovascular disease [1]. A likely reason for this obesity is the choice of refined food products with high caloric density, which makes over-consumption of food inevitable [2, 3].

Several hormones, neuropeptides and metabolites regulate feeding. There is also a macronutrient-specific appetite regulation, dietary protein the most satiating and dietary fat the least satiating nutrient [3]. The satiety effects have been explained by various factors released during intake of fat, carbohydrate and protein, respectively. Since dietary fat is the least satiating macronutrient, the mechanism for regulation of fat intake has been much studied. The studies have shown that fat-induced satiety is intestinally mediated, i.e. as long as fat stays in the intestine, satiety is promoted [4]. This occurs through the release of fat-released gut hormones, the best known being cholecystokinin, CCK. CCK is released to blood from the intestine by fatty acids formed during digestion. It is widely accepted that CCK causes satiety, both in rats and humans [5]. We therefore hypothesised that retarded fat digestion with prolonged time for delivery of fatty acids without causing steatorrhea would promote satiety.

The enzyme mainly responsible for intestinal fat digestion is pancreatic lipase and its obligatory cofactor, pancreatic colipase. They form a 1:1 molar complex, which is necessary for intestinal hydrolysis [6]. Here we report that isolated biological membranes inhibit the pancreatic lipase/colipase mediated hydrolysis of dietary fat. The inhibition is inherent in the hydrophobic, trans-membrane, alpha helices of the intrinsic membrane proteins. Moreover, we show that biological membranes, represented by chloroplast membranes, when added to refined food, suppresses food intake in rats, lowers blood lipids and increase the release of the satiety-inducing hormone, CCK.

EXPERIMENTAL

Preparation of membranes and membrane proteins.

Thylakoids for use in the lipase assay were isolated as described [7]. For preparing food, the thylakoids were isolated as follows: Leaves were homogenised in a blender and filtered through four layers of Monodur polyester mesh (20 μ m). The filtrate was centrifuged, the

rotor operating at 5000g for 10 min, to collect the thylakoids. These were washed by re-suspension in water and recentrifuged as above.

Removal of lipids: 4 ml thylakoid suspension (3.8 mg chlorophyll/ml) was mixed with 40 ml chloroform/methanol and incubated for 1 hr on ice. After centrifugation, the rotor operating at 4000g for 10 min, the pellet was extracted for a second time and centrifuged as above. The pellet was dried in air and extracted, on ice, with 10 ml of the buffer solution used for thylakoid isolation to remove water-soluble proteins. The mixture was centrifuged, the rotor operating at 4000g for 10 min, and the pellet collected. The pellet is named “membrane protein fraction” (Fig.1C).

Trypsin treatment was carried out by incubating the thylakoids with 300 µg trypsin (Sigma type III) /mg chlorophyll, in 20 mM phosphate buffer (pH 7.4), for 45 min at 37°C. After adding 1mM phenylmethylsulphonyl fluoride (PMSF), to inhibit the trypsin, the thylakoids were collected by centrifugation for 10 min at 900g.

Chlorophyll was determined as described [8] and protein according to Bradford [9]. *Mitochondria* from potato tubers [10] was a gift from Per Gardeström, Umeå University; Chicken heart mitochondria were prepared according to Tang et al [11] with some modifications: Hearts were trimmed of fat, washed and cut into small pieces, on ice, with a pair of scissors and further by a mixer in a medium of 300 mM sucrose, 50 mM phosphate buffer, pH 7.4, 1 mM NaCl, 5 mM Mg Cl₂, then homogenised with a Potter homogeniser for 1 min and centrifuged; the rotor operating for 10 min at 900g. The supernate was centrifuged once more at the same time and speed. Then, the supernate was centrifuged, the rotor operating for 15 min at 10000g. The pellet was washed 2 times at the same speed and time in the same medium. The pellet was collected.

Plasma membranes from spinach leaves [12] was a gift from Christer Larsson, Lund University; and membranes from *Synechosystis* [13] a gift from Birgitta Norling, Stockholm University. Intracytoplasmic membrane fragments, chromatophores, from *Rhodospirillum rubrum* [14] was a gift from Agneta Norén, Stockholm University; before use, extrinsic water soluble proteins were removed by washing with 0.5 M NaCl, 25 mM Tris-HCl, pH 7.8 followed by two washings with the Tris buffer only, as described [14].

Membrane proteins: Light harvesting complex II (LHCII) was prepared as described [15]. Transhydrogenase [16] from E.coli was a gift from Jan Rydström, Gothenburg University and cytochrome b₆f from spinach leaves [17] was a gift from Jörgen Ström, Lund University.

The synthetic polypeptide VIHCRWAMLGALGCVFPELL was obtained from Innovagen AB, Lund.

Binding studies

15 ml of lipase or lipase/colipase, at the same concentrations and in the same buffer as used in the lipase assay (see below) were mixed with increasing amounts of thylakoids at room temperature for 1 minute. The thylakoids were pelleted by centrifugation, the rotor operating at 6800g for 10 minutes in the experiments without NaTDC and 8600g for 10 minutes when NaTDC was present. The clear chlorophyll free supernatants were collected; triglyceride (0.5 mL) was added and the lipase activity assayed with the pH stat (see below).

Partition of thylakoids in aqueous/oil two-phase systems

Thylakoids containing 1 mg chlorophyll was added to a phase system of 9 ml water and 1 ml of rapeseed oil. After mixing using a homogenizer the phases were allowed to settle for two hours at room temperature. The upper layer was a stable oil in water emulsion containing thylakoids attached to the droplets.

Electron microscopy

The rapeseed oil in water emulsion (described in the previous paragraph) was fixed with 2.5 % glutaraldehyde in 0.15 M cacodylate buffer, embedded in Epon, and stained with 3% uranyl acetate and lead citrate.

Calculation of surfaces of triglyceride and of thylakoids

Thylakoids consist of 50% protein and 50% lipids. 26% of the lipids are pigments of which chlorophyll (a+b) account for 85% i.e. 22% of the lipids. 74% of the lipids, or 3.36 mg/mg chlorophyll, are membrane - forming lipids i.e. MGDG and DGDG. With a density of 0.8 for the lipids their volume will be 4.2 μ /mg chlorophyll. Calculation of the bilayer surface, assuming a thickness 40Å, gives 1 m²/mg chlorophyll. Together with the membrane proteins the thylakoid surface will be about 2 m²/mg chlorophyll. This is in agreement with the value obtained by Flores et al. [18]. They measured the volume occupied by a known amount of membranes and dividing this figure by the membrane thickness, estimated to be 60 Å from electron micrographs. This gave a value of 1.85 m²/mole chlorophyll i.e. about 1.7 m²/mg chlorophyll, assuming an average molecular weight for chlorophyll to be 900 (Chlorophyll a/b ratio=3).

Feeding experiments

Female Sprague-Dawley rats (200 g) from B&K, Sollentuna, Sweden were housed in a temperature-controlled room ($22 \pm 1^\circ\text{C}$) under a 12-h light (6:00 – 18:00)/12-h dark cycle, given free access to water, and fed ad libitum on a standard chow until the start of experiment as detailed below. All procedures using animals were approved by the Local Animal Ethics Committee Lund, Lund, Sweden. For measurement of food consumption rats were individually housed in cage and given a high-fat diet for one week before the start of the study. The high-fat diet consisted of a diet, containing by energy 42.1 % fat , 23.9 % protein and 34.0 % carbohydrate with a caloric density of 4.7 kcal/g as described [19]. The high-fat diet containing thylakoids were prepared as for the high-fat diet with the addition of purified thylakoids at a concentration of 2 mg chlorophyll per gram of food. Food intake was measured daily and body weight at the start and end of the feeding period. Cages were carefully monitored for evidence of food spillage.

Blood lipids, glucose and CCK analysis

Blood was drawn from the intra-orbital bulbar plexus under isoflurane anaesthesia and collected in ice-cold tubes. Serum was obtained and stored at -20°C until analysis. Serum free fatty acids were measured by a NEFAC kit (Wako Chemicals GmbH, Neuss, Germany). Serum triglycerides were measured by a GPO-Trinder kit (SIGMA Diagnostics, Steinheim, Germany). Serum glucose was analyzed by Infinity™ Glucose Oxidase Liquid Stable Reagent (Thermo AB). Plasma CCK concentrations were measured using a highly specific antiserum (no. 92128) that does not bind any of the homologous gastrin peptides [20].

Pancreatic colipase-lipase activity measurement

Colipase-dependent lipase activity was determined with pH-stat titration (Mettler Components DK 10, DK 11, DV11) using 0.2 N NaOH. The substrate was prepared in a vial by adding 0.5 ml of tributyrine to 15.0 ml buffer of 2 mM Tris maleate buffer, pH 7.0, 4 mM in NaTDC, 1 mM in CaCl_2 and 0.15 M in NaCl. The incubation was performed at 25°C . Stirring was maintained with a magnetic rod under standardized conditions. Lipase (0,1 mg/ml) was added (10 μl) followed by colipase (0,1 mg/ml; 10 μl) as described [21]. The activity was recorded during a few minutes, thereafter the various membrane and protein solutions were added and the activity recorded.

Western blot analysis of pancreatic lipase

Pancreas from rats was homogenized and 10 μ g total protein was applied on a 10% SDS-polyacrylamide gel. Proteins were transferred to nitrocellulose membranes and lipase protein was detected by immunostaining using a polyclonal lipase antiserum diluted 1/2000 (a rabbit anti-porcine-lipase which recognizes both rat and mouse pancreatic lipase) and developed with chemo-luminescence [22].

Statistics

The StatView Software program was used for statistical analysis. The data were analyzed using two-way analysis of variance (ANOVA) followed by post hoc tests for comparison of individual differences or Student's t-test (paired comparison). All data are presented as mean \pm SEM. The difference was considered significant if $p < 0.05$.

RESULTS AND DISCUSSION

Inhibition of pancreatic lipase

We first assessed if thylakoid membranes – the photosynthetic membranes of chloroplasts - were able to affect lipolysis by pancreatic lipase/colipase *in vitro*. Thylakoid membranes powerfully suppressed the lipolytic activity in a dose-dependent way during *in vitro* hydrolysis of an emulsion of triglycerides dispersed in bile salt (Figure 1A). Other biological membranes, such as mitochondria, plasma membranes and bacterial membranes also inhibited lipolysis (Figure 1B). This effect, therefore, seems to be a general property of biological membranes.

We next separated the proteins from the lipids in the thylakoid membranes by methanol-chloroform extraction and found that the inhibiting component was contained in the protein fraction (Figure 1C). Membrane proteins are either intrinsic or extrinsic, treatment with trypsin being able to remove most of the extrinsic as well as the exposed loops of the intrinsic proteins. We found that thylakoids treated with trypsin were still able to inhibit lipolysis (Figure 1D), suggesting that the membrane spanning region of the intrinsic proteins possessed the inhibitory effect.

We therefore isolated one of the major intrinsic membrane proteins of thylakoids, the light harvesting chlorophyll a/b protein (LHCII), which constitutes about half of the protein mass of the thylakoid, and found that this protein alone inhibited lipolysis (Figure 1E). LHCII contains three hydrophobic membrane-spanning alpha helices [23] and we hypothesised that the hydrophobicity of these is responsible for the inhibition. If so, a synthetic polypeptide with the same sequence as one of the helices of LHCII should inhibit the lipolysis. Addition of the synthetic peptide inhibited the lipolysis (Figure 1E), although not as powerful as the three-spanning helices of LHCII, suggesting that the number of membrane spanning helices, i.e. the length of the protein molecule, was important for the inhibition. Two intrinsic membrane protein complexes, transhydrogenase [16] and cytochrome b_6f [24, 25] both having several hydrophobic alpha helices, were also found to inhibit lipolysis (Figure 1F). By contrast, water soluble proteins, like serum albumin, did not affect lipolysis (Figure 1E), in agreement with previous observations using the same assay including NaTDC as used here [26]. Thylakoids were found to inhibit lipase activity also in the absence of colipase and NaTDC (Figure 1G).

Mechanism of lipase inhibition by thylakoids

In order to gain information on the mechanism behind the inhibition of thylakoids on lipase/colipase activity we investigated if thylakoids bound lipase/colipase directly and if thylakoids bound to the triglyceride interface.

Binding of lipase to thylakoid membranes

In the absence of bile salt, both lipase and the lipase/colipase complex bound to isolated thylakoids, the lipase/colipase complex having the strongest affinity for the thylakoids (Figure 2). This is probably due to the larger surface and exposure of more exposed hydrophobic groups of the lipase/colipase complex compared to lipase alone. It is known that lipase is a rather hydrophilic protein while colipase is hydrophobic [26-31]. The binding of lipase and the lipase/colipase complex to thylakoid probably involves both ionic and hydrophobic interactions. In the presence of bile salt, the colipase/complex seems to bind weaker to the thylakoids than in the absence of bile salt (Figure 2). However, at a concentration of thylakoids equivalent to 1 mg of chlorophyll, about 80 % of the lipase/colipase complex is bound to the thylakoids, both with and without bile salts, suggesting this to be one reason for the observed inhibition of lipase/colipase activity by thylakoids shown in Figure 1A.

Partitioning of thylakoids in an oil-water two-phase system

Thylakoid membranes were partitioned in an aqueous/triglyceride water two-phase system. The thylakoids were all found at the interface. Electron microscopy shows how the thylakoids are firmly bound to the interface of the lipid drops, with no thylakoid membranes being visible in the aqueous phase (Figure 3A) indicating a strong affinity for the oil phase. That cell particles in general have a strong tendency to collect at liquid/liquid interfaces has been described and theoretically explained earlier [32].

A proposal for the mechanism of inhibition

In vitro, triglycerides are readily hydrolysed by pancreatic lipase even in the absence of its cofactor colipase. During intestinal fat digestion, however, dietary lipids are dispersed in bile salts, and in this situation colipase is obligatory for lipolysis by anchoring the lipase-colipase complex to the lipid-water interface. This attachment involves hydrophobic as well as ionic interactions [26-31]. For reviews on the catalytic mechanism of lipase/colipase, see references [30, 31] and references therein.

The conventional lipolysis system involves heterogeneous catalysis involving the lipase/colipase complex, bile salt and the surface of a lipid or micellar phase. This is in itself a rather complex system. By addition of biological membranes the system becomes even more complex, involving two different surfaces together with bile salt and the lipase/colipase complex, which can all interact with each other. Based on our data we propose that the inhibition of lipolysis by biological membranes can be due to two types of mechanisms working together:

1. Binding of the membranes, or the membrane proteins, to the lipase/colipase complex thereby blocking its active site and preventing the enzyme complex to come in contact with the substrate. The binding involves both ionic and hydrophobic interactions. It is known that the lipase/colipase complex exposes on its surface several hydrophobic amino acid residues [28-31]. This type of mechanism is supported by the binding experiments (Figure 2).

Similar arguments can be applied on the inhibitory effect of intrinsic membrane proteins on lipolysis. Due to their hydrophobic alpha helices, these can bind to the lipase/colipase

complex and thereby inhibit its enzyme activity. Intrinsic membrane proteins can be considered as block polymers with strings of hydrophobic, trans-membrane alpha helices, comprising 20-25 hydrophobic amino acids residues, separated by hydrophilic polypeptide stretches. In the flanking region of a trans-membrane helix there is often inserted one or two amino acid residue with charged side chains. This is the case with one of the transmembrane helices of LHC II used in the experiment shown in Figure 1E. Near the N-terminus you find one arginin and near the C-terminus one glutamic acid. Thus, the long hydrophobic string in the middle of the alpha-helix together with the charges has a strong potential for binding to the lipase/colipase complex, which exposes a large hydrophobic domain on its surface close to charged residues, see [31] and references therein.

2. Binding of the membranes or membrane proteins to the triglyceride/water interface, thereby covering the substrate surface and blocking the access of the lipase-colipase complex to the lipid-water interface. This type of mechanism is supported by the interfacial binding shown in Figure 3A. A similar mechanism is proposed for intrinsic membrane proteins. The hydrophobic strings are adsorbed on the surface of the lipid-bile salt micelles while the hydrophilic stretches, loops and tails, protrude into the aqueous phase (Figure 3B). Together, the entire membrane protein sterically hinders the anchoring of the lipase-colipase complex onto the lipid substrate.

With this mechanism of action an obvious question is if thylakoids could cover the lipid/water interface. In the lipase assay we use 0.5ml triglyceride. If we assume that the size of the triglyceride droplets in the lipase assay is 1 μm in diameter then the total interfacial surface area of the droplets is 3 m^2 . Using two independent estimations (see methods) a thylakoid surface area was found to be about 2 m^2 /mg chlorophyll. This means that the surface of the thylakoids and the surface of the oil droplet are of the same order of magnitude. It is therefore reasonable to assume that the thylakoids can inhibit lipase activity by binding to the oil/water interface thereby preventing the access of lipase/colipase to its substrate. When bile salts are added the membranes will be unfolded and the membrane proteins more or less exposed and these can cover a much larger area than the intact membrane vesicles.

The two mechanisms proposed above do not exclude each other, they rather complement each other and together they can explain the generality of the inhibitory action of biological membrane on lipase/colipase.

Effects of thylakoids added to food

Having found that biological membranes inhibit lipolysis we investigated if the retarded fat digestion could promote satiety in an animal model for diet-induced obesity using high-fat feeding. We found that thylakoid membranes, added to high-fat food, significantly suppressed food intake in rat (Figure 4) and caused a reduced body weight (Table 1). The reduction of food intake started a few days after the thylakoids had been given in the food. This suggests that thylakoids do not have any aversive effect, which otherwise should have acted immediately. Instead there seems to be a gradual inhibition of food intake, suggesting satiety signalling to be gradually upregulated. Moreover, circulating levels of triglycerides were significantly reduced in the high-fat fed, thylakoid-treated, animals compared to high-fat fed controls (Table 1).

We also found the satiety hormone CCK to be significantly elevated in the thylakoid-treated animals (Table 1). The time course for elevation of CCK is not known but may occur coincidentally with the satiety effect observed after day four. We finally collected intestinal content and found an increased lipase/colipase activity (Table 1) in the thylakoid-treated animals compared to control as well as a significantly elevated pancreatic lipase protein expression (Table 1, Figure 5). Since the animals had been fasted for 24 hours before the collection of intestinal content there were no thylakoids in the intestinal content, explaining the perhaps counterintuitive observation. The increased lipase activity in the thylakoid-treated animals is rather explained as a consequence of an increased lipase expression in the pancreas, the secreted pancreatic juice hence containing more lipase protein (Figure 5). The increased pancreatic enzyme activity may well be a consequence of elevated CCK levels, the increased CCK concentrations in plasma being due to a prolonged process of fat digestion. Thus, the thylakoid-treated animals appear to respond, in a compensatory way, to a thylakoid induced suppression of lipase/colipase activity by raising the endogenous secretion and production of lipase/colipase.

It should be emphasized that steatorrea was not observed in the thylakoid-treated animals. This indicates that the satiety effects probably are due to a retardation of fat digestion. The membrane proteins added to inhibit lipase activity will be degraded by intestinal proteases and, with time, lose their inhibitory action on lipolysis. The fact that thylakoid membranes promoted the release of CCK is of particular interest since it means that fat digestion was

retarded in such a way so as to promote, by a feed-back mechanism, a compensatory secretion of lipolytic enzymes. This is a novel mechanism and does not occur with the lipase inhibitor orlistat, which inhibits lipase to 80 % but causes undigested fat to leave the intestine through the faeces with no release of CCK [33]. A similar phenomena was however observed by the use of another inhibitor for lipase/colipase, namely dimaele – a synthetic positively charged ether, that was found to inhibit lipase/colipase activity in vitro, suppress feeding in vivo and to increase intestinal CCK secretion [34]. Whether the thylakoids act on a standard diet is not known and needs further studies. That CCK is important for the satiety of duodenal fat has been demonstrated in various animal models, including the Otsuka Long-Evans Tokushima fatty rats lacking CCK-A receptors, showing a decreased responsiveness to the satiating effect of duodenal fat [35].

The elevated lipase/colipase activity induced by thylakoids infers that also enterostatin is elevated. Enterostatin is a pentapeptide produced from procolipase, in equivalent amounts to colipase in the intestine. It promotes satiety for fat and also induces thermogenesis [36,37]. The receptor for enterostatin has been demonstrated to be the F1-ATPase beta subunit, which when targeted causes a transient inhibition of ATP-production and an increased thermogenesis [37,38]. The observed body weight loss induced by the thylakoid membranes may thus be both an effect of a suppressed appetite and an increased thermogenesis. It has been demonstrated that obese subjects often have a reduced diet-induced thermogenic response [39]. The addition of compounds into the food that raises thermogenesis is thus a valuable tool for promoting energy balance in obese subjects.

In summary, we have found that thylakoids, the photosynthetic membranes of chloroplasts, isolated from green leaves, suppress appetite in rat during intake of a typical refined food containing 42 % fat, a level of fat found in the every day energy intake in the Western diet. In addition the concentration of circulating triglycerides was reduced. We propose that the appetite suppression occurred through the retardation of intestinal fat digestion without causing steatorrea. The absence of steatorrea is probably due to the fact that the thylakoids will eventually be hydrolysed just like any cell membrane by intra-intestinal proteases, phospholipases and galactolipases. The lipolysis is thus only temporarily blocked.

The inhibitory effect on lipolysis appeared to be a general phenomenon associated with all intrinsic membrane proteins having hydrophobic alpha helices. Biological membranes in

general have a favourable nutritional composition. They are composed of “functional” proteins like enzymes, transport proteins and receptors, and bilayer lipids i.e. phospholipids, other charged lipids, sterols and glycolipids. The reason that we focused our interest in thylakoids is that their isolation is relatively simple and could easily be scaled up. Thylakoids are the most abundant of all biological membranes on earth. Thylakoids contain more than hundred different membrane proteins; most of them are found in the major protein complexes [40-42] which, together with their bound pigments chlorophyll and carotenoids [43], account for about 70% of the thylakoid mass. Galactolipids with a dominance of omega-3 polyunsaturated fatty acids [44] account for the remaining 30 %.

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Figure legends

Figure 1. Inhibition of pancreatic lipase by biological membranes and membrane proteins (Figure A-F in the presence of colipase and bile salt).

(A) Thylakoids isolated from spinach (circles), clover (squares), *Arabidopsis thaliana* (triangles). (B) Mitochondria from potato tuber (squares), mitochondria from chicken heart (circles), plasma membrane from spinach leaf (squares), membranes from *Synechocystis* (crosses) and chromatophores of *Rhodospirillum rubrum* (triangles). (C) Membrane protein fraction obtained after removing lipids and soluble proteins from thylakoids (crosses), untreated thylakoids (circles). (D) Thylakoids before (circles) and after (crosses) treatment with trypsin. (E) LHCII a membrane protein isolated from spinach thylakoids (crosses), synthetic polypeptide with the same sequence as one of the membrane spanning alpha helices of LHCII i.e. VIHCRWAMLGALGCVFPELL (triangles), bovine serum albumin (squares). (F) Transhydrogenase (crosses) and cytochrome b_6f complex (circles). (G) Thylakoids from spinach in the absence of colipase and bile salt.

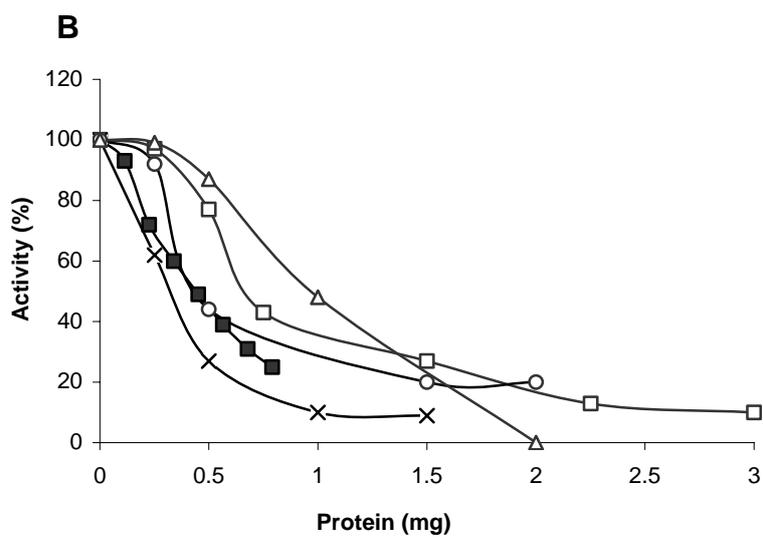
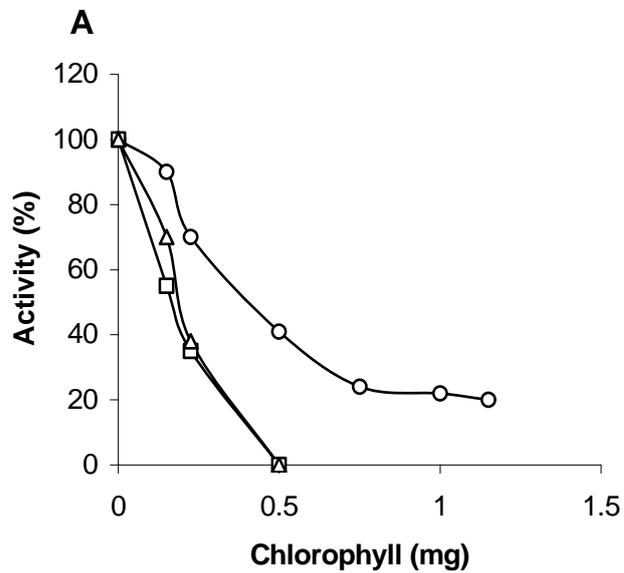
Figure 2. Binding of lipase to thylakoids as function of added thylakoids (expressed as mg chlorophyll). Lipase alone (squares), lipase/colipase in the absence (triangles) and in the presence of NaTDC (circles). Concentration of lipase, colipase, and NaTDC, see Methods.

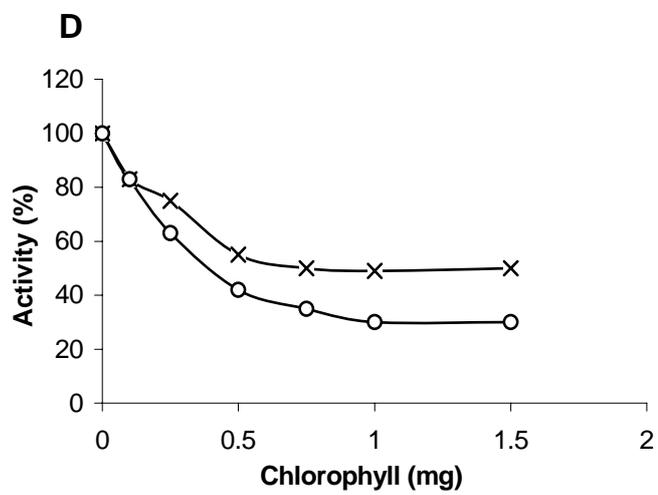
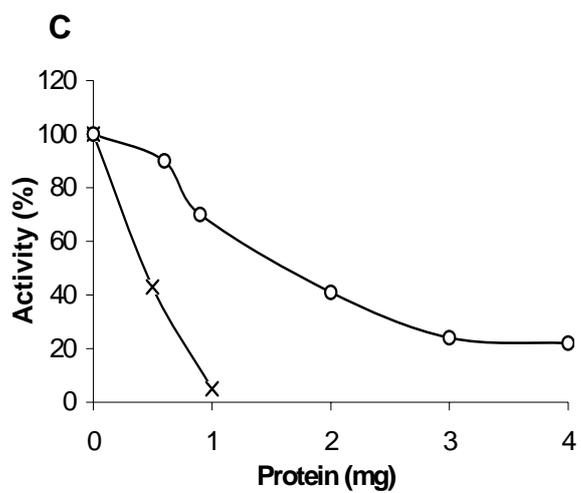
Figure 3. (A) Electron microscopy of thylakoid membranes (arrows) covering the entire interface of an oil droplet in water. No thylakoids were found in the surrounding aqueous phase (B) Schematic model of how an intrinsic membrane protein chain, adsorbed at the surface of a bile-salt (BS) covered triglyceride (TG) droplet, sterically hinders the anchoring of the lipase (L)-colipase (CL) complex to its triglyceride substrate. The hydrophobic helices are embedded in the lipid-bile salt surface layer and together with the hydrophilic loops and tails they hinder the contact between lipase/colipase complex and the lipid.

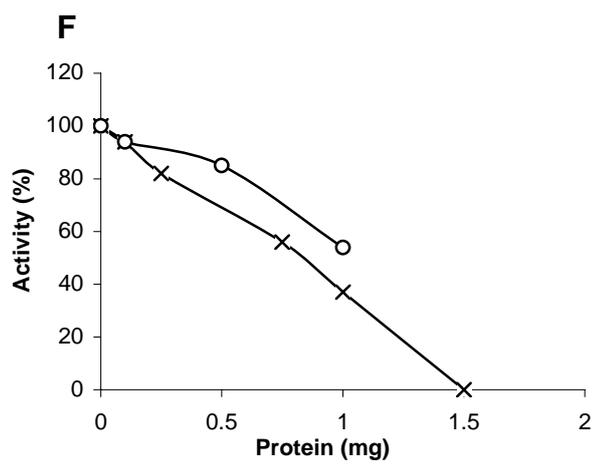
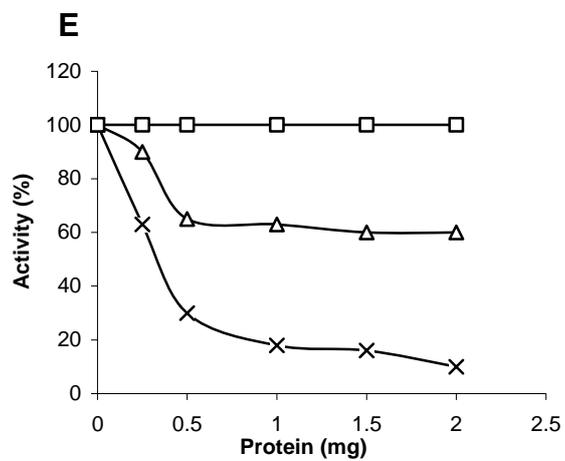
Figure 4. Effect of added thylakoids in high-fat diet for eleven days in Sprague-Dawley rat. Food intake with thylakoids (squares) and without (triangles). The daily food intake is given as means \pm SEM from eight animals in each group (n=8). Treatment with thylakoids shows a significant suppression of food intake (two-way ANOVA; $p=0.017$).

Figure 5. Effect of thylakoids on pancreatic lipase expression. Representative Western blot of pancreatic lipase expression from pancreatic homogenate. Treatment with thylakoids during high-fat diet resulted in a significant increased expression of lipase in the pancreas compared to control.

Figure 1







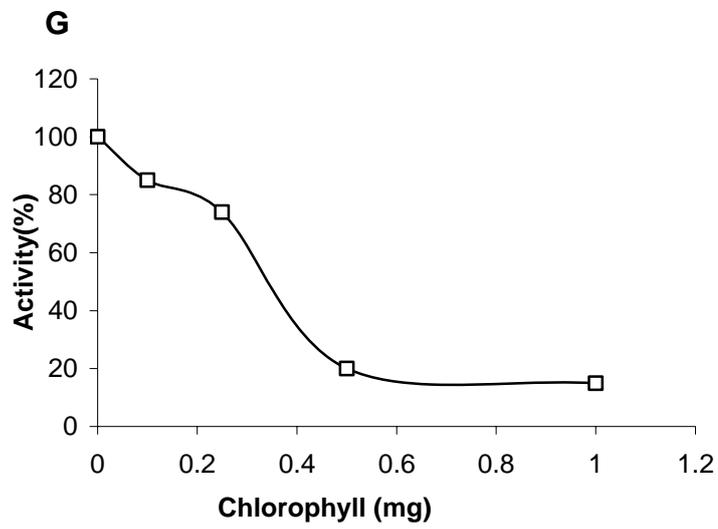


Figure 2

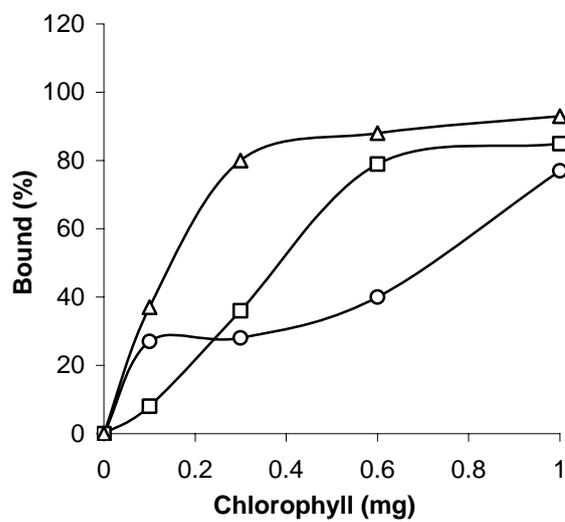
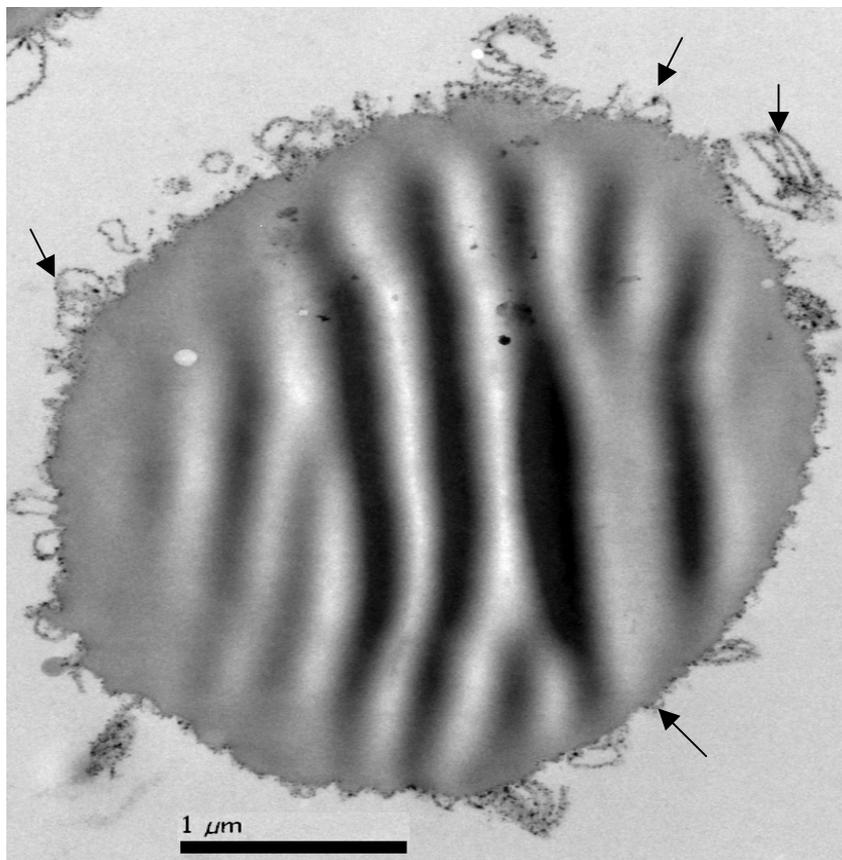


Figure 3

A



B

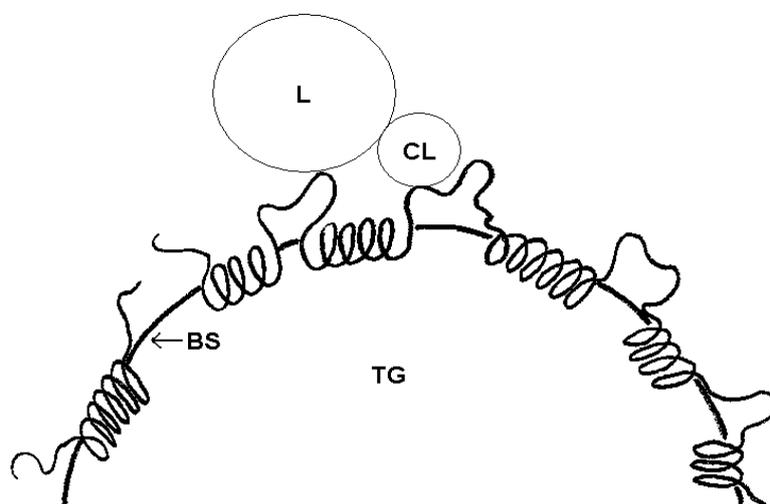


Figure 4

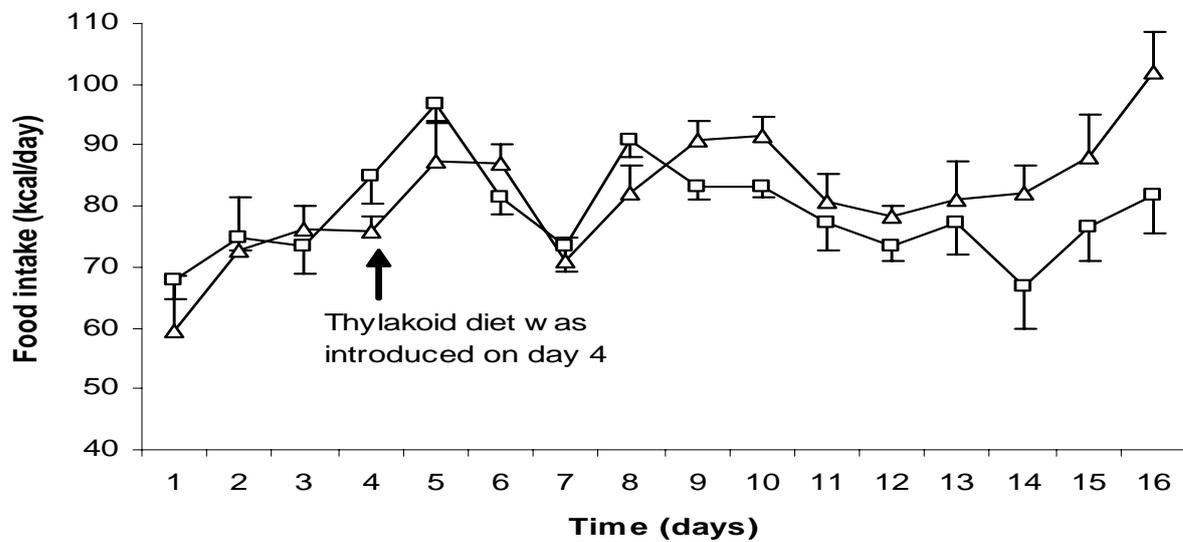


Figure 5

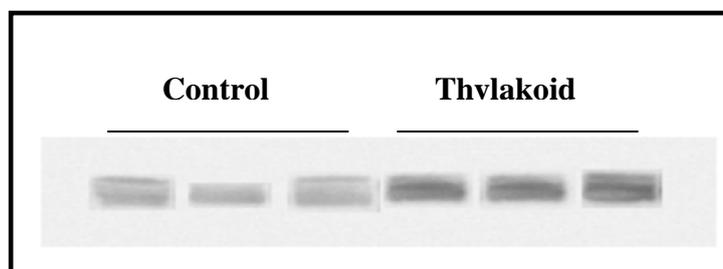


Table 1

Effect of treatment with thylakoids during high-fat diet for eleven days in Sprague-Dawley rat

	Control diet	Thylakoid
Body weight gain (g)	60.5 ± 3.55	49.9 ± 3.05*
Serum TG (mmol/L)	1.02 ± 0.13	0.62 ± 0.04*
Lipase/colipase activity (U/mg)	130.5 ± 18.6	222.1 ± 37.5*
Lipase protein expression (arbitrary units/mm ²)	2269 ± 539	5305 ± 809**
Plasma CCK (pmol/L)	0.675 ± 0.08	0.862 ± 0.12*

Values are means ± SE with *Significance level of $P < 0.05$ and **significance level of $P < 0.01$ between control diet and thylakoid treatment.