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# A Randomized, Double-Blind, Placebo-Controlled Study Examining the Effects of a Rapidly Soluble Chitosan Dietary Supplement on Weight Loss and Body Composition in Overweight and Mildly Obese Individuals

Rebecca N. Schiller, MS, CN,<sup>1</sup> Eleanor Barrager, RD,<sup>2</sup> Alexander G. Schauss, PhD,<sup>1</sup>  
Everett J. Nichols, MSPH, PhD<sup>3\*</sup>

1. American Institute for Biosocial and Medical Research, Inc., Tacoma, Washington
2. GENESIS Center for Integrative Medicine, Graham, Washington
3. Vanson Inc., Redmond, Washington

## ABSTRACT

**Goal:** The goal of this study is to evaluate the efficacy of a rapidly-soluble chitosan (LipoSan Ultra™) in facilitating weight loss and reducing body fat in overweight and mildly obese individuals consuming a high-fat diet. This study also examines functional changes in gastrointestinal and elimination symptoms caused by supplementation of this compound.

**Methods:** 59 overweight, mildly obese, otherwise healthy females with a history of daily dietary fat consumption greater than or equal to 30% of total calories participated in a randomized double-blind placebo-controlled trial. Subjects received either three capsules of a rapidly-soluble chitosan (LipoSan Ultra™) or matched placebo twice daily for eight weeks. Supplementation was divided into two doses, 1.5 g each, and was taken just prior to the two largest meals of the day. No food restrictions or modifications were assigned, and subjects were instructed to continue their regular caloric intake. Dietary calorie and fat intake was monitored during the study by three-day diet diaries completed at baseline and at weeks four and eight. Anthropometric measurements and functional gastrointesti-

nal and elimination symptoms were measured at baseline and week eight.

**Results:** Mean weight, body mass index (BMI), and percent body fat increased significantly in the placebo group as compared to baseline ( $p < 0.01$ ,  $0.001$ , and  $0.05$  respectively); additionally, percent lean body mass decreased significantly within the placebo group ( $p < 0.005$ ). Within the treatment group, both mean weight and BMI decreased significantly ( $p < 0.005$  and  $0.05$  respectively) compared to baseline. The mean weight loss within the treatment group was 1.0 kg over an 8 week period. This was in contrast to the placebo group which experienced a mean weight gain of 1.5 kg over the same time period. When comparing study groups, endpoint values for mean weight and BMI were significantly higher in the placebo group ( $p < 0.0001$  and  $0.01$  respectively). Chi-square analysis for weight loss between groups indicates significantly more subjects lost weight within the treatment group than within the placebo group (63% and 17% respectively,  $p < 0.001$ ). No significant changes in functional gastrointestinal and elimination symptoms occurred in either group.

## CONCLUSION

Our findings suggest that this rapidly-soluble chitosan (LipoSan Ultra™) is efficacious in facilitating weight loss and reducing body fat in overweight and mildly obese individuals. In addition, we found no significant changes in gastrointestinal and elimination symptoms with the use of this polysaccharide biopolymer.

### \* Correspondence:

Everett J. Nichols, MSPH, PhD  
Vanson Inc.  
8840 152 Avenue NE  
Redmond, WA 98052  
Phone: 425-881-6464 Fax: 425-882-2476  
e-Mail: enichols@vanson.com

## INTRODUCTION

Obesity, defined as a body mass index of at least 30.0, is associated with a significant increase in risk for a number of chronic diseases, including cardiovascular disease, type-II diabetes, and some cancers.<sup>1-5</sup> Analysis of the Third National Health and Nutrition Examination Survey (NHANES III) indicates that obesity has increased by 6.6% in women and 7.6% in men since 1980.<sup>6</sup> In addition, it is estimated that 97 million Americans are currently overweight or obese.<sup>7</sup> Given the increasing prevalence and public health implications of obesity, it is important to identify efficacious clinical treatments for weight management.

Current clinical approaches to weight management include caloric restriction, exercise, behavior modification, and pharmaceutical and/or nutritional supplementation. Pharmaceutical agents generally fall into one of three categories: lipase inhibitors, appetite suppressants, and metabolic stimulants. None of these are without side effects. For example, the appetite suppressants fenfluramine and dexfenfluramine have been shown to be associated with cardiac abnormalities and neurotoxicity.<sup>8-12</sup> Additionally, while pharmaceutical lipase inhibitors have been shown to significantly reduce body fat and improve a number of obesity-related disease risk factors, reported side effects include increased flatulence, steatorrhea, oily spotting, and fecal incontinence and urgency.<sup>13,14</sup>

Chitosan is a popular dietary supplement often used to prevent dietary fat absorption as a means for controlling weight.<sup>15-18</sup> It is a cationic polysaccharide produced from the biopolymer chitin, which is derived from the cuticles of crustaceans such as shrimp, crab, and lobster.<sup>19</sup> The cationicity of chitosan is due to the non-acetylated amines of the polyglucosamine residues that make up the polymer chains.<sup>19</sup> A number of *in vitro*, pre-clinical, and clinical studies have demonstrated that chitosan binds dietary lipids and bile acids.<sup>17,20-34</sup> The lipid-binding activity has been attributed to a combination of chitosan's unique pH-dependent solubility characteristic<sup>16,17,19</sup> and fat emulsification properties.<sup>17,35</sup>

While a number of randomized double-blind placebo-controlled trials have shown significant weight loss with chitosan supplementation in conjunction with a hypocaloric diet,<sup>36-43</sup> this effect has not been observed in the few trials where the diet was unrestricted.<sup>44-46</sup> The ability of chitosan to bind fat depends on its solubility rate, molecular weight, and concentration;<sup>47</sup> variations in these properties may account for the disparities observed in clinical trial results.

LipoSan Ultra™ is a form of chitosan possessing both enhanced solubility properties and high density.<sup>49</sup> The combination of rapid solubility, high density, and molecular weight contributes to LipoSan Ultra's effective fat-binding activity. *In vitro* studies have shown LipoSan Ultra™ to be more effective in binding triglycerides than chitosan

itself.<sup>47</sup> Furthermore, preliminary clinical studies evaluating serum triglycerides suggest that LipoSan Ultra™ binds significantly more dietary fat than regular chitosan.<sup>50</sup> As weight gain is positively associated with dietary fat intake,<sup>51</sup> LipoSan Ultra™ may prove beneficial as an effective weight-loss management supplement.

The goal of this study was to evaluate the efficacy of LipoSan Ultra™, (Vanson, Inc., Redmond, WA) in facilitating weight loss and reducing body fat in overweight and mildly obese individuals consuming a high-fat diet. We also examined potential functional changes in gastrointestinal and elimination symptoms that might be caused by supplementation with this substance.

## METHODS

### Materials

The chitosan used in this study (LipoSan Ultra™) consisted of >90% chitosan and <10% succinic acid. The chitosan exhibited a deacetylation value of >78%, a Brookfield viscosity of approximately 155 mPas (1% in 1% glacial acetic acid), and a molecular weight average of >100,000 daltons as determined by multi-angle light scattering. *In vitro* triglyceride binding was determined according to Vanson's Standard Method, ALL-STM-0161, rev. 01. LipoSan Ultra™ has been shown to bind in excess of 50 g of triglycerides per gram of product (according to STM-0161, rev. 01). The LipoSan Ultra™ capsule formulation was identical to that used for ChitoSense™ (NutraSense Company, Lenexa, KS) and contained 2% talc by weight. Placebo consisted of a maltodextrin-semolina flour blend (25% and 75% by weight respectively). Both LipoSan Ultra™ and placebo were encapsulated in "00" gelatin capsules using talc as the only excipient.

### Subjects

Sixty-nine overweight and mildly obese but otherwise healthy females between the ages of 21 and 55 were recruited for this study. Subject population included only females in order to eliminate any gender-related variables. Subjects were recruited via a research pamphlet and notices placed on display at two clinical sites. Inclusion parameters included a stable weight history of at least six months, a BMI between 27 and 40, and a routine intake of at least 30% of calories as dietary fat per day.

Individuals were excluded if they 1) had a history of metabolic, hepatic, renal, autoimmune, gastrointestinal, or neurological disease; 2) consumed supplements, OTC (over the counter) products and/or pharmaceutical agents that might influence the outcome of this study; 3) had a history of eating disorders, bipolar disorder, or severe depression; 4) had experienced or were experiencing significant perimenopausal or menopausal symptoms; and 5) were pregnant or nursing.

## Study Design

The study protocol and design were approved by the Human Subjects Review Committee of the American Institute for Biosocial and Medical Research (Puyallup, WA). Prior to treatment, and in addition to obtaining informed consent, subjects were required to complete a personal and medical history survey, the Beck Depression Inventory, and the Medical Outcome Survey (Short Form 36). The Beck Depression Inventory and the Medical Outcome Survey (Short Form 36) were also completed at the end of the study. Routine calorie and dietary fat intake were measured by food frequency questionnaire and diet diary recordings and were assessed by the software program, Nutritionist IV (Version 4.0, First DataBank, San Bruno, CA). Functional gastrointestinal and elimination symptoms were assessed by a Symptom Observational Survey questionnaire (SOS) (GENESIS Center for Integrative Medicine, Graham, WA). Body composition was measured by bioelectrical impedance (RJL Systems Inc., Clinton, MI) according to the National Institutes of Health Technology Assessment Conference Statement<sup>56</sup> and included percent body fat and percent lean body mass. Additional anthropometric measurements assessed included subject weight, BMI, and waist-to-hip ratio. Fasting serum lipid levels were monitored during the course of this study, and were assessed at baseline and week 8. Fecal fat was assessed at baseline and at week eight in a randomly selected subset of subjects from both placebo (n=3) and treatment groups (n=4). A limited number of fecal fat assessments were performed due to budget constraints. Twenty-four hour fecal collections were individually analyzed for total fat content by an independent lab (Quest Diagnostics-Nichols Institute), using the gravimetric method utilizing organic extraction (petroleum ether) following acidification with hydrochloric acid.

Subjects were randomly assigned to either the treatment or placebo group using a random numbers table.<sup>51</sup> Both subjects and investigators were blinded to group assignment. Subjects consumed either three capsules of a rapidly soluble chitosan (LipoSan Ultra™) or matched placebo immediately prior to each of the two largest meals of each day for a period of eight weeks. Each chitosan capsule contained 500 mg of the active compound; each placebo capsule contained 500 mg of the maltodextrin-semolina flour blend.

Subjects were instructed to not deviate from their normal dietary intake or exercise routines. Compliance was monitored by assessing supplement containers for any remaining capsules at week eight and by reviewing diet and exercise diary recordings. SOS, body composition, BMI, and waist-to-hip ratio were measured at baseline and week eight.

## STATISTICAL ANALYSIS

Statistical analyses were performed with 2-tailed paired student t-tests to compare baseline values of all study

parameters to endpoint values (Microsoft® Excel 97, Redmond, WA). Differences between groups were analyzed with 2-tailed student t-tests (for independent samples). Chi-squared analysis was performed on subject weight measurements in order to compare weight loss between groups. A *p* value less than or equal to 0.05 was considered statistically significant.

## RESULTS

Fifty-nine subjects completed the study, 29 within the treatment group and 30 within the placebo group. Ten subjects (5 from the treatment group and 5 from the placebo group) did not complete the study. Out of the 10 subjects who did not complete the study, 7 were dropped due to non-compliance with study protocol, 1 was dropped due to non-study related illness, and 2 were lost during follow-up. There were no significant variations between the placebo and treatment groups for baseline values in demographics, anthropometrics, and dietary fat and caloric intake (Table 1).

### Dietary Composition

Analysis of baseline and endpoint values for both caloric and dietary fat intake showed there were no significant changes, in either group, during this study. Analysis of subject diet diaries showed the mean daily caloric intake at eight weeks was 2003 kcal for the placebo group and 2086 kcal for the treatment group; mean daily dietary fat consumption at week eight was 76 g and 81 g for the placebo and treatment groups respectively.

### Symptom Observation Survey (SOS)

Although there were no statistically significant changes in total SOS or elimination subsection scores in either the treatment or the placebo group, a higher number of subjects

**Table 1.** Mean baseline values for study subjects (n=59).

	Placebo (n=30)		Treatment (n=29)	
	Mean	SD	Mean	SD
<b>Demographics</b>				
Age (y)	40	8.3	41	8.2
<b>Anthropometry</b>				
Height (cm)	166.1	5.0	166.5	5.4
Weight (kg)	87.8	12.8	89.7	14.2
BMI	31.8	4.2	32.2	4.8
Body Fat (%)	39.1	0.1	38.1	0.1
Lean Mass (%)	60.9	0.1	61.6	0.1
<b>Diet History</b>				
Caloric Intake (kcal)	2101	647.9	2171	473.0
Dietary Fat Intake (g)	81.4	34.3	89.9	25.2

in the treatment group experienced an increase in several symptoms associated with gastrointestinal discomfort, including flatulence, increased stool bulkiness, bloating, mild nausea, and heartburn. These symptoms, commonly experienced by individuals who increase their dietary fiber intake, were alleviated by increasing water consumption. It is interesting to note that the treatment group also experienced a reduction in straining required during bowel movements and a decreased incidence of diarrhea.

### Anthropometry

Analysis of the anthropometric data (Table 2) shows that within the placebo group, the mean weight increased significantly by 1.5 kg ( $p<0.01$ ), while in the treatment group, mean weight decreased significantly by 1.0 kg ( $p<0.005$ ). BMI, percent body fat, and percent lean body mass increased significantly in the placebo group ( $p<0.001$ ,  $p<0.005$ ,  $p<0.005$  respectively), while BMI decreased significantly ( $p<0.05$ ) in the treatment group. When comparing study groups, endpoint values for mean weight and BMI were significantly higher in the placebo group ( $p<0.0001$  and  $0.01$  respectively). Chi-square analysis for weight loss between groups indicated significantly more subjects lost weight within the treatment group than within the placebo group (62.8 % and 17.0 % respectively,  $p<0.001$ ). Of the subjects who lost weight ( $n=18$ ) within the treatment group, the mean weight loss was 1.7 kg as compared to 1.2 kg in the placebo group ( $n=5$ ).

### Fecal Fat

Total fecal fat was analyzed at baseline and week eight in a small subset of subjects ( $n=7$ , 3 placebo and 4 treatment) (see Table 3). No significant changes were observed for either group, though treatment subjects did experience an increase in fecal fat elimination. The sample size was too small to draw any statistically significant conclusions.

## DISCUSSION

This study demonstrates that supplementation with a rapidly soluble form of chitosan (LipoSan Ultra™) results in a significant mean weight loss of 1 kg and a 1% reduction in BMI without adhering to a hypocaloric diet over an eight week period. The observed weight loss in the LipoSan Ultra™ group was in contrast to the 1.5 kg weight gain in the placebo group. Also significant was the higher percentage of subjects in the LipoSan Ultra™ group that lost weight compared to the placebo group (62.8 % and 17.0 % respectively). This trial was conducted during the holiday season (November and December); thus the weight gain observed in the placebo group was not unexpected even after a stable weight history of at least six months. It is quite common to see a weight gain during the November – December holiday season due to an increase in caloric intake and/or a lack of exercise. Despite no significant changes in dietary intake in both calories and fat there was a significant weight increase in the placebo group. A gain of one pound of fat requires an extra intake of 3,500 cal.

**Table 3.** Fecal fat excretion as measured by total fecal fat analysis ( $n=7$ ).

Subject #	Group	Total fecal fat (g) excreted/24 h	
		Baseline	8-week
32	LipoSan Ultra	3.3	17.0
36	LipoSan Ultra	0.3	5.2
38	LipoSan Ultra	1.2	2.4
42	LipoSan Ultra	6.9	11.0
	<i>Mean</i>	2.93	<i>Mean</i> 8.90
	<i>SD</i>	2.93	<i>SD</i> 6.48
34	Placebo	4.7	3.4
37	Placebo	10.1	3.2
43	Placebo	3.1	4.3
	<i>Mean</i>	5.97	<i>Mean</i> 3.63
	<i>SD</i>	3.67	<i>SD</i> 0.59

**Table 2.** Mean values for significant study results ( $n=59$ ).

	Placebo ( $n=30$ )				Treatment ( $n=29$ )			
	Baseline		Week-8		Baseline		Week-8	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
<b>Anthropometry</b>								
Weight (kg)	87.8	12.8	89.3 <sup>↑d*</sup>	14.0	89.7	14.2	88.7 <sup>↓c*</sup>	13.9
BMI	31.8	4.7	32.4 <sup>↑b*</sup>	5.3	32.2	4.8	31.9 <sup>↓b*</sup>	4.8
Body Fat (%)	39.1	0.1	40.1 <sup>↑c</sup>	0.05	38.1	0.38	38.7	0.39
Lean Body Mass (%)	60.9	0.1	60.2 <sup>↓c</sup>	0.05	61.6	0.62	61.1	0.61

↑= significant increase, ↓=significant decrease.

a)  $p<0.05$ , b)  $p<0.01$ , c)  $p<0.005$ , d)  $p<0.001$  (2-tailed t-test, 95% CI).

\* At week 8, mean weight and BMI were significantly higher in the placebo group as compared to the treatment group ( $p<0.0001$  and  $0.05$  respectively).

Consuming just 80 additional calories (approximately equivalent to one slice of bread) per day over an eight week period would contribute 4,480 calories resulting in a 1-1.5 pound increase in fat deposit. This might explain the weight gain observed in the placebo group. Within the treatment group, mean waist-to-hip ratios were lower at eight weeks as compared to baseline, and were increased within the placebo group. These results were significant when the analysis was performed using a 1-tailed paired student t-test.

Individuals who consumed LipoSan Ultra™ reported increased stool bulkiness and a reduction in straining required during bowel movements, though these results were not statistically significant. This is consistent with results from previous chitosan clinical trials, where treatment subjects report a greater ease in bowel elimination.<sup>37,38,40,41,43</sup> Fecal fat analysis was limited in scope and design, and while the results were not significant ( $p=0.069$ ), treatment subjects ( $n=4$ ) did experience an increase in fecal fat elimination as compared to baseline values (Table 3). Within this subset of subjects, mean fecal fat excretion increased by 5.97 g/24 h. In two cases within this subset, at least five- to ten-fold higher values were obtained at eight weeks compared to baseline.

Our weight loss findings are in agreement with previous studies<sup>36-43</sup> that have examined chitosan supplementation in conjunction with a hypocaloric diet; these results are further supported by a recent meta-analysis study.<sup>53</sup> The results of our study are noteworthy in that we found significant weight loss in subjects adhering to a non-restrictive diet while similar trials have not.<sup>44-46</sup> One hypocaloric trial that included a subset of subjects adhering to a non-restrictive diet did find significant weight loss in the non-restrictive diet treatment group; however there was no corresponding placebo group.<sup>39</sup> The lack of measurable weight loss in non-calorie restricted trials has been attributed to an insufficient dosage of chitosan,<sup>44,46</sup> non-compliance,<sup>46</sup> and failure to follow guidelines to not modify usual dietary intake.<sup>45,46</sup> Analysis of the chitosan-containing supplement used in the study by Pittler et al,<sup>44</sup> found that the capsules contained only 42% chitosan, which was less than the stated 71% (250 mg). Based on the reported number of capsules consumed, subjects would have ingested approximately 0.59 g of chitosan twice a day. A study by Ho et al, used a chitosan salt (Absorbitol™) at an average dose of 2.48 g and 2.36 g of chitosan salt per day in females and males respectively.<sup>46</sup> After comparing their study to a successful lipid reduction study that used 3-6 g of chitosan per day, the authors suggested that their chitosan dose may have been sub-therapeutic.<sup>34</sup> Although the amount of chitosan present in the salt form was not stated, it was likely significantly lower than the reported total amount consumed by the subjects in the study. Since effective *in vitro* triglyceride binding is concentration-dependent, with an optimal chitosan concentration of 1-2%, the lack of weight loss

observed in these trials may be due to insufficient dosage.<sup>47</sup> The importance of dosage is supported in a recent study conducted for a period of six months.<sup>48</sup> The study was a randomized, double-blind, placebo-controlled trial comparing 4.5 grams of chitosan per day to a placebo for a period of six months while incorporating a low calorie diet. The chitosan dose was 1.5 grams per meal three times a day. Following up in six months, the chitosan-supplemented group experienced a statistically significant mean body weight decrease of 15.9 kg compared to a mean decrease of 10.9 kg in the placebo group. Although the number of chitosan capsules ingested per day was reported in some studies,<sup>44,46</sup> the timing of chitosan relative to meals was not. The timing of chitosan ingestion before meals may be important in its ability to bind fat and prevent lipid absorption. It is typically recommended that chitosan supplements be ingested approximately 30 minutes to 1 hour prior to a meal in order to allow the chitosan sufficient time to dissolve in the stomach acid. This recommendation is likely based on previous studies with ascorbic acid that suggested that the solubility of chitosan might be important in decreasing fat digestibility.<sup>32</sup> These findings are supported by *in vitro* studies demonstrating that chitosan is ineffective in binding triglycerides unless it is first dissolved.<sup>47</sup> Chitosan ingested too close to a meal may not become sufficiently soluble to emulsify and subsequently bind fat as the contents of the meal exits the stomach.

In another study, subjects with a BMI greater than 30 received 0.40 g of microcrystalline chitosan just before lunch and dinner for eight weeks.<sup>45</sup> This study showed a significant decrease in LDL-cholesterol in the microcrystalline chitosan group as compared to the placebo group at 4 weeks ( $p<0.05$ ); however, no significant change in body weight was observed in either group. Microcrystalline chitosan treatment tended to slightly increase serum triglycerides compared to the placebo. Interestingly, after 8 weeks we also observed an increase in serum triglycerides in both the LipoSan Ultra and placebo group ( $p<0.05$ ,  $p<0.05$  respectively, 1-tailed student t-test), and a decrease in LDL-cholesterol in the LipoSan Ultra group ( $p<0.05$ , 1-tailed student t-test). These findings indicate that further lipid studies are warranted.

The ability of chitosan to bind triglycerides *in vitro* can be inhibited in the presence of certain excipients used in the encapsulation or tableting process.<sup>54</sup> The nature and type of excipients used in the microcrystalline chitosan study was not defined and may have contributed to the results observed. A variety of commonly used excipients has been evaluated for their effect on the *in vitro* triglyceride binding performance of LipoSan Ultra.<sup>TM54</sup> The results show that certain lubricants such as magnesium stearate and binders including calcium phosphate) can reduce chitosan's ability to bind triglycerides, an effect that may be due to interference with the polymer solubility required for triglyceride

binding. Polyelectrolyte complex formation of chitosan with anionic polymers such as carboxymethylcellulose has also been shown to prevent fat binding due to insolubilization of chitosan. Based on the *in vitro* observations, these tableting and encapsulation excipients present in chitosan formulations may negatively influence chitosan's ability to bind fat *in vivo*. Therefore, excipient-related effects should be taken into consideration when evaluating the results of chitosan clinical trials.

Chitosan polymer chains must also be of sufficient molecular weight in order to bind and entrap triglycerides. It has been observed that commercially available forms of high density chitosan lack sufficient molecular weight to bind and entrap triglycerides *in vitro*.<sup>47</sup> The commercial demand for these high density chitosans is motivated, in part, by the need to increase the amount of chitosan per capsule to reduce supplement size. The density of chitosan is typically increased by extensive grinding, which reduces particle size and allows a larger amount of material to be encapsulated. One significant consequence of grinding is the dramatic reduction in the average polymer molecular weight resulting in short polymer chain lengths that are ineffective in entrapping triglycerides.<sup>47</sup> Preclinical studies have shown that the entrapment of chitosan-emulsified fat is necessary for it to be excreted, thereby reducing its digestibility.<sup>17,30,31,32,55</sup> It has been proposed that the observed hypolipidemic activity of chitosan is due to the polar entrapment of micelles or the disruption and/or inhibition of fatty acid, cholesterol, and monoglyceride micelle formation which occurs near chitosan's isoelectric point of pH 6.0–6.5.<sup>17,20</sup> Gelation of chitosan-emulsified fat has been observed in the intestine of animals, and is likely to result from a reduced solubility of chitosan owing to the higher pH of the intestine, a pH higher than the isoelectric point of most chitosans.<sup>30</sup>

The physical gelation and complexation of lipids with chitosan may render it somewhat resistant to emulsification with bile acids and subsequent hydrolysis by pancreatic lipases. In fact, a recent study showed that chitin-chitosan was effective in preventing an increase of body weight in mice fed a high-fat diet, and that chitin-chitosan inhibited pancreatic lipase-mediated hydrolysis of lecithin-emulsified triglyceride.<sup>56</sup> The inhibitory activity was thought to be due to binding of the substrate and not the inhibition of the lipase enzyme.

The long term safety of chitosan supplementation has been questioned due to its observed ability to decrease serum vitamin E levels and absorption of certain minerals such as calcium, magnesium, and iron in rats.<sup>62</sup> The rats in this study were fed diets that contained chitosan in an amount equal to 50 g of chitosan per 1000 g of food or 5% (wt./wt.). The massive intake of chitosan resulted in decreased absorption of calcium, magnesium, and iron. Supplementation with sodium ascorbate prevented the

observed decrease in iron and magnesium and restored their levels to that of the controls, and although calcium absorption improved, it did not recover to the control levels. A human consuming an equivalent amount of chitosan (5%) based on 500 grams of food intake per day would ingest approximately 25 g of chitosan or 50 capsules containing 0.5 g of chitosan per capsule. This is approximately 8 times the effective dose used in the study reported here. The effect of chitosan ingestion on the reduction of serum iron was not observed in another study involving rats which also showed no reduction in hemoglobin.<sup>28</sup> A number of human trials cited earlier examined the effect of chitosan supplementation on blood chemistry including fat soluble vitamins (A,D,E) and minerals (sodium, potassium, calcium, magnesium, iron, zinc, copper) uptake. These studies showed no significant difference compared to the placebo over a period of 4 or 8 weeks.<sup>38,40-45</sup> Longer term studies have not been performed and may be valuable in view of the observed reductions in the fat soluble vitamins D and E following long term use of an FDA-approved pharmaceutical lipase inhibitor.<sup>13,14</sup> Daily consumption of a vitamin supplement taken at a time when chitosan is not ingested has been suggested in an effort to address the fat soluble vitamin and mineral depletion concern.<sup>16</sup> The concern over potential calcium and iron depletion following chitosan supplementation is interesting in view of the known iron and strong calcium chelating properties of FDA-approved food additives such as alginates, pectins, and citric acid which are commonly used in a variety of foods.<sup>63,64</sup> Chitosan intake has been shown to have an effect on the intestinal microbiota in humans.<sup>65</sup> The putrefactive activity of intestinal microbiota was inhibited by chitosan following a two week ingestion period. The only microbiota that was significantly depressed in number was the lecithinase-negative clostridia. Chitosan is approved as a food additive in Japan, Italy, and Finland,<sup>58-60</sup> and is approved for use in livestock feed up to 0.1% by the Association of American Feed Control Officials.<sup>61</sup>

Liposan Ultra™ is a form of chitosan that has incorporated approximately 5-10% succinic acid during the proprietary manufacturing process.<sup>49</sup> This chitosan exhibits both a higher tap density (without sacrificing polymer molecular weight) and rapid acid solubility compared to other chitosans. *In vitro* studies have shown that LipoSan Ultra™ dissolves faster in 0.16 N hydrochloric acid compared to typical chitosans.<sup>47</sup> The rapid solubility property suggests that LipoSan Ultra™ may be ingested just prior to a meal rather than one half hour to one hour before. Preliminary studies in a limited number of human subjects have shown that ingestion of LipoSan Ultra™ just prior to a meal reduces serum triglycerides over a period of time following the meal.<sup>50</sup> The results of the trial presented here demonstrate that LipoSan Ultra™ may be an effective weight loss supplement and may prove beneficial for weight maintenance in some individuals.

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