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The Combination of a High-protein Formula Diet and Polyglucosamine Decreases Body Weight and Parameters of Glucose and Lipid Metabolism in Overweight and Obese Men and Women

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Research Article

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ABSTRACT

Aims: To examine the efficacy of a weight loss strategy using a high-protein formula diet in combination with a lipid adsorbing fibre, polyglucosamine, over 12 weeks.
Study Design: Randomised, double-blind, placebo-controlled, parallel group study.
Place and Duration of Study: Institute of Food Science, Unit of Nutrition Physiology and Human Nutrition, Leibniz University of Hannover, Germany, between February 2010 and July 2010.
Methodology: 120 overweight and obese subjects participated in this study and ingested a protein-rich formula diet as a meal replacement once daily. Half of the participants (n = 60) additionally took two lipid-adsorbent tablets (F+LA), polyglucosamine, once daily, while the other half (n = 60) received two placebo tablets (F+P). Measurements were taken at weeks 0, 6 and 12 to determine the response to intervention.
Results: Both groups achieved a highly significant ($P < 0.001$) weight loss (F+LA: -5.5 ± 3.8 kg vs. F+P: -4.7 ± 3.9 kg, Full Analysis Set (FAS) population). There was a significant decrease in HbA1c ($P < 0.01$), total cholesterol ($P < 0.001$), LDL cholesterol ($P = 0.002$), and triacylglycerol ($P = 0.001$) in the F+LA group, while the F+P group experienced no changes.
Conclusion: The investigation demonstrated that a formula diet alone or combined with polyglucosamine were both effective in weight reduction. The additional administration of polyglucosamine was more effective on the reduction of glucose and lipid parameters than the formula diet alone.

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Keywords: High-protein formula diet; polyglucosamine; obesity; weight reduction; lipid profile.

1. INTRODUCTION

Cardiovascular diseases have become one of the most common and significant health issues of our time. Within the framework of the pathogenesis of cardiovascular diseases, various causes are thoroughly discussed in the literature in which obesity plays a special key role as a dependent and an independent risk factor. In this regard, (abdominal) obesity is considered a prerequisite and a central component of the metabolic syndrome which is characterized by the coexistence of key cardiovascular disease risk factors including obesity, hyperglycemia, hypertension and dyslipidemia (Kloting et al., 2007).

Overweight and obesity in children and adults is a major problem affecting a very significant proportion of populations worldwide (Mensink et al., 2005; Gellner and Domschke, 2008; Apfelbacher et al., 2008; Moura and Claro, 2011). Therefore, an integral part of the strategy with regard to the preventive and therapeutic measures against diseases of the cardiovascular system and even in the presence of metabolic syndrome, is the reduction of body weight, particularly visceral fat mass (Case et al., 2002).

Different nutrition and lifestyle-related treatment methods are currently available for stabilisation of weight and weight loss (Shaw et al., 2006; Brunner et al., 2007; Rolland et al., 2009). Meal replacement strategies (partial and full meal replacements) using formula diets are widely used to attain initial weight loss (Keogh and Clifton, 2005; Carbajo et al., 2010). This has already been confirmed as a health claim by the European Food Safety Authority (EFSA, 2011a).

Due to its easy, customer-friendly features and comparatively low costs, as well as the predetermined serving size, formula diet products are especially suitable for people who have difficulty losing weight. Apart from weight loss, various different studies have shown improvements in metabolic risk factors (glucose, insulin, lipid profile including blood pressure and waist circumference) when using formula diet products as partial meal replacements (Ditschuneit et al., 1999; Flechtner-Mors et al., 2000; Ditschuneit and Flechtner-Mors, 2001; Ashley et al., 2001a, 2001b; Rolland et al., 2009; Smith et al., 2010).

This concept has proved efficacious even in overweight patients with type 2 diabetes (Yip et al., 2001; Redmon et al., 2005; Hamdy and Zwiefelhofer, 2010). In this context, several studies have proved the short-term effectiveness (e.g. 12 weeks) of a meal replacement strategy using formula diet products (Rothacker et al., 2001). Moreover, other studies also showed that, together with long-term follow-ups (e.g. 1 year), formula diets may support weight stabilisation (Rothacker et al., 2001; Noakes et al., 2004).

Lipid adsorbing substances, such as polyglucosamine, can be considered as a further supplementary strategy to support weight loss via increased excretion of faucal fat. Polyglucosamine is derived from the shells of crustaceans, such as prawns, lobsters and crabs. Polyglucosamine fibres have fat adsorbing properties and are, therefore, able to bind therapeutically relevant amounts of fat (Rodriguez and Albertengo, 2005; Yao and Chiang, 2006). Polyglucosamine is protonated in the stomach. In this state, polyglucosamine can bind monomeric fatty acids and bile acids with high affinity in the duodenum by means of

electrostatic attraction (the positively charged amino groups of the glucosamine residues (cations) bind the negatively charged carboxyl groups of fatty acids and bile acids (anions)), forming a molecular gel structure (Kanauchi et al., 1995). The fats mainly bound to polyglucosamine can no longer be resorbed and are excreted in the stool.

The absorption of lipids through the intestinal mucosal cells proceeds at a much slower rate in the presence of polyglucosamine, as the bile acids, including the mono fatty acids, the nonpolar lipids and undigested fats (lipophilic interactions), needed for the emulsification are primarily bound to polyglucosamine (Kaats et al., 2006). Due to this mechanism, the use of polyglucosamine can be theoretically useful for weight loss and weight control, as well as for the treatment of hypercholesterolemia (Pittler et al., 1999; Schiller et al., 2001; Ylitalo et al., 2002; Mhurchu et al., 2004, 2005; Kaats et al., 2006; Jull et al., 2008; Cornelli et al., 2008).

Within this context, the treatment effect of a weight loss programme using a high-protein formula diet (formoline protein diet) in combination with a lipid adsorbing (LA) fibre, polyglucosamine L112, compared with the treatment group without polyglucosamine L112 was investigated in this parallel-group trial. The dose of polyglucosamine L112 (0.8 g/d) was taken according to weight stabilization. Hence, we put forward the hypothesis with regard to weight loss that the strategy combining (F+LA) was more effective than the combination, formula diet with placebo (F+P). Changes in different laboratory parameters (lipid profile, carbohydrate metabolism) were additionally assessed in the overweight and obese participants.

2. MATERIALS AND METHODS

2.1 Study Design and Population

This clinical trial was designed as a 12-week, single centre, randomised, placebo-controlled, double-blind, and parallel group study. The trial protocol was developed with methods according to the guidelines for Good Clinical Practice (GCP) and approval of the Freiburg Ethics Commission International (FEKI) was received on 30th November 2009. Written informed consent was obtained from all participants. The clinical investigation was registered in the German Clinical Trials Register (DRKS) with the identification number DRKS00000325.

The study was carried out at the Institute of Food Science, Leibniz University Hannover, Germany. One hundred and twenty overweight (16.7%) and obese (83.3%) participants (61 males and 59 females, all of them Caucasian) were enrolled in the trial in February 2010. The trial participants were recruited through advertisements in the daily newspapers. Study criteria were assessed in advance via structured questionnaires over the telephone. Inclusion criteria for this study were a body mass index (BMI) between 28 and 35 kg/m², age between 30 and 60 years. Major chronic diseases (e.g. cancer diseases, manifest cardiovascular disease (CVD), insulin-dependent type 1 or 2 diabetes, severe renal or liver diseases, endocrine and autoimmune diseases), gastrointestinal disorders (e.g. ulcers, chronic inflammatory bowel diseases, coeliac disease, pancreatitis), prior gastrointestinal surgical procedures (e.g. gastrectomy, short bowel syndrome, gastric bypass, gastric banding, stomach balloon), lactose intolerance, pregnancy, breastfeeding, and alcohol or drug addiction were defined as exclusion criteria. Participants were excluded if they were currently following a diet or taking any supplements that could interfere with the preparations given.

After returning the completed admission questionnaire sent by post, participants who met the inclusion criteria without the presence of any of the exclusion criteria were included and invited to the first examination. Patients (stratified according to their gender) were assigned to their respective intervention groups by means of block randomisation appropriate to the sample size, sorted in ascending order of the patient numbers. The chief investigator, investigators, study staff, and patients were all blinded to the treatment allocation in accordance with the double-blind design.

All 120 participants consumed one serving of protein-rich formula diet (formoline protein diet, Certmedica International GmbH, Aschaffenburg, Germany) a day (either lunch or dinner). It was administered daily as a drink replacement meal. The formula diet was made by mixing three tablespoons (approximately 23 g) of powder plus 300 ml milk (low fat) plus 5 g vegetable oil together. The nutritional composition of the formula diet is presented in Table 1.

Table 1. Nutrient and energy content of the formula diet with recommended preparation*

	Preparation per serving	**Preparation per 100 ml**
Energy [kJ]	1319 (313 kcal)	389 (92 kcal)
Protein [g]	29.3	8.6
Carbohydrate [g]	25.6	7.6
Fat [g]	10.2	3.0
Vitamin A [µg]	339	100
Vitamin D [µg]	1.55	0.5
Vitamin E [mg]	7.2	2.1
Vitamin C [mg]	30.1	8.8
Vitamin B1 [mg]	0.7	0.2
Vitamin B2 [mg]	2.1	0.6
Niacin [mg]	7.8	2.3
Vitamin B6 [mg]	1.0	0.3
Folate [µg]	131.0	38.6
Vitamin B12 [µg]	3.3	1.0
Biotin [mg]	0.04	0.01
Pantothenic acid [mg]	3.6	1.1
Calcium [mg]	604.0	178.2
Phosphor [mg]	384.0	113.3
Iron [mg]	4.9	1.4
Zinc [mg]	4.0	1.2
Copper [mg]	0.34	0.1
Iodine [µg]	50.0	14.7
Sodium [g]	0.301	0.089
Magnesium [mg]	92.0	27.1
Manganese [mg]	0.3	0.1
Potassium [mg]	530.0	156.3
Selenium [µg]	20.0	5.9

**Recommended preparation: 300 ml milk (low fat) + 23 g powder + 5 g vegetable oil*

In addition to the formula diet, participants in group F+LA (n = 60) received two lipid adsorbent tablets (polyglucosamine L112, Certmedica International GmbH, Aschaffenburg, Germany), which were taken once a day with the main meal of the day. In the specification of polyglucosamine L112, the main active ingredient in the lipid adsorbing verum preparation

is a natural, indigestible fibrous material (β-1,4 polymer of D-glucosamine and N-acetyl-D-glucosamine). One tablet of the verum preparation contains approximately 400 mg of the active ingredient, polyglucosamine. The intake of two tablets per day is the manufacturer's recommended amount for weight maintenance. Further excipients are cellulose from plants, vitamin C, tartaric acid, silicon dioxide, and magnesium stearate (vegetable origin). The preparation does not contain flavour enhancers, colours, preservatives, gelatine, gluten, lactose, iodine, or cholesterol.

In addition to the formula diet, the participants in group F+P were given two placebo tablets which mainly contained cellulose (245 mg/2 tablets), calcium hydrogen phosphate (745 mg/2 tablets) and magnesium stearate (10 mg/2 tablets). There were no apparent differences in colour, size, shape, smell, and taste between the LA and placebo tablets. The participants were instructed to replace either lunch or dinner (depending on the daily routine) with the protein-rich formula diet. They also received recipe suggestions for the meal replacement drinks to have variation in the preparation. Breakfast and every other meal were prepared according to the principles of a balanced wholefood diet and based on national recommendations (German Nutrition Society). A recipe book adapted to suit the clinical trial was given to all the study participants to encourage healthy eating habits. Two tablets of either LA or P should be taken together with the correspondingly other main meal of the day (which could be either lunch or dinner). The required amount of investigational products in neutral packaging was provided to the study participants prior to the trial commencement and after six weeks. At the end of the study, the remaining tablets were counted to check compliance. Participants also received two sessions of nutrition education and six sessions of physical activity training as an adjunct to the intervention. Furthermore, all the study participants were given elastic bands (Therabands) including exercise handouts (information sheets and workout DVDs) to promote and support physical activity at home.

2.2 Questionnaires, Anthropometric Measurements and Body Composition

Anthropometric measurements were taken at each time point, in particular at baseline (week 0) and after 6 and 12 weeks (t_0, t_6, t_{12}). Height was measured using a stadiometer (SECA, Model 217, Seca Gmbh & Co Kg, Germany). Weight was measured with the participants dressed in light clothes and without shoes. An amount of 1 kg was deducted from the weight measured as an allowance for the clothing. Waist circumference (WC) was measured at the midpoint between the lower border of the rib cage and the top of the iliac crest. Hip circumference (HC) was defined as being the widest circumference over the buttocks. The measurements of the WC and HC were taken with the participants standing relaxed, breathing normally, with the measuring tape placed horizontally. Body composition was determined by means of bioelectrical impedance analysis (BIA) using a calibrated device, the Nutriguard-M (Data Input GmbH, Darmstadt, Germany).

2.3 Blood Sampling and Laboratory Procedures

Fasting blood samples (approximately 45 ml at each time point week 0, 6, 12) were collected using sealed Blood Collection Tubes and System S-Monovettes® (Sarstedt, Germany). The blood samples were centrifuged at 2000 x g for 10 minutes immediately after collection to prepare the plasma. Blood samples were allowed to clot for 20-30 minutes in the refrigerator and were subsequently centrifuged under the above-mentioned conditions to separate the serum. The serum triacylglycerol (TAG) concentration, total cholesterol (TC) and HDL cholesterol (HDL-C) levels were measured enzymatically (Beckman Coulter, Inc.). LDL

cholesterol (LDL-C) was calculated using the Friedewald equation. HbA1c was measured in EDTA whole blood using ion exchange chromatography (Bio-Rad Laboratories, Germany), which is a method of high-performance liquid chromatography (HPLC). Fasting plasma glucose measurements were performed using the hexokinase method, an ultraviolet (UV) enzymatic (*in vitro*) assay (Beckman Coulter, Inc.). Serum insulin concentrations were determined by immunoassays (cobas®, Roche Diagnostics, Mannheim, Germany).

2.4 Detection of Cardiovascular Risk Factors

The systolic (SBP) and diastolic blood pressures (DBP) were measured at rest using a digital device, Tensoval (Paul Hartmann AG, Heidenheim, Germany). Readings were taken three times at an interval of three minutes and at each time point (week 0, 6, 12). The mean value was calculated from this set of readings. Those whose blood pressure was ≥ 130 mmHg (SBP) and/or ≥ 85 mmHg (DBP) were defined as hypertensive. Those study participants with lower blood pressure levels and already diagnosed with hypertension and/or were taking antihypertensive medications (n = 22) were also classified as hypertensive. In total, 104 (86.7%) participants were hypertensive (n = 53 male, n = 51 female). The criteria used in the present study for defining the metabolic syndrome (MetS) according to Alberti et al. (2009) are shown in Table 2.

Table 2. Criteria for identification of the metabolic syndrome, defined by at least three of the five risk factors from Alberti et al. (2009)

Risk factor	Defining level
Elevated waist circumference	Men ≥ 102 cm
	Women ≥ 88 cm
TAG	≥ 150 mg/dl (1.7 mmol/l)
HDL-C	Men < 40 mg/dl (1.0 mmol/l)
	Women < 50 mg/dl (1.3 mmol/l)
Fasting glucose	≥ 100 mg/dl
Blood pressure	Systolic ≥ 130 and/or diastolic ≥ 85 mmHg

TAG: triacylglycerol; HDL-C: HDL cholesterol

2.5 Statistical Analysis

The statistical data analysis was carried out by using the Statistical Package for Social Sciences SPSS 19.0 (SPSS Inc., Chicago, Illinois, USA). The results were shown as the mean value ± standard deviation (s.d.). Differences between groups F+LA and F+P were calculated using the Mann-Whitney U test. The changes in the parameters in comparison with baseline were analysed using the Wilcoxon test. The chi-square test was used to compare the difference between the frequencies of the two groups. P values < 0.05 were interpreted as statistically significant. For data analysis within the framework of the population analysed, missing data were replaced by the last available values, provided at least one data value and the baseline value (t_0) could be obtained.

The Full Analysis Set (FAS) population (n = 106) comprised those participants with at least a baseline value (t_0) and at least one further measurement value. All 120 randomised participants were assessed on the basis of the Intention-To-Treat (ITT) analysis. Missing values were constantly updated in participants without any further values after the baseline measurements.

3. RESULTS

3.1 Baseline Characteristics

The data sets collected within the framework of this intervention study were obtained from 120 participants who were included in the study during the time period between February 15 and 26, 2010 (basic analysis). Subjects from the waiting list moved up if others did not fulfil the BMI criteria at the baseline visit. Sixty participants were randomised to group F+LA and another 60 participants to group F+P (ITT-Population). Fourteen participants (11.7%) who dropped out of the study before week 6 (t_6) because of intolerance to the study medication (n = 3), acute illnesses (n = 7) or other reasons were (n = 4) were excluded from the FAS evaluation.

Consequently, 106 participants were included in the FAS analysis: 52 of them received LA tablets (n = 27 male, n = 25 female) and 54, P tablets (n = 27 male, n = 27 female). A flowchart is depicted in Figure 1.

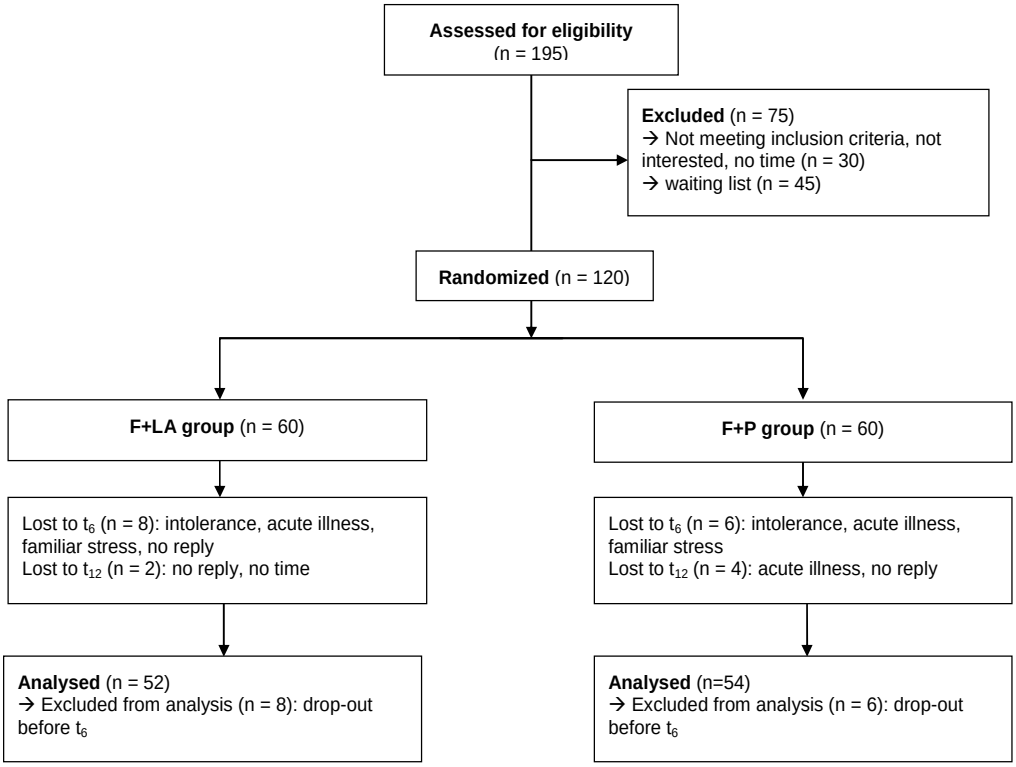

Figure 1. Flowchart

F+LA: formula diet + lipid-adsorbent tablets; F+P: formula diet + placebo tablets

There were no statistically significant differences between groups F+LA (n = 52) and F+P (n = 54) at the beginning of the trial with respect to the reported baseline parameters listed in Table 3. With trend significance, the F+P group has a higher age and DBP compared to the F+LA group. In view of the blood parameters, there were no significant differences between the groups at baseline (Table 5).

Table 3. Baseline participant's characteristics (full analysis set)

	Total group	F+LA group	F+P group	P*
Number of participants [n]	106	52	54	
Sex distribution [m/f]	54 / 52	27 / 25	27 / 27	0.843**
Age [years]	46.9 ± 7.2	45.4 ± 7.0	48.3 ± 7.1	0.061
Initial weight [kg]	97.0 ± 11.7	96.8 ± 12.8	97.1 ± 10.7	0.865
Height [m]	174.7 ± 9.8	174.5 ± 9.5	175.0 ± 10.1	0.884
BMI [kg/m²]	31.7 ± 1.95	31.7 ± 2.02	31.7 ± 1.9	0.987
WC [cm]	106.1 ± 8.1	105.6 ± 7.8	106.5 ± 8.3	0.702
HC [cm]	114.6 ± 4.8	114.1 ± 4.8	115.0 ± 4.9	0.390
SBP [mmHg]	138.5 ± 15.7	135.8 ± 13.1	141.0 ± 17.5	0.227
DBP [mmHg]	93.3 ± 11.0	90.9 ± 9.1	95.6 ± 12.3	0.060
MetS [%]	63.2 (n = 67)	63.5 (n = 33)	63.0 (n = 34)	

**Mann-Whitney U test; **chi-square test; BMI: body mass index; WC: waist circumference; HC: hip circumference; SBP: systolic blood pressure; DBP: diastolic blood pressure; MetS: prevalence of metabolic syndrome; F+LA: formula diet + lipid-adsorbent tablets; F+P: formula diet + placebo tablets*

3.2 Changes in Anthropometric Data

The anthropometric measurements observed in both the FAS and ITT populations for the two groups after an intervention period of twelve weeks confirmed the highly significant changes ($P < 0.001$; Wilcoxon test) (Table 4). Weight loss in the F+LA group was 0.74 kg more than in the F+P group, although the difference between the two groups turned out not to be statistically significant. Men decreased significantly more weight than women (-5.3 ± 4.5 kg vs. 3.6 ± 3.4 kg, $P < 0.05$; Mann-Whitney U test). Altogether, 95% of the participants in the allocated group (n = 101) in the FAS lost weight during the study period, 41% (n = 43) achieved a weight loss of around 5 kg or more. The proportion of about 46% (n = 24) in group F+LA was higher than in group F+P (35%, n = 19). However, this difference was not significant ($P = 0.283$; chi-square test). There were trends for greater improvements in further anthropometric parameters (e.g. BMI, WC) in the F+LA group relative to the F+P group.

After an intervention period of 12 weeks, a highly significant ($P < 0.001$; Wilcoxon test) reduction in systolic and diastolic blood pressures and in heart rate were seen in the whole study population (SBP $\Delta\ t_{12}$-t_0: -10.9 ± 11.0 mmHg; DBP $\Delta\ t_{12}$-t_0: -6.6 ± 7.0 mmHg; heart rate $\Delta\ t_{12}$-t_0: -5.7 ± 7.9 beats/min): in group F+LA (SBP $\Delta\ t_{12}$-t_0: -11.7 ± 10.0 mmHg; DBP $\Delta\ t_{12}$-t_0: -5.7 ± 6.8 mmHg; heart rate $\Delta\ t_{12}$-t_0: -4.5 ± 7.2 beats/min) and in group F+P (SBP $\Delta\ t_{12}$-t_0: -10.2 ± 12.0 mmHg; DBP $\Delta\ t_{12}$-t_0: -7.6 ± 7.2 mmHg; heart rate $\Delta\ t_{12}$-t_0: -6.7 ± 8.5 beats/min). There were no significant differences between the two groups.

3.3 Changes in Blood Parameters

The HbA1c levels ($P < 0.01$; Wilcoxon test), TC ($P < 0.001$; Wilcoxon test), LDL-C ($P = 0.001$; Wilcoxon test), and TAG levels ($P = 0.001$; Wilcoxon test) in group F+LA, as well as the FAS and ITT populations, were significantly reduced compared to baseline levels, while no significant changes were experienced in group F+P. When the intervention phase ended, group F+LA demonstrated significantly lower TC and LDL-C levels than group F+P ($P < 0.01$; Wilcoxon test). A significant reduction in insulin levels of around 30% ($P < 0.001$; Wilcoxon test) was measured in both groups. The changes in the measurement of biochemical parameters are shown in Table 5.

The percentage of study subjects with elevated TC levels (≥ 6.2 mmol/l) in the FAS population of group F+LA at the time of study entry, dropped from 53% (n = 26) to 25% (n = 12) during the course of the study, whereas the proportion in group F+P rose slightly (48%; n = 24 vs. 54%; n = 27, respectively). Similar changes were observed in the concentrations of LDL-C and TAG. The percentage of participants in group F+LA with elevated LDL-C (≥ 4.1 mmol/l) and TAG concentrations (≥ 1.7 mmol/l) dropped from 35% (n = 16) to 24% (n = 11) and from 49% (n = 24) to 35% (n = 17), respectively. No significant change was observed in the elevated LDL-C levels in group F+P. The percentage of participants with an elevated TAG level (≥ 1.7 mmol/l) in group F+P dropped from initially 52% (n = 26) to 44% (n = 22).

With regard to the metabolic syndrome (MetS, at least three out of five criteria), a reduction in the prevalence could be achieved in the whole study population (n = 106). The prevalence in group F+LA fell from 64% (n = 33) to 35% (n = 18) and the incidence could therefore be halved. The prevalence of MetS in group F+P decreased from an initial 63% (n = 34) to 44% (n = 24). The difference between the frequency in the two groups was not statistically significant (P = 0.301; chi-square test).

3.4 Adverse Events and Side Effects

Incidences of all kinds of adverse events (e.g. common cold) in both treatment groups were comparatively similar. However, there were no statistically significant differences between groups F+LA and F+P in the proportion of participants reporting adverse events (F+LA: 15 out of 60; 25% vs. F+P: 12 out of 60; 20%; P = 0.512; chi-square test).

At week 6 there were statistically significant differences between the F+LA and the F+P group in the proportion of participants with flatulence, diarrhea and constipation. While flatulence (n = 4) and diarrhea (n = 4) only occurred in the F+P group (P < 0.05; chi-square test), complaints of constipation were significantly more frequent in the F+LA group (n = 10 vs. n = 3; P = 0.032; chi-square test). Multiple answers were possible here. At the visit t_{12} no statistically significant differences were seen between the F+LA und F+P group. Effects such as bloating, nausea or eructation were rarely mentioned.

Table 4. Changes of anthropometric data in the FAS and ITT population after 12 weeks of intervention

	FAS					ITT				
	Participants	**t_0 mean ± SD**	**t_{12} mean ±SD**	**Δ t_{12}-t_0 mean ± SD**	**P#**	**Participants**	**t_0 mean ± SD**	**t_{12} mean ±SD**	**Δ t_{12}-t_0 mean ± SD**	**P#**
Weight	F+LA [n = 52]	96.8 ± 12.8	91.4 ± 12.2	-5.46 ± 3.83	**< 0.001**	F+LA [n = 60]	96.8 ± 12.2	92.1 ± 11.8	-4.73 ± 4.03	**< 0.001**
[kg]	F+P [n = 54]	97.1 ± 10.7	92.3 ± 10.1	-4.72 ± 3.88	**< 0.001**	F+P [n = 60]	96.9 ± 10.6	92.6 ± 10.1	-4.24 ± 3.94	**< 0.001**
P*		0.865	0.702	0.125			0.929	0.912	0.362	
BMI	F+LA [n = 52]	31.7 ± 2.0	29.9 ± 2.2	-1.76 ± 1.20	**< 0.001**	F+LA [n = 60]	31.8 ± 2.0	30.2 ± 2.3	-1.53 ± 1.27	**< 0.001**
[kg/m²]	F+P [n = 54]	31.7 ± 1.9	30.2 ± 2.2	-1.50 ± 1.17	**< 0.001**	F+P [n = 60]	31.9 ± 1.9	30.5 ± 2.3	-1.44 ± 1.23	**< 0.001**
P*		0.987	0.422	0.159			0.753	0.391	0.376	
WC	F+LA [n = 52]	105.6 ± 7.8	99.8 ± 7.3	-5.83 ± 3.34	**< 0.001**	F+LA [n = 60]	105.9 ± 7.8	100.9 ± 7.8	-5.05 ± 3.69	**< 0.001**
[cm]	F+P [n = 54]	106.5 ± 8.3	100.9 ± 8.5	-5.59 ± 3.82	**< 0.001**	F+P [n = 60]	106.3 ± 8.2	101.2 ± 8.4	-5.03 ± 4.00	**< 0.001**
P*		0.702	0.588	0.516			0.854	0.823	0.796	
HC	F+LA [n = 52]	114.1 ± 4.8	109.5 ± 4.8	-4.56 ± 3.32	**< 0.001**	F+LA [n = 60]	114.1 ± 5.0	110.2 ± 5.2	-3.95 ± 3.46	**< 0.001**
[cm]	F+P [n = 54]	115.0 ± 4.9	111.0 ± 5.0	-3.98 ± 3.32	**< 0.001**	F+P [n = 60]	115.2 ± 5.1	111.6 ± 5.5	-3.58 ± 3.37	**< 0.001**
P*		0.390	0.179	0.255			0.281	0.183	0.491	
WHR	F+LA [n = 52]	0.93 ± 0.07	0.91 ± 0.06	-0.02 ± 0.03	**< 0.001**	F+LA [n = 60]	0.93 ± 0.07	0.92 ± 0.06	-0.01 ± 0.03	**< 0.001**
[WC/HC]	F+P [n = 54]	0.93 ± 0.08	0.91 ± 0.07	-0.02 ± 0.03	**< 0.001**	F+P [n = 60]	0.92 ± 0.08	0.91 ± 0.07	-0.02 ± 0.03	**< 0.001**
P*		0.783	0.830	0.769			0.805	0.707	0.619	

**Mann-Whitney U test; #Wilcoxon test; FAS: Full Analysis Set (n = 106); ITT: intention-to-treat (n = 120); BMI: body mass index; WC: waist circumference; HC: hip circumference; WHR: waist-to-hip ratio; F+LA: formula diet + lipid-adsorbent tablets; F+P: formula diet + placebo tablets*

Table 5. Changes of blood parameters in the FAS and ITT population after 12 weeks of intervention

	FAS					ITT				
	Participants	**t_0 mean ± SD**	**t_{12} mean ± SD**	**Δ t_{12}-t_0 mean ± SD**	**P#**	**Participants**	**t_0 mean ± SD**	**t_{12} mean ± SD**	**Δ t_{12}-t_0 mean ± SD**	**P#**
Glucose	F+LA [n = 52]	5.16 ± 0.47	5.09 ± 0.55	-0.07 ± 0.43	0.109	F+LA [n = 60]	5.21 ± 0.50	5.15 ± 0.58	-0.06 ± 0.40	0.109
[mmol/l]	F+P [n = 54]	5.48 ± 0.85	5.54 ± 1.80	0.06 ± 1.26	0.264	F+P [n = 60]	5.43 ± 0.83	5.48 ± 1.71	0.06 ± 1.19	0.264
P*		0.115	0.084	0.769			0.442	0.354	0.722	
HbA1c	F+LA [n = 52]	5.54 ± 0.25	5.46 ± 0.20	-0.08 ± 0.18	**0.006**	F+LA [n = 60]	5.57 ± 0.28	5.50 ± 0.25	-0.07 ± 0.17	**0.006**
[%]	F+P [n = 53]	5.68 ± 0.48	5.67 ± 0.68	-0.004 ± 0.34	0.398	F+P [n = 59]	5.67 ± 0.47	5.68 ± 0.65	-0.003 ±0.32	0.398
P*		0.223	**0.027**	0.222			0.341	0.061	0.251	
Insulin	F+LA [n = 52]	95.5 ± 38.1	68.1 ± 35.6	-27.4 ± 29.9	**< 0.001**	F+LA [n = 60]	96.7 ± 36.3	72.9 ± 36.1	-23.7 ± 29.3	**< 0.001**
[pmol/l]	F+P [n = 54]	106.2 ± 61.3	74.6 ± 41.3	-31.6 ± 42.6	**< 0.001**	F+P [n = 60]	108.7 ± 60.6	80.2 ± 45.4	-28.5 ± 41.5	**< 0.001**
P*		0.517	0.509	0.820			0.493	0.558	0.644	
TC	F+LA [n = 52]	6.12 ± 1.00	5.66 ± 0.82	-0.45 ± 0.75	**< 0.001**	F+LA [n = 60]	6.09 ± 0.97	5.69 ± 0.80	-0.39 ± 0.72	**< 0.001**
[mmol/l]	F+P [n = 54]	6.43 ± 1.27	6.39 ± 1.34	-0.04 ± 0.79	0.783	F+P [n = 60]	6.37 ± 1.28	6.33 ± 1.34	-0.04 ± 0.74	0.783
P*		0.236	**0.007**	**0.011**			0.301	**0.017**	**0.021**	
HDL-C	F+LA [n = 52]	1.40 ± 0.32	1.37 ± 0.30	-0.03 ± 0.15	0.050	F+LA [n = 60]	1.39 ± 0.31	1.36 ± 0.29	-0.03 ± 0.14	0.050
[mmol/l]	F+P [n = 54]	1.42 ± 0.32	1.42 ± 0.29	0.00 ± 0.21	0.936	F+P [n = 60]	1.43 ± 0.32	1.43 ± 0.29	0.00 ± 0.20	0.936
P*		0.578	0.359	0.172			0.303	0.177	0.161	
LDL-C	F+LA [n = 50]	3.86 ± 0.87	3.56 ± 0.73	-0.30 ± 0.60	**0.002**	F+LA [n = 58]	3.85 ± 0.83	3.59 ± 0.71	-0.26 ± 0.56	**0.003**
[mmol/l]	F+P [n = 51]	4.13 ± 1.02	4.14 ± 1.07	0.01 ± 0.60	0.866	F+P [n = 57]	4.07 ± 1.05	4.08 ± 1.10	0.01 ± 0.56	0.866
P*		0.216	**0.047**	**0.013**			0.339	**0.023**	**0.022**	
TAG	F+LA [n = 52]	1.95 ± 1.13	1.68 ± 1.19	-0.27 ± 0.78	**0.001**	F+LA [n = 60]	1.92 ± 1.09	1.69 ± 1.14	-0.24 ± 0.73	**0.001**
[mmol/l]	F+P [n = 54]	2.10 ± 1.59	1.86 ± 1.09	-0.23 ± 1.32	0.167	F+P [n = 60]	2.05 ± 1.55	1.84 ± 1.09	-0.21 ± 1.25	0.167
P*		0.719	0.084	0.076			0.908	0.150	0.127	

**Mann-Whitney U test; #Wilcoxon test; FAS: Full Analysis Set (n = 106); ITT: Intention-to-treat (n = 120); TC: total cholesterol; HDL-C: HDL cholesterol; LDL-C: LDL cholesterol; TAG: triacylglycerol; F+LA: formula diet + lipid-adsorbent tablets; F+P: formula diet + placebo tablets*

4. DISCUSSION

The study results show that substantial weight loss, on average about 5 kg, can be achieved within twelve weeks by using the investigational product (formula diet) plus a lifestyle and nutritional programme. The administration of the lipid-adsorbent tablets (0.8 g polyglucosamine/day) with the concurrent use of a protein-rich formula diet (F+LA) led to significant changes in biochemical lipid and glucose parameters compared with placebo. There were also significant improvements in the parameters of the BMI, waist circumference, hip circumference, and blood pressure of both groups during intervention. However, the changes were not significantly different between the groups. The changes were also more favourable here for group F+LA, however without significant differences between the groups. The evaluations of all the results from the FAS population were principally confirmed by the more conservative approach of ITT.

Even though men had a significantly higher weight loss than women, the overall weight loss in both groups was comparable. This mainly arises from the stratification by gender which led to an equal distribution.

Both the usage of the formula diet on its own or taken in combination with polyglucosamine were effective for weight loss within the framework of this clinical investigation. It is probable that an increased intake of polyglucosamine L112, 1 x 2 tablets initially, then 2 x 2 tablets a day, may result in a more pronounced effect compared with placebo. It should be noted, however, that a higher dose could also cause more side effects such as constipation, mild nausea or flatulence.

According to the manufacturer's specifications, the recommended daily intake is 2 x 2 tablets (1.6 g polyglucosamine/day) for weight reduction. The number of lipid-adsorbent tablets (0.8 g polyglucosamine/day) ingested throughout the study is the recommended amount for weight maintenance.

The efficacy of polyglucosamine at different doses and also when administered concomitantly with a calorie-restricted diet was investigated in various studies (Sciutto and Colombo, 1995; Giustina and Ventura, 1995; Macchi, 1996; Colombo and Siutto, 1996; Veneroni et al., 1996; Schiller et al., 2001; Zahorska-Markiewicz et al., 2002; Mhurchu et al., 2004; Cornelli et al., 2008). The results showed that the additional administration of ß-1,4-poly-D-glucosamine within the scope of the common treatment for overweight and obesity (Caloric restriction) led to significantly more weight loss than with caloric restriction alone. In contrast, Pittler et al. (1999) reported that oral chitosan (1 g/day) did not reduce body weight in the absence of dietary alteration after 28 days of treatment.

In a Cochrane systematic review by Mhurchu et al. (2005), fourteen intervention studies including a total of 1131 participants were considered to assess the effects of chitosan on weight loss. Trials were only included if they were randomised controlled trials for a minimum of four weeks' duration. Study preparations containing chitosan achieved a significantly greater weight (-1.7 kg; $P < 0.001$), TC levels (-0.2 mmol/l; $P < 0.001$), SBP (-5.9 mmHg; $P < 0.001$) and DBP (-3.4 mmHg; $P < 0.001$) loss compared with placebo (Mhurchu et al., 2005). In previous clinical trials, the doses of polyglucosamine or chitosan administered were between 0.24 and 15.0 g per day (mean 3.7 g/day) and were, therefore, much higher than those provided during the intervention here (Macchi, 1996; Williams, 1998; Ho et al., 2001; Schiller et al., 2001; Zahorska-Markiewicz et al., 2002; Mhurchu et al., 2005; Mhurchu et al., 2004; Cornelli et al., 2008; Tapola et al., 2008; Anraku et al., 2009). In the study at hand, the

administration of polyglucosamine (0.8 g/day) showed a further slight (-0.74 kg) but not significant weight loss compared with placebo. This is not consistent with the findings in previous studies in which higher dosages were given. However, it should be noted that only a few publications are available that indicate a dose-response relationship (Jaffer and Sampalis, 2007).

According to Jull et al. (2008), there is some evidence that chitosan is more effective than placebo in the short-term treatment of obesity. But it was widely criticized that many trials mentioned above have been limited by poor quality (Mhurchu et al., 2005; Jull et al., 2008). Overall results from high quality trials only demonstrated minimal effect from chitosan on body weight. In this study, weight reduction may be mainly due to the effect of the high-protein formula diet. This confirms established findings that meal replacements are a valid alternative dietary strategy in the treatment of obesity (Keogh and Clifton, 2005).

However, the add-on intake of polyglucosamine had a more favourable effect on the parameters of the glucose metabolism compared with placebo in this clinical trial. While the insulin levels were significantly lowered in the two groups, a significant decrease in HbA1c levels was detected in group F+LA but not in the placebo group. This verifiable effect of polyglucosamine should be seen as a protective effect with regard to diabetes risk and the risk of developing metabolic syndrome. Additionally, it should be mentioned that baseline HbA1c levels were within normal range (4.1-6.2% laboratory reference value) for both groups. It is worth considering whether there are any benefits of HbA1c reduction within the normal range. In patients with higher initial HbA1c values, each 1% decrease in mean HbA1c was associated with risk reduction for diabetic complications of 21% for any diabetes endpoint (Stratton et al., 2000).

The combined approach to weight loss (F+LA) demonstrated in particular its favourable effect on blood lipid levels. The present intervention study showed that the additional intake of polyglucosamine had a significant hypocholesterolemic effect. TC, LDL-C and TAG levels were significantly lowered.

The participants in group F+LA benefited from the intake of polyglucosamine more, especially with regard to the cardiovascular risk, compared with group F+P. This additional benefit is indeed highly relevant with respect to the metabolic syndrome and related disorders. An improvement of the lipid profile with the use of polyglucosamine had already been observed in previous clinical studies (Ylitalo et al., 2002; Bokura and Kobayashi, 2003; Mhurchu et al., 2005; Cornelli et al., 2008). Therefore, the intake of polyglucosamine led to a significant lowering of the TC and LDL-C levels, a reduction of TAG concentrations and to an increase of HDL-C levels. Compared with Mhurchu et al. (2005) the reduction of total cholesterol levels in this study was apparently higher (-0.39 mmol/l vs. -0.15 mmol/l) despite a lower dose.

According to the EFSA, the cause and effect relationship between the consumption of chitosan and the maintenance of normal blood LDL-cholesterol concentrations has been established. The EFSA Panel considers that in order to achieve the claimed effect, 3 g of chitosan should be consumed daily (EFSA, 2011b).

The study is limited in its findings in the following ways:

There was no estimation of the energy and nutrient intake at baseline and after twelve weeks. Although physical activity has been monitored throughout the study, the actual

influence cannot be quantified. These factors could be act as confounders. The remaining powder of the formula diet was not weighed as there would have been great variability. To control compliance to the intake of the formula diet, concentration of 25-OH-D3 (Vitamin D) and folic acid were determined in the serum (data not shown). The nutrient supply for vitamin D and folic acid improved significantly in both groups with the intake of the formula. The changes in the two groups were not significantly different. Due to the number of dropouts, the sample size and possibly the effect size decreased. Probably more subjects should have been included at baseline.

This study may represent only a short-term effect over twelve weeks. With a view to lasting weight maintenance, long-term effects are required.

5. CONCLUSIONS

The clinical investigation demonstrates that the moderate application of a meal replacement strategy led to a significant loss of clinically relevant body weight within twelve weeks. The additional administration of lipid-adsorbent tablets containing polyglucosamine with a meal a day showed a further slight but not significant weight loss compared to placebo. More important than weight loss ought to be the fact, that this treatment method had favourable effects on carbohydrate and lipid metabolism and led to a significant reduction of HbA1c, insulin, TC, LDL-C, and TAG. Further examinations concerning a dose-response relationship are needed.

COMPETING INTERESTS

The authors declared no conflict of interest. The preparations used in this study were provided by Certmedica International GmbH, Aschaffenburg, Germany.

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