

Can Thylakoids Replace Bariatric Surgery for Long Term Maintenance of Weight Loss in Obesity Giving A More Physiological Approach

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Abstract

We reviewed the literature regarding thylakoids as the naturally physiologically occurring agents from green leafy food plants like spinach. We carried out a search in PUBMED DATABASE for detailed information using MeSH terms like satiety, fat metabolism, weight loss, glucose metabolism, lipid metabolism, gut microbiota for studying the efficacy of these thylakoids in utilizing for treatment for medical management of obesity Results- We found 70-80 articles pertaining to the same relating to obesity, diabetes, weight loss. Of these we used 65 articles excluding the duplicate articles. No meta-analysis was carried out.

Since bariatric surgery acts by acting on various targets and remain the most effective therapy for obesity management in contrast to current pharmacotherapeutic agents, use of thylakoids appears to be a promising method for tackling both obesity and preventing its associated comorbidities like type 2 diabetes mellitus (T2DM), non-alcoholic fatty liver disease (NAFLD), decreasing both appetite as well as hedonic aspect of hunger reducing the cravings which make an obese patient revert to snacking in between meals and find it very difficult to control food intake simply by lifestyle interventions like diet control and exercise. Most modern medicines have not been found to be useful for long term maintenance of weight loss. In view of their cardiovascular (CVS) side effects a lot had to be withdrawn. The only drug approved is the lipase inhibitor orlistat for long term obesity treatment which has its limitations because of steatorrhea which is very cumbersome for the patient. Thylakoids seem to be acting on multiple targets like preventing hedonic hunger, improve fat digestion along with causing loss of weight along with fat mass as measured by DEXA, besides increasing transit time in intestine by delaying fat digestion and thus mimicking the effect of natural satiety hormones like increasing GLP1, CCK, decreasing ghrelin, an appetite stimulating hormone and thus does the work of multiple anti-obesity drugs like orlistat, GLP1 agonists like liraglutide, with its probiotic effect it further promotes the weight lowering effect although whether SCFA increase is part of this mechanism is still not clear. Still Appethyl™ the patented form of spinach thylakoids offers a great option for treatment of obesity and metabolic syndrome. Greater BMI experiments are needed in humans than the ones carried out till now to study if these effects seem to translate into practice in humans with morbid obesity.

Keywords: obesity; metabolic syndrome; hedonic control; satiety; GLP1; CCK; Ghrelin; probiotic effect; weight loss; fat loss

Introduction

There is an increase in an obesity epidemic currently extending globally. We had previously reviewed the aetiopathogenesis, medical treatment of obesity followed by further updates and latest the novel pathways to be targeted medically [1-8]. In this review we stress on more physiological approach in targeting various aetiopathogenetic factors by the more physiologically obtained thylakoids from natural green leafy foods like spinach which act by multiple mechanisms like acting on fat digestion,

involving satiety, interacting with various factors like glucagon like peptide 1 (GLP1), cholecystokinin (CCK), as well as act as probiotics along with improving glucose and lipid metabolism.

The various mechanisms which contribute to this obesity epidemic include

1. Greater consumption of energy dense and nutrient poor food which contain high levels of fat and sucrose [9]
2. Two systems apparently control appetite namely the

homeostatic regulation and the hedonic systems [10]. They not only modulate hunger and satiety, but also choice and liking of certain food items as explained by Bridge et al [11].

3. Loss of appetite control could be secondary to a disturbance in the homeostatic pathway and /or an improper sensitization of the hedonic pathway, reviewed in ref [2].

An interaction exists between the homeostatic and hedonic systems [12]. They not only affect hunger and satiety but also whether a particular food item is liked or not. For getting appetite control and influence body weight regulation it is thus important to consider how energy homeostasis is achieved besides the hedonics of eating [13]. One important factor contributing to appetite control is the bacterial flora in the intestine.

Thylakoids which are the photosynthetic membranes in green leaves suppress hunger and promote satiety. This occurs through the modulation of gastrointestinal (GIT) appetite peptides like GLP1, CCK, besides changing gut micro flora, longterm studies have shown that thylakoids produce weight loss besides a decrease in blood lipids and blood glucose. Subjects who receive thylakoids also have decreased liking for fat and sweet.

Physical Properties and Composition of Thylakoids

Thylakoids are the membranes in the chloroplast of green leaves responsible for the light reaction in photosynthesis [14,15]. Possibly they are the most complex biological membranes having different composition and function. They have over 100 different proteins which are both intrinsic and extrinsic, together with membrane lipids and pigments. These thylakoids are located in droplets of green leaves and are at a higher concentration in dark green leaves as compared to light green leaves. Characteristics of the proteins is that they are membrane spanning which means they are hydrophobic and attract to hydrophobic surface like lipids. Thylakoids have an isoelectric point of 4.7 [16] (figure 1). This suggests they are positively charged at a pH < 4.7 and negatively charged above that pH. In the stomach the pH is approximately 2 until during fasting conditions. During a meal, the pH of gastric contents increases to 6-7 depending on the food composition and remains much > 2 until most of the meal is emptied. In the intestine pH is 6.5 in the lumen which implies that they are negatively charged and can bind positively charged ions. In the lumen of the intestine they form large swollen structures which adhere to mucosal surface [17]. The pH in mucosal surfaces of the intestine is acidic i.e. Around 5.3 [18].

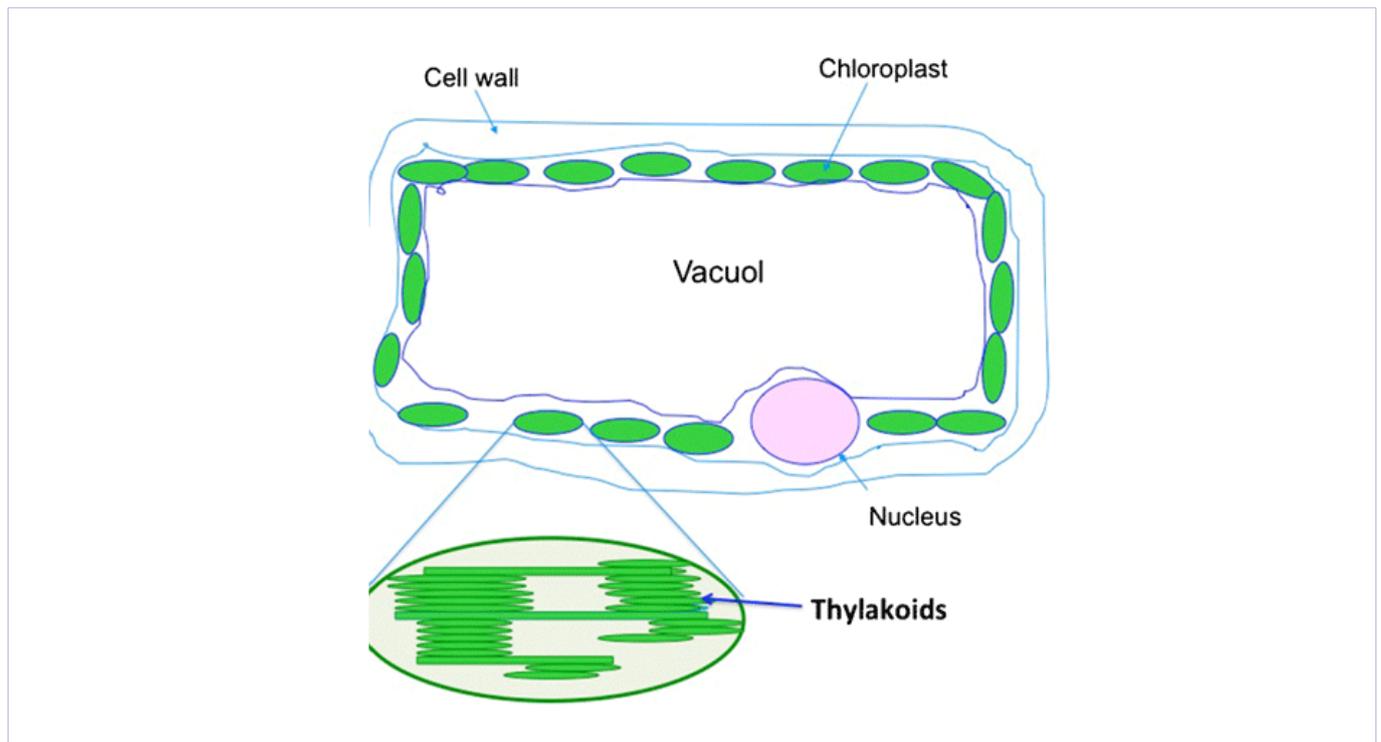


Figure 1: Courtesy –ref number 66- A plant cell consists of chloroplasts, where the photosynthesis takes place. The cell is surrounded by a cell wall, resistant to digestion by humans. A vacuole with water takes up 80 % of the volume of a cell. The thylakoids are membranes building up the photosynthetic apparatus in the chloroplast. The thylakoids consists of galactolipids, proteins, pigments, vitamins and antioxidants

Hence thylakoids are is electric at the mucosal surface and hence adhere to this as has been studied by Monthlies et al2011 [17] and Rachmeyer 1986[18].

Besides proteins thylakoids contain phospholipids, galactolipids and pigments building up the membrane. The thylakoids are built as stack which connects to each other through a grand structure. Together they form a tightly compressed and stable 3 dimensional structure ,which is able to withstand other factors from the environment yet retaining a great flexibility .They are resistant to degradation by gastric and pancreatic enzymes in the stomach and intestine[19]. Because of this these thylakoids remain in intestine for several hrs before they get totally degraded .Through this stability food digestion in the presence of thylakoids goes on for a long time which =>digestive products to reach the distal intestine to release satiety hormones .Pancreatic lipase related protein 2 is a lipase not dependent on colipase ,it hydrolyses galactolipids present in vegetables[20,21]and degrade the thylakoids gradually in the intestine. Thylakoids also contain vitamin E and K as well as certain pigments and anti oxidants like chlorophyll, carotenoids , oxidation induced by light and may after consumption protect against disease .The bioavailability of lutein and zeaxanthin is high[22],which implies antioxidant may protect against disease .Chlorophyll is a green pigment which adsorbs energy from light during the photosynthesis process present in green plants and algae .It has a structure similar to haemoglobin having a carbon chain named phytol.Many health benefits have been attributed to chlorophyll,although evidence has not been there.It still has been shown to protect against various chemicals which can induce cancer[23].

Benefits of Thylakoids

Inhibition of Lipase/Colipase Activity

Albertson PA et al in 2007 first described the effects of thylakoids as a strong inhibitor of lipase/colipase, which catalyzed hydrolysis of fat [24].Pancreatic lipase, with its protein cofactor colipase is the main enzyme responsible for hydrolysis of dietary fat in the intestine [25]. If there is lack of either lipase or colipase, it impairs fat digestion and steatorrhea results [26].Further the discovery of an oral compound, which inhibited pancreatic lipase /colipase, simultaneously decreased food intake in rat, gave further strength to this idea [27].

This idea of thylakoids causing lipase/colipase inhibition was on the fact that galactolipids are powerful inhibitors of lipase /colipase, with thylakoids being a rich source of galactolipids. Pancreatic lipase and colipase adsorb these galactolipids in monolayer's [28],which slows down lipolysis,helping in promoting satiety[29].Besides galactolipids there are other protein suppressing components of thylakoids which are proteins also believed to be light harvesting complexes and antioxidants. Basically the complete macromolecular structure of thylakoids is required for a full effect on appetite suppression. Thylakoids show a dose dependent inhibition of pancreatic lipase /colipase activity in vitro.Extraction of the lipids from thylakoids still had the capability to inhibit this pancreatic lipase /colipase. Hence the hydrophobic proteins in the thylakoids may be responsible for the inhibition seen [24].(figure 2)

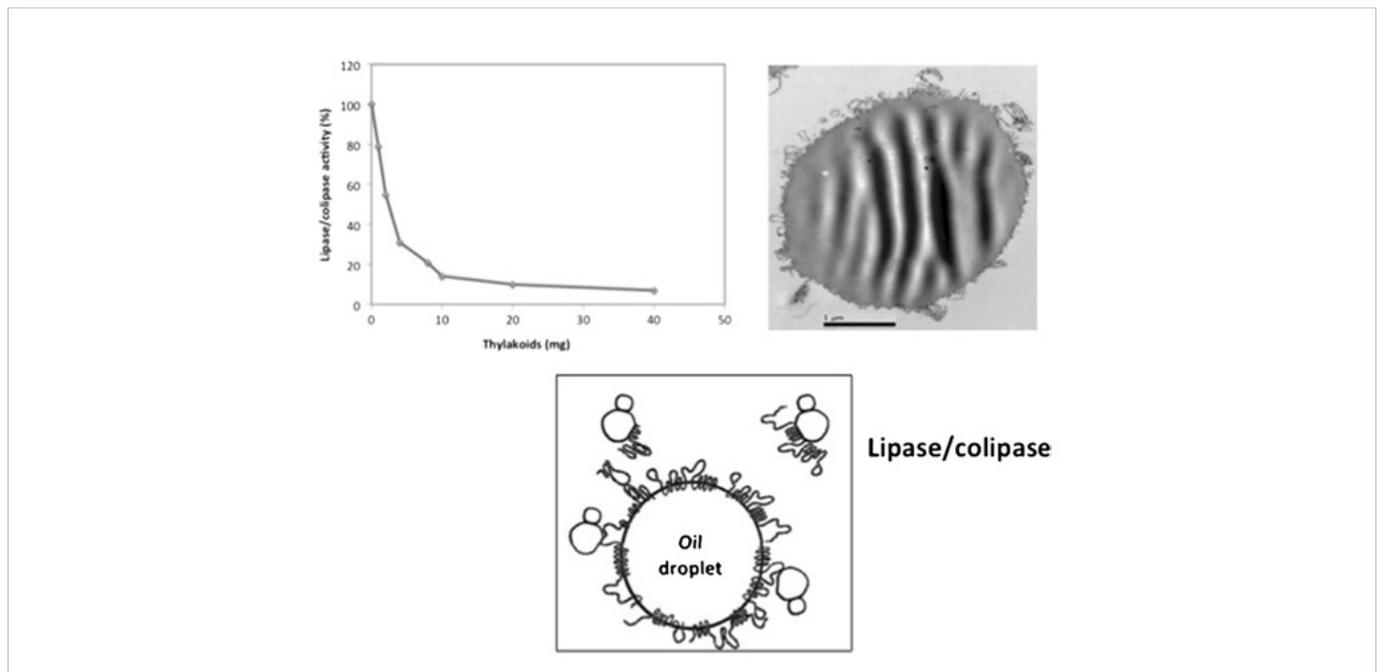


Figure 2: Courtesy ref 66-Thylakoids and fat digestion. Thylakoids inhibit lipase/colipase activity in dose dependent way [18]. The inhibition is due to the binding of thylakoids to the triglyceride interface, thus covering the substrateto be hydrolysed [18, 19]. The thylakoids also bind the pancreatic lipase/colipase complex [20]. The hydrolysis of the oil droplet thus occurs more slowly. Intestinal enzymes gradually break down the thylakoids, which allows fat digestion to be completed. Therefore there is no steatorrhea.

Pancreatic lipase and colipase is inhibited by many proteins, but in the presence of bile salt and colipase, as occurs during intestinal fat digestion under physiological conditions, no inhibition occurs [30]. Ability of thylakoids to inhibit lipase during the bile salts presence along with that of colipase was a new phenomena seen. This inhibition was not unique to thylakoids membranes, but was seen with other membranes as well like plasma membranes from animal cells, mitochondria from animal cell along with bacterial membranes [24]. Advantage of thylakoids is their resistance to gastric and pancreatic juice enzymes proteolysis [19]. This resistance is conferred by high content of membrane pigments like chlorophyll a and b in thylakoids besides carotenoids, which are strongly bound to the intrinsic membrane proteins. Thus enzymes are unable to reach the intrinsic membrane proteins. This helps in actions of thylakoids in view of stability. Adding mitochondria for inhibition of pancreatic lipase and colipase couldn't suppress food intake and body weight in experiments carried out in rats by Albertson PA et al [not published]. Also milk protein casein, which strongly inhibits pancreatic lipase and colipase could not suppress food intake in rats.

Diethylaminomethyl ether, which is a synthetic compound inhibited both pancreatic lipase and colipase and simultaneously inhibited appetite [27]. No steatorrhea was seen which was explained by an increased synthesis of pancreatic lipase which led to a persistent fat digestion at a low rate [27]. Various inhibitors of pancreatic lipase like orlistat interact with the active site of lipase and thus block its activity. This inhibition gets partially reversed in the presence of an emulsion [31]. Clinically it caused intermediate weight loss i.e. 5kg/year. It has a side effect of steatorrhea [32]. In contrast to centrally acting antiobesity drugs this is not a serious side effect making it approved by FDA for treatment of diabetes and decreasing blood lipids [33]. Main difference between thylakoids and orlistat is the release of satiety hormones like CCK by thylakoids [24, 34-36] but not by orlistat [37]. Reason behind it being that fatty acids, the end products of triacylglycerol (TAG) hydrolysis cause the release of these satiety hormones. Thylakoids => a 100% conversion of TAG to fatty acids in contrast to only 25% fat leaving intestine undigested with orlistat. Hence complete fat digestion seems to be a requisite for satiety hormone release.

Different polyphenols extracted from plants, fungi, algae, wine, green tea and coffee have been shown to inhibit pancreatic lipase in vitro [38].

Mechanism of Action of thylakoids in fat digestion

Different ways of improving fat excretion are there by which thylakoids act. 1) they temporarily inhibit pancreatic lipase/colipase during fat digestion. This delay in fat digestion causes lipids to reach the distal end of intestine, where they get absorbed. Thus food is pushed to reach distal part of intestine. 2) This causes release of satiety hormones. 3) Mechanism for inhibition of pancreatic lipase is binding of thylakoids to lipid surface and thus covering the triglyceride substrate which has

to be hydrolyzed [24,39]. 4) Also binding of pancreatic lipase / colipase to thylakoids occurs [40], although very small percentage of pancreatic lipase / colipase is bound to thylakoids, this implies that inhibition of lipase, colipase activity is due to the triglycerides getting covered by thylakoids. Further thylakoids have emulsifying properties i.e. they get attracted to lipid surfaces forming stable emulsions. For thylakoids to act as appetite suppressant it is important for them to be dissolved in liquid formula, which thus makes them properly spread to form a single layer around lipid particles as well as on surfaces.

Further Steenbok et al in 2016 found an increased expression of fat oxidative enzymes in the gut and increased fat oxidative enzymes in rats fed high fat diets with thylakoids supplementation [41], which suggests other mechanisms might explain the fat reduction. This could be faecal fat excretion and/or recruitment of new adipocytes which have a more active metabolism. When storing energy, initially adipocytes grow in size, which is followed by differentiation and recruitment of new adipocytes [42,43]. Special genes have been identified, the peroxisome proliferator activated receptor gamma (ppary) genes, which on activation, increase the number of adipocytes, to direct the energy to small adipocytes which are less inflammatory [44]. In this study the authors were interested in understanding the mechanism of reduction of fat mass at the adipose cell level following thylakoids supplementation, by focusing on cell size distribution of adipocytes [45]. Also they wanted to know if there was fecal fat excretion which was not full scale steatorrhea.

Thus they randomized mice to receive HFD or ethyl HFD for 14 days for the control group and 16 for thylakoids group. The effect of thylakoids on body fat distribution, fecal and liver fat content and adipose tissue metabolism was investigated following high fat feeding. Administration of thylakoids for 14 days caused an increased fecal fat content without compensatory fat eating compared to control. Due to this thylakoids treated animals had a reduced fat mass depots and reduced liver fat accumulation, as compared to controls. The size distribution of adiposities isolated from visceral adipose tissue was narrowed and the cell size decreased. Adipocytes from the thylakoids treated mice displayed a significant increased lipogenesis and protein expression of peroxisome proliferator activated receptor gamma (ppary), downstream target FAS as well as transcriptional co activators, PPAR gamma co activator 1alpha (PGC 1 alpha), and lapin 1 were unregulated in adipose tissue from thylakoids fed mice. Thus they concluded that thylakoids supplementation reduces body fat and fat cell size by binding to dietary fat and increasing its faecal excretion thus decreasing the fat available for absorption [46]. Various investigators have found several natural agonists for PPAR gamma, bioactive compounds being plant antioxidants like luteolin, quercetin, catechin and resveratrol [47]. Thylakoids contain antioxidants in large amounts like lutein, carotenoids and zeaxanthin. Thus it was also hypothesized that these antioxidants may be responsible for an interaction with the PPAR system.

Further in this study ethyl HFD fed mice also accumulated less fat in the liver as compared to HFD fed mice. Earlier studies have shown decreased levels of triacylglycerol in the blood by thylakoids [35], but this was the 1st time that it was observed that there is reduction in fat accumulation in liver. This finding might be of importance in non alcoholic fatty liver disease (NAFLD), which is a metabolic disorder and has a multisystem involvement, and is strongly associated with obesity, insulin resistance and type 2 diabetes mellitus (T2DM) [48]. Because of its effects on hepatic oxidative capacity, circulating triglycerides and free fatty acid levels, lipin 1 has been suggested as a potential therapeutic target for obesity related dyslipidemia and NAFLD [49]. Possibly, thylakoids treatment increases lipin expression in liver, similar to adipocytes, which may reduce fat accumulation.

Increasing Satiety and Suppressing Hunger

Once given thylakoids inhibit eating along with promoting satiety as shown in rats [24,50], mouse [35] and human [34,36,51,52]. On giving them chronically body weight gets effectively decreased besides glucose/lipid metabolism in overweight patients. Thylakoids were mixed with food in animal studies while in human's studies they were mixed with food ingredients as part of a complete meal or served as a juice just before a meal. Thylakoids suppress food intake, be it a high fat diet [24, 34], or a high carbohydrate, high fat diet [36, 50-52]. This suppression of food intake was a direct effect in human observed within the same meal [36, 52].

Serving a high fat meal with increasing concentrations of thylakoids from 5 to 50g in humans [34], thylakoids were mixed with rapeseed oil (26g) and cashew nuts (25g), lime, salt and basil and was eaten as a pesto sauce on bread with tomatoes. Fat percentage was 65 energy%, carbohydrate 25E%, and protein 10 E%. CCK got released in a dose dependent manner, with such a meal, which was significantly different from control at a time point 4h and 6h after the start of a meal. With the control meal optimal CCK release at time point 30' after the start of meal occurred while with use of thylakoids, an early release at time point 30' occurred besides a new peak after 4h. This time course suggested that the stimulus for CCK release is continued for up to 6h. Because this was a high fat meal one could conclude that it was dietary fatty acids which caused the CCK release and that these fatty acids are formed during a longer time period as compared to control. This suggests that thylakoids slow down degradation of dietary fats in a way which promotes the release of CCK during a longer time period. Further late appearance of fatty acids in blood as compared to control supports delayed fat digestion. Leptin was significantly raised during these studies as compared to control at 6h time point period [34]. Since lipase is inhibited by dilapidated thylakoids as effectively as by normal thylakoids [24], one needed to study their potential appetite suppressive effect. CCK release was similar as for normal thylakoids, though no release of leptin occurred [34]. Thus dilapidated thylakoids which lack pigments and membrane lipids are not efficient in regulating appetite like the untreated ones.

Now one needed to check if thylakoids have appetite suppressive effects in carbohydrate rich meals as well like the fat meals. Thus overweight women received carbohydrate rich breakfast with or without thylakoids [36]. This meal consisted of muesli, white bread, butter, cheese, ham, black currant jam, yoghurt, orange juice and banana with coffee following 4h. This meal had 71E% carbohydrates, 11E% fat and 18E% protein as energy source. Thylakoids were mixed with jam and eaten with yoghurt and muesli. This study had a crossover design. Suppression of hunger along with motivation to eat occurred in overweight women following consumption of thylakoid mixed breakfast as compared to controls [36]. Significant rise of CCK was seen 3h onwards as compared to controls. The hunger got suppressed with satiety coming in as early as 2h after the start of meal, when measured using a standard carbohydrate based breakfast [52].

Thus with satiety promotion even with carbohydrate meal having low fat suggested that other mechanisms of thylakoids unrelated to a decreased rate of fat digestion may be operating. Possible was effect of thylakoids on glucose homeostasis and insulin secretion. There was a biphasic response on glucose in thylakoids enriched breakfast while controls showed a single rise of glucose followed by hypoglycemia postprandial [36]. The hypoglycemia in control breakfast correlated with increased hunger as compared to satiety with increased blood glucose following thylakoids enriched breakfast [53]. A 2nd explanation may be release of CCK and GLP1. Release of CCK had been postulated only after fat and protein rich meals. Even carbohydrate rich meal increased CCK levels [54]. GLP1 was significantly stimulated by thylakoids during consumption of breakfast in overweight women [51]. GLP1, which is produced by intestinal cells both in response to carbohydrate and fat causes satiety. Especially fatty acids are potent releasers of GLP1. GLP1 is being developed as an anti-obesity drug in the form of liraglutide and is approved by FDI for the same purpose. Advantage of thylakoids is that they cause endogenous release of GLP1 which is a more physiological method of increasing satiety as well as achieving energy balance [55].

Also suppression of ghrelin by thylakoids was seen after the high dose thylakoids in a single meal containing 60% fat and where thylakoids were added to pesto sauce [34]. Further ghrelin was suppressed in pigs receiving pure carbohydrate by thylakoids as compared to control [56].

Further Rebelled et al 2015 studied 60 overweight and obese individuals enrolled in a double cross over study who consumed the spinach extract or placebo in a random order of at least a week apart. Lipid and glucose was assessed before a standard breakfast following 4h later by a 5g dose of the extract and a standard lunch. Visual Analogue scale were administered before lunch and at intervals until an ad libitum pizza dinner served 4h later. 2h after lunch a 2nd blood was drawn. Mixed model were used to analyze response changes. They found as compared to placebo, consumption of spinach extract decreased hunger ($p < 0.01$) and longing for food over 3h ($p < 0.01$) and increased postprandial

plasma glucose concentration ($p < 0.01$). No difference in plasma lipids and energy intake at dinner occurred, but males showed a trend towards decreased energy intake ($p = 0.08$). Thus they concluded that spinach extract containing thylakoids increases satiety over 2 h period. Hence thylakoids intake might affect gender specific food cravings [57].

Further Steenbok et al 2016 studied if thylakoids affected gastrointestinal (GI) passage in rats. They studied 16 rats who were gavages fed a HFD or ethyl supplemented HFD (thylHFD), 30' before receiving Evans blue. Another 16 rats were fed a control HFD or ethyl HFD for 2 weeks before challenge with Evans Blue. The amount of Evans Blue in the stomach and the distance of migration in the intestine after 30' were used as measurement of gastric emptying and intestinal transit. These were decreased by supplementation in the acute study, however not significantly also after the 2 week diet study. Thus concluding dietary supplementation with thylakoids affects satiety both by affecting GI fullness along with satiety hormones, along with affecting microbial composition (details later section) without causing GI side effects like steatorrhoea [58].

Suppression of Hedonic Hunger

Hedonic Hunger implies that

- i) Thinking of food, although one might have just eaten
- ii) Uncontrollable urge to eat highly palatable food
- iii) Overeating

Overweight people have an increased liking for palatable food [12] especially sugar [59] and fat [60]. So one needs to find strategies to suppress this. Studies by Montelius et al 2014, and Steenbok et al 2014, 2015 [51, 52, 61] showed that thylakoids suppressed hedonic hunger [51, 52, 61].

A single breakfast with or without thylakoids was given to overweight women. Liking for salt, sweet, fat and sweet/fat was estimated utilizing a visual analogue score (VAS) combined with pictures (chips, goodies, cheese on fat and sweet was shown after a single shot of thylakoids, the same day [13, 52, 50]). The liking decreased further after 12 weeks of daily 5g thylakoids [51]. Thus it is concluded that thylakoids have the ability to suppress urge for sweet and fat. This appears to last the whole day following consumption of a thylakoids enriched juice in the morning [52]. Importance of this is craving for sweets usually start in the afternoon. Thus thylakoids, when consumed this way, provides good strategy to suppress the urge for palatable food. In the long term studies, GLP1 was significantly increased by thylakoids, both at the 1st day, but even more on the last day of thylakoids treatment. Since GLP1 is a strong suppressant of urge for sweet food, the increase in GLP1 may well explain the observed decrease in sweet urge.

The mechanism may also involve the satiety hormone CCK. Another explanation maybe the stabilization of serum glucose by thylakoids to prevent postprandial hyperglycemia, which otherwise triggers cravings for rewarding foods.

Effects on gut microbiota

Monthlies et al 2013 investigated the effects of feeding spinach thylakoids on the gut microbiota, mainly the taxa of lactobacilli and bifidobacteria in rats fed either a thylakoids enriched diet or control diet for 10 days. Also they found oral GTT test 10 days after ethyl/control diet significantly decreased plasma insulin levels in the thylakoids fed rats as compared to controls, although no difference was found in blood glucose levels. Analysis of gut bacteria showed a significant increase of lactobacilli on the ileal mucosa, specifically lactobacillus reuteri in the rats fed thylakoids diet as compared to control diet while faecal lactobacilli decreased. No difference in bifidobacteria was seen between thylakoids and control group rats. Analysis using terminal restriction fragment length polymorphism and principal component analyses of faeces showed different microbial populations in the thylakoids and control animals thus suggesting thylakoids modulate gut microbial composition [50] whose importance is already known in regulation of body weight and energy metabolism, reviewed in ref [2, 62, 63].

Further Steenbok et al 2016 studied effect on gut microbiota in thylakoids supplementation in healthy human volunteers ($n = 34$) who received thylakoids/placebo treatment for 3 months. Microbiota was analyzed using 16S rRNA genes sequencing and qPCR. The total bacteria and specifically Bacteroides fragilis group were increased by thylakoids treatment versus placebo [58].

Loss of Body Weight

It has been seen that thylakoids cause weight loss in rats, mouse and man. In rats a loss of 17(5%) body weight ($p < 0.05$) was seen after 13 days of treatment with thylakoids when added to a HFD [24]. Loss of weight was associated with decrease in serum TAG by 40% ($p < 0.05$). In mice body weight loss with thylakoids was 17% ($p < 0.001$) along with increased release of satiety hormone, CCK by 52% ($p < 0.05$). The loss in body weight was evident from day 28. This time delay suggests that genes are gradually unregulated which promote satiety signaling. If there is altered thermo genesis is not clear.

The thylakoids diet given to mice also reduced body fat mass by approximately 33% ($p < 0.001$) and serum leptin by 44% ($p < 0.001$) [25]. Loss in body weight was thus effectively a loss of fat mass as shown by dual energy X-ray Absorptiometry (DEXA)-Scanner and leptin measurements for the amount of fat mass [64]. This loss of body fat suggests that there is an increased fatty acid oxidation by thylakoids.

S .TAG was at same time decreased by 25% ($p < 0.05$) and serum glucose by 17% ($p < 0.05$), which supports coupling between body weight loss, fat mass and reduction of serum lipids, glucose values [25]. Surprisingly serum free fatty acids (FFA) were also decreased by thylakoids treatment by 16% ($p < 0.05$). Normally a restricted diet is often associated with increase in FFA. These FFA's have a potential of leading to inflammation in pancreas, liver and brain [29]. So increase of FFA's by thylakoids by weight loss might have important health promoting property.

In humans thylakoids were shown to promote body weight loss along with decrease in blood lipids ,significantly different from controls($p<0.02$).38women (40-65 yrs of age)with a BMI of 25-33were randomly designed to consume a glass of blueberry juice before breakfast with thylakoids(5g/day)or without for 12weeks .They were recommended to eat three meals a day and exert 30'of physiological activity daily .By the end of study both groups lost body weight but significantly in the thylakoids -treated group,-5kg vs 3.5kg in the control group respectively($p<0.01$).Besides weight loss the most other important change was a reduction of LDL cholesterol($p<0.05$) levels. This reduction occurred already after 3weeks; i.e prior to weight loss .This gives a suggestion those thylakoids lower blood lipids by mechanisms other than as a weight loss consequence.

Conclusions

Thus thylakoids are biological membranes membranes which are derived from green plants .They reversibly inhibit pancreatic lipase by binding dietary fat droplets during fat digestion thereby preventing access of the enzymes present in the GIT. Increased expression of fat oxidative enzymes in the gut and increased fat oxidation in rats fed a HFD with thylakoids supplementation.Thylakoid supplementation decreased body fat and fat cell size by binding to dietary fats ,decreasing fats and increasing faecal excretion which is not full scale steatorrhoea ,which is associated with increased PPAR γ ,PGC1,and lipin1. Also because delayed fat digestion forces the food to reach distal

end of satiety simultaneously the satiety hormones like CCK and GLP1 get released. Also gherkin release was suppressed in both humans and pigs .Further hedonic hunger is decreased thereby overeating by thinking or urge for palatable foods both sugar and fat was reduced, which prevents lot of snacking in between meals which causes difficulty in weight loss maintainance in overweight patients. Further they helped in improving lipid and glucose metabolism, and improve the gut microbiota profile.

Thus by multiple mechanisms causing weight loss (figure 3) as is seen with bariatric surgery thylakoids might become a strategy for treating obesity, T2DM, NAFLD, metabolic syndrome, although more studies in greater numbers of obese women need to be carried out for them to become an acceptable longterm medical therapy for obesity replacing the currently approved drugs approved like orlistat, or liraglutide which act on a single mechanism. The only problem in using natural green leafy vegetables will be the loss of the ability to stabilize emulsions having increased lipid droplet sizes, decreased emulsification capacity and elevated load by heat treatment along with chlorophyll degradation, lipase inhibiting capacity [65].Thus right now one may need to use Appethyl™, which is the currently available form of thylakoids patented by the group of Erlanson-AlberssonC, and group from Sweden. No studies comparing thylakoids with bariatric surgery regarding obesity or T2DM was foundon either of the search engines like pubmed, google scholar. One suggests a randomized clinical trial comparing the 2 in future.

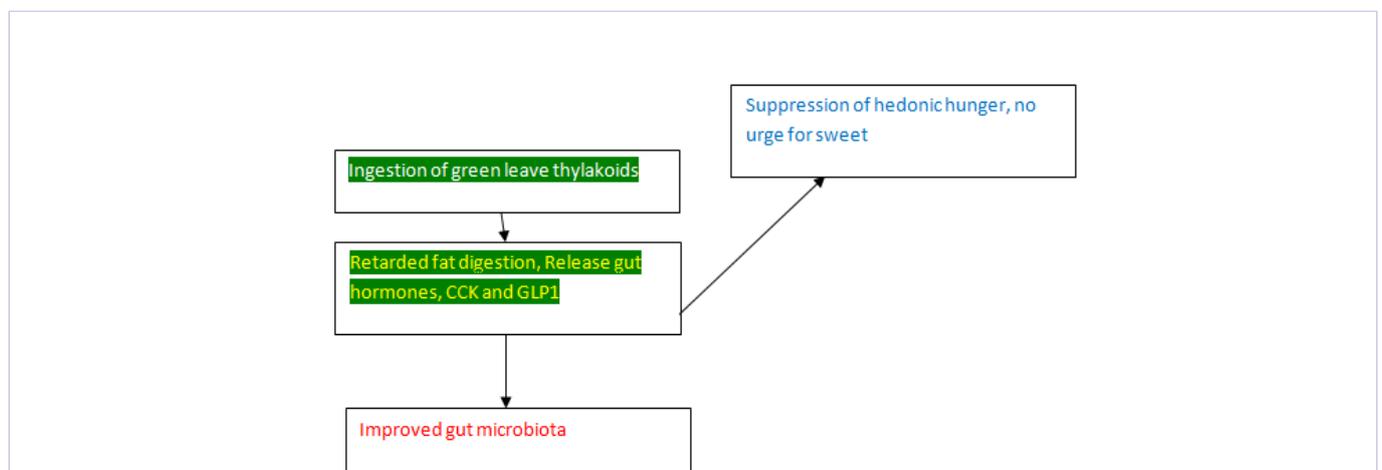


Figure 3: Mechanism of action of thylakoids in appetite regulation. When thylakoids get consumed they retard fat digestion, and release the gut hormones cholecystokinin and glucagon like peptide1. These hormones promote satiety and suppress hunger: Urge in particular hedonic hunger i.e. urge for sweet and fat. Thylakoids also change bacteria of intestine in a prebiotic way releasing lactobacillus reuteri. Combined these effects are important for prevention of obesity and metabolic syndrome.

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