Effects of Weight Loss and Long-Term Weight Maintenance With Diets Varying in Protein and Glycemic Index on Cardiovascular Risk Factors

The Diet, Obesity, and Genes (DiOGenes) Study: A Randomized, Controlled Trial

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Background—We sought to separately examine the effects of either weight loss or diets varying in protein content and glycemic index without further changes in body weight on cardiovascular risk factors within the Diet, Obesity, and Genes study (DiOGenes).

Methods and Results—DiOGenes is a pan-European controlled dietary intervention study in 932 overweight adults who first lost body weight on an 8-week low-calorie diet and were then randomized to 1 of 5 ad libitum diets for 26 weeks. The diets were either high or low protein or high or low glycemic index in 4 combinations or control. Weight loss (−11.23 kg; 95% confidence interval, −11.54 to −10.92; $P<0.001$) reduced high-sensitivity C-reactive protein (−1.15 mg/L; 95% confidence interval, −1.30 to −0.41; $P<0.001$), low- and high-density lipoprotein cholesterol, triglycerides, and blood pressure. During the 26-week weight maintenance period in the intention-to-treat analysis, the further decrease of high-sensitivity C-reactive protein blood levels was −0.46 mg/L greater (95% confidence interval, −0.79 to −0.13) in the groups assigned to low-glycemic-index diets than in those on high-glycemic-index diets ($P<0.001$). Groups on low-protein diets achieved a −0.25 mg/L greater reduction in high-sensitivity C-reactive protein (95% confidence interval, −0.59 to −0.17) than those on high-protein diets ($P<0.001$), whereas lipid profiles and blood pressure were not differently affected.

Conclusions—This large-scale intervention study clearly separates weight loss from dietary composition–related effects. Low-glycemic-index carbohydrates and, to a lesser extent, low-protein intake may specifically reduce low-grade inflammation and associated comorbidities in overweight/obese adults.

Clinical Trial Registration—http://www.clinicaltrials.gov. Unique identifier: NCT00390637.

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Key Words: cardiovascular diseases ■ cardiovascular risk factors ■ C-reactive protein ■ diet ■ inflammation

Dietary composition significantly influences cardiovascular risk factors and outcomes under ad libitum (free access to food/diets and type of nutrients) conditions, as reported in several observational and intervention studies.[1–4] Although the role of different types of fat has been addressed in numerous studies, the roles of carbohydrate quality and of protein intake are less well established.

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Although a high glycemic index is related to postprandial hyperglycemia and a greater release of insulin, which may lead to an unfavorable impact on other cardiovascular risk factors,[5] a lower glycemic index was correlated with favorable effects on blood lipids, particularly low-density lipoprotein cholesterol and triglycerides and a lower level of high-sensitivity C-reactive protein (hsCRP) as a marker of low-grade inflammation.[6–8] hsCRP was shown to correlate with the risk of cardiovascular events and type 2 diabetes mellitus in prospective studies.[9,10]

Dietary protein improves postprandial satiety,[11] and higher intakes may be associated with improved weight maintenance.[12] There is an ongoing controversy about the effects of dietary protein on glucose metabolism. Several studies showed that dietary protein, apart from saturated fatty acids, which may accompany animal protein in meat and cheese, itself seems to cause insulin resistance in both cross-sectional[13] and experimental studies.[14–16] Other studies showed that an increased protein content of up to 30% of energy intake has beneficial effects on postprandial and fasting plasma glucose concentrations, particularly in insulin-resistant subjects.[17,18] Favorable effects of high-protein intake on blood pressure and blood lipids were described in subjects with elevated cardiovascular risk factors,[1] but effects on inflammatory markers were not investigated in large human studies.

The role of the glycemic index and protein intake on cardiovascular risk markers in healthy but overweight and obese subjects under non–energy-restricted conditions has not been investigated in a large cohort. The design of the Diet, Obesity, and Genes study (DiOgenes), a multicenter, pan-European study, imposed an initial weight loss period of at least 8% of body weight in overweight and obese subjects followed by a 26-week dietary intervention period serving as a weight maintenance phase. Four different diets were compared with either high or low glycemic index or protein content, and an additional healthy diet according to national guidelines served as a background control. Table 1 shows the targeted macronutrient composition of the diets. The participants were provided with commercially available food, recipes, cooking and behavioral advice, and a point-based teaching system to achieve the targeted macronutrient compositions.[19] The primary hypothesis of the study was that one of these diets may be more beneficial for weight maintenance after the initial weight loss period than the others. As recently published, the participants in the low-glycemic-index/high-protein group were able to maintain their weight most successfully during the 26-week diet intervention phase.[20]

Weight loss is known to be associated with substantial improvements in blood lipids and inflammatory biomarkers.[21] Our DiOgenes study design clearly separates effects from weight loss from those due to different dietary intakes in the weight maintenance period. Therefore, the aim of the present study was to investigate whether improvements in hsCRP, triglycerides, total cholesterol, high-density lipoprotein cholesterol, and low-density lipoprotein cholesterol as well as blood pressure after the initial weight loss period can be maintained or even further improved with diets differing in protein content and glycemic index and whether these diets elicit additional weight loss–independent effects.

Methods

Participants

The study protocol was approved by the local ethical committees of each center, and all subjects provided written informed consent. Volunteer families from 8 European countries (Netherlands, Denmark, United Kingdom, Greece, Spain, Germany, Bulgaria, and the Czech Republic) were enrolled from November 2005 to April 2007 by various recruitment strategies. Families (2-parent or single-parent) who were generally healthy with at least 1 parent overweight (body mass index $\geq$27 kg/m^2) and aged $<$65 years and with at least 1 child aged between 5 and 18 years were eligible for the study. Exclusion criteria for adults were body mass index $>$45 kg/m^2, liver or kidney diseases, cardiovascular diseases, diabetes mellitus (type 1 or type 2), special diets/eating disorders, systemic infections/chronic diseases, cancer within the last 10 years, weight change $>$3 kg within the previous 3 months, and other clinical disorders or use of prescription medication that might interfere with the outcome of the study. A detailed description of inclusion and exclusion criteria has been published.[19]

Study Design

After the first clinical investigation day (pre–low-calorie diet) with baseline measurements, eligible adults followed an 8-week low-calorie diet (Modifast, Nutrition et Santé, France) consisting of 800 kcal/d. In addition, 200 g of vegetables per day was allowed. Weight loss, compliance, and adverse events were checked at regular intervals during the low-calorie diet. Adults who achieved a weight loss of $\geq$8% after 8 weeks underwent the second clinical investigation day (post–low-calorie diet), and the respective family was randomized to 1 of 5 ad libitum diets differing in protein content and glycemic index: (1) low protein, low glycemic index; (2) low protein, high glycemic index; (3) high protein, low glycemic index; (4) high protein, high glycemic index; and (5) control diet according to accepted national dietary guidelines. Because we sought to specifically investigate the effects of low- versus high-glycemic-index diets as well as low- versus high-protein diets, the control diet served as a background healthy diet only. At the 8 participating study centers (Maastricht, Copenhagen, Cambridge, Heraklion, Potsdam, Pamplona, Sofia, and Prague), the participating families received dietary instruction for a 26-week period. Subjects were invited to the clinical investigation days after at least 10-hour overnight fasts and were asked to eat normally the day before the respective clinical investigation day (no alcohol consumption or exercise). A detailed description of the study design has been published.[19,20,22]

Dietary Intervention

Subjects were advised to maintain their achieved weight during the intervention. All subjects had to complete a 3-day dietary record at weeks 4 and 26 of the dietary intervention period and were given careful, intensive, and regular dietary and behavioral guidance in regard to both the macronutrient composition and the glycemic index of their diets. The targeted macronutrient compositions are shown in Table 1. To obtain the correct protein/carbohydrate ratios and glycemic indexes, a point-based system was developed.[19] The control diet is according to the guidelines of the respective national associations for nutrition. During the whole intervention period (including the low-calorie diet), the average amount of plant protein from total protein intake was 36%, with very small changes over time: 37% at screening, 36% at week 4, and 36% at week 26 of the dietary intervention. The urinary nitrogen excretion was measured to control for adherence to the targeted protein intake. The study was ad libitum concerning the individual choice of food from

Table 1. Targeted Composition of Nutritional Intake in the 5 Diet Groups During the 26-Week Diet Intervention Phase

	Low Protein		High Protein		
Component	Low Glycemic Index	High Glycemic Index	Low Glycemic Index	High Glycemic Index	Control (Healthy) Diet
Protein	10–15		23–28		12–15
Carbohydrate	57–62		45–50		55–63
Difference in glycemic index*	15 points		15 points		
Fat	23–28				25–30

Values, except points, are percentages of total energy intake.

*The difference in glycemic index in points is the targeted difference between the low-glycemic-index and the high-glycemic-index diet groups.

food lists/recommendations but was carefully controlled concerning the macronutrient composition of the respective diets and adherence to the dietary protocol. Detailed information on the strategy to manipulate ad libitum macronutrient intake and glycemic index has been published.[22]

Anthropometric Measurements, Blood Pressure, and Blood Parameters

Detailed descriptions of measurement of anthropometric parameters, blood pressure, and blood parameters are given in Methods in the online-only Data Supplement and a recent report of the Diogenes consortium.[19]

Statistical Analysis

Detailed information on the statistical analysis is given in Methods and Table II in the online-only Data Supplement.

In brief, results are presented as mean±SD. Statistical significance was defined as $P<0.05$. Statistical significance of changes in hsCRP, lipid profile parameters, and blood pressure was tested by applying 2-tailed Student *t* tests for unpaired samples. In the case of hsCRP, the matching of the diet groups during the low-calorie diet phase was tested by ANOVA without adjustment.

For hsCRP, triglycerides, and total, high-density lipoprotein, and low-density lipoprotein cholesterol concentrations as well as blood pressure, intention-to-treat analyses were performed including data from all 773 participants who underwent randomization by fitting linear mixed models, taking into account missing values from participants who dropped out and missing records.

For a sensitivity analysis on hsCRP data, records from all 773 randomized participants were included. For missing data from participants who withdrew during the dietary intervention, the baseline data at the time of randomization were carried forward. The same model as in the intention-to-treat analysis was calculated.

A completion analysis was performed including all 487 participants with data available at randomization and at the end of the intervention. Dietary effects on hsCRP during intervention were calculated by ANCOVA.

In the case of hsCRP, in which the diet term was significant for the predicted model (intention-to-treat, sensitivity, and completion analyses), significance between the main effects (glycemic index and protein) was tested by applying 2-tailed Student *t* tests for unpaired samples on the already adjusted model predictors.

The statistical analysis was performed with the use of IBM SPSS Statistics version 18.0 (IBM, Somers, NY).

Results

Study Progress

A total of 1209 adults (mean age, 41 years; body mass index, 34 kg/m^2) were screened. The participant flow through the study is presented in Figure 1. Of 773 adults who were randomized to the 5 different diets, 546 completed the study (71%). Details about dropouts are shown in Results in the online-only Data Supplement. The characteristics of the participants of the 5 dietary groups were not significantly different either at randomization or during the low-calorie diet phase (Table I in the online-only Data Supplement). Detailed information about dietary intake is given in the online-only Data Supplement.

Weight Change

Detailed data on the influence of the different diets on weight maintenance have been published.[20] In brief, the participants showed a substantial and significant reduction in body weight during the low-calorie diet phase of the study (−11.23 kg; 95% confidence interval [CI], −11.54 to −10.92; $P<0.001$). Only the low-protein, high-glycemic-index diet was associated with a subsequent weight regain (1.67 kg; 95% CI, 0.48–2.87; $P<0.05$), whereas in an intention-to-treat analysis, the weight regain was 0.93 kg less in high-protein versus low-protein diet groups (95% CI, 0.31–1.55; $P=0.003$) and 0.95 kg less in low-glycemic-index versus high-glycemic-index groups (95% CI, 0.33–1.57; $P=0.003$).

Blood Parameters

High-Sensitivity C-Reactive Protein

Before the low-calorie diet phase, hsCRP (mg/L) was slightly increased compared with routine reference clinical values in all diet groups (ie, hsCRP >3 mg/L; Table I in the online-only Data Supplement). The hsCRP values decreased significantly in all groups during the low-calorie diet period (low-protein, low-glycemic-index group, −1.41±5.55; low-protein, high-glycemic-index group, −0.87±3.55; high-protein, low-glycemic-index group, −1.29±2.84; high-protein, high-glycemic-index group, −1.23±2.90; control, −0.92±1.76; unadjusted $P<0.001$ each; Table I in the online-only Data Supplement) but did not differ significantly between diets, demonstrating that the groups were well matched (unadjusted $P=0.703$; Table I in the online-only Data Supplement). The subsequent 26-week randomized intervention period led to a further decrease in hsCRP, which appeared to be different in the distinct diet groups (Figure 2 and Table 2).

Intention-to-Treat Analysis

A linear mixed model was fitted. During the dietary intervention, the decrease in hsCRP of participants in the low-glycemic-index groups was −0.46 mg/L (95% CI, −0.79 to −0.13) greater than in the high-glycemic-index groups ($P<0.001$) and was −0.25 mg/L (95% CI, −0.59 to −0.17) greater in the low-protein groups than in the high-protein groups ($P<0.001$). There was no significant

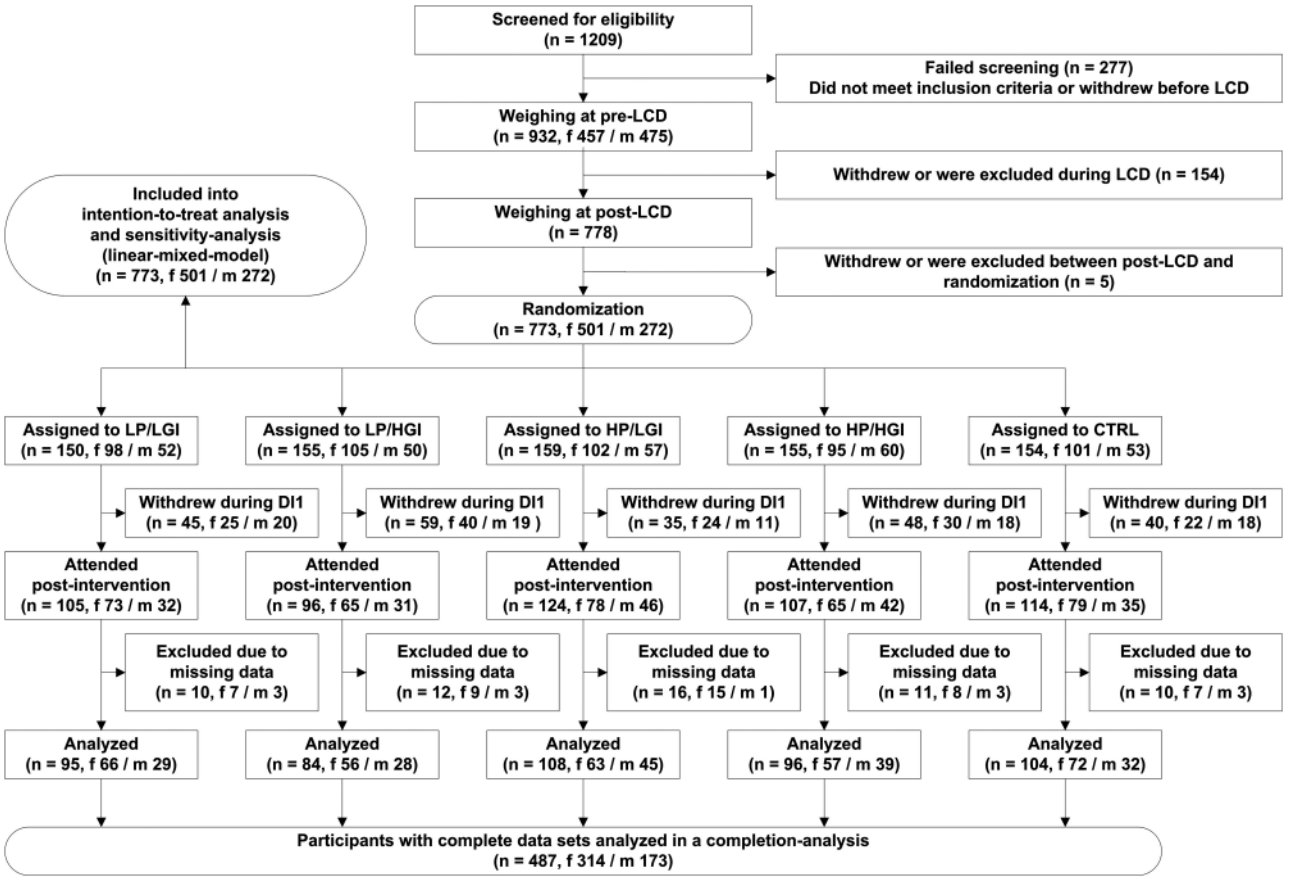

Figure 1. Organizational chart of participant flow through the study. Participants entering subsequent stages of the study as well as dropouts are indicated in total and separated by gender. LCD indicates low-calorie diet; f/m, female/male; HP, high protein; LP, low protein; HGI, high glycemic index; LGI, low glycemic index; CTRL, control, and DI1, diet intervention.

interaction between the low-glycemic-index and the low-protein groups (Table 3). A summary of all fitted models is shown in Table II in the online-only Data Supplement.

The low-glycemic-index groups were more likely to achieve an additional reduction of hsCRP concentration of >15% of the value at randomization than the high-glycemic-index groups (odds ratio, 1.57; 95% CI, 1.13–2.17; $P=0.007$). By contrast, achieving an additional decrease in hsCRP >15% by varying the protein content of the diet was unlikely (low protein: odds, 1.05; 95% CI, 0.79–1.39; high

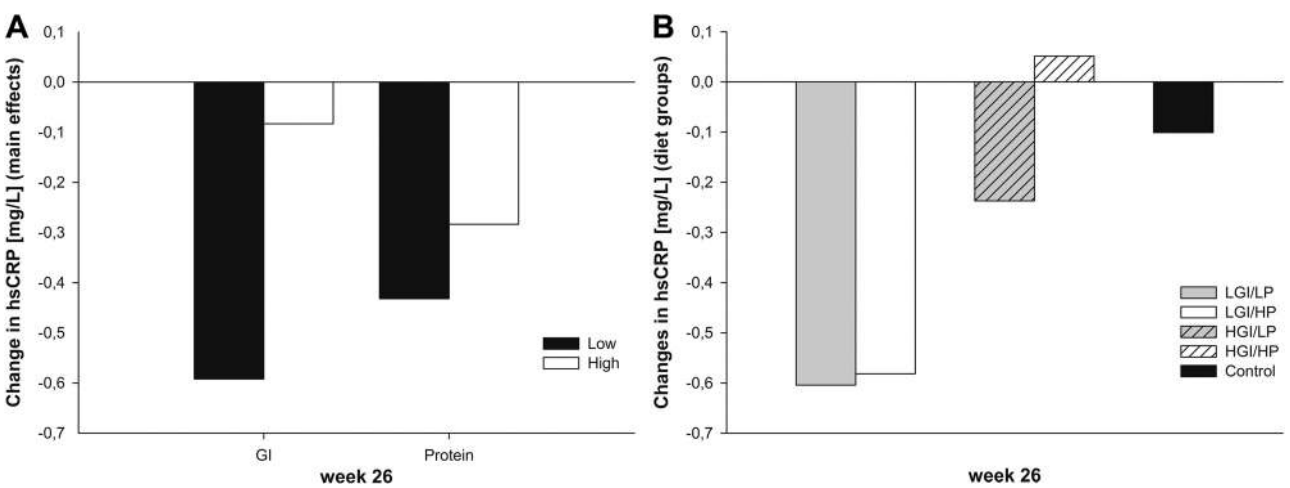

Figure 2. A, Changes of high-sensitivity C-reactive protein (hsCRP) between post–low-calorie diet (week 0) and postintervention (week 26). The values were normalized to post–low-calorie diet. For absolute values, see Tables I and II in the online-only Data Supplement. **B**, Changes of hsCRP between post–low-calorie diet (week 0) and postintervention (week 26) for the combined low-glycemic-index (LGI) diets (HP/LGI and LP/LGI) vs high-glycemic-index (HGI) diets (HP/HGI and LP/HGI) and for the combined high-protein (HP) (HP/HGI and HP/LGI) vs the low-protein (LP) diets (LP/LGI and LP/HGI).

Table 2. Blood Parameters and Anthropometric Measurements and Unadjusted Changes Between Randomization and Week 26 of the Diet Intervention Period

	Low Protein				High Protein					
	Low Glycemic Index		High Glycemic Index		Low Glycemic Index		High Glycemic Index		Control	
Variable	No. of Participants	Change From Randomization to Week 26	No. of Participants	Change From Randomization to Week 26	No. of Participants	Change From Randomization to Week 26	No. of Participants	Change From Randomization to Week 26	No. of Participants	Change From Randomization to Week 26
Blood parameters										
hsCRP, mg/L	95	−0.60±2.84*	84	−0.24±2.40	108	−0.58±1.81*	96	0.05±2.26	104	−0.10±2.01
Cholesterol, mmol/L	95	0.70±0.72*	84	0.79±0.87*	107	0.80±0.75*	95	0.64±0.66*	103	0.75±0.71*
High-density lipoprotein, mmol/L	95	0.23±0.21*	84	0.23±0.26*	107	0.21±0.24*	96	0.20±0.21*	104	0.19±0.21*
Low-density lipoprotein, mmol/L	93	0.40±0.53*	83	0.52±0.74*	108	0.50±0.63*	95	0.37±0.60*	104	0.47±0.61*
Triglycerides, mmol/L	93	0.13±0.53*	82	0.13±0.52*	107	0.19±0.51*	94	0.14±0.38*	102	0.19±0.41*
Systolic blood pressure, mm Hg	92	4.47±12.81*	80	5.12±10.67*	105	4.24±14.27*	93	2.72±13.39	101	4.50±12.90*
Diastolic blood pressure, mm Hg	92	1.01±7.89	80	3.55±7.68*	105	1.94±8.01*	93	0.96±7.99	102	3.49±8.02*
Fasting insulin, mU/L	81	0.79±8.28	69	3.17±9.30*	82	1.56±3.49*	80	1.32±5.23*	85	1.30±3.70
Insulin 120-min OGTT, mU/L	85	−7.73±31.38*	77	−4.31±32.91	104	−3.02±28.74	90	−7.62±32.01*	98	−3.40±30.36*
Fasting glucose, mmol/L	92	0.11±0.58	83	0.24±0.46*	103	0.10±0.60	94	0.08±0.71	103	0.14±0.44*
Glucose 120-min OGTT, mmol/L	85	−1.28±1.84*	79	−0.93±1.75*	102	−1.01±1.73*	88	−1.06±1.79*	98	−0.89±1.65*
Anthropometric measures										
Body weight, kg	95	0.27±5.01	84	1.45±5.34*	108	−0.38±6.28	96	0.36±5.41	104	0.55±4.46
Fat-free mass, kg	79	0.80±3.73	67	0.75±5.87	91	0.05±4.41	78	1.79±5.82*	86	1.58±3.83*
Fat mass, kg	79	−0.92±5.54	67	−0.40±4.96	91	−1.00±5.65	78	−0.20±7.52	86	−1.14±4.73*
Waist circumference, cm	93	0.09±7.07	81	0.89±6.84	104	0.49±6.82	95	−0.07±7.09	103	−0.67±6.45
Hip circumference, cm	93	−0.64±6.54	81	−0.15±6.45	104	−0.46±5.90	95	−0.47±6.23	103	−0.59±5.36
Sagittal diameter, cm	91	0.07±1.60	80	0.15±2.14	102	−0.12±1.98	91	−0.10±2.42	100	0.20±2.35

Values shown are mean±SD. Participants were included in this analysis if they had completed the diet intervention period and had complete records for high-sensitivity C-reactive protein (hsCRP) at the beginning (randomization) and the end of the diet intervention period. OGTT indicates oral glucose tolerance test.
*Significant changes (Student *t* test for paired samples, $P<0.05$).

protein: odds, 0.87; 95% CI, 0.66–1.15; odds ratio, 1.20; 95% CI, 0.87–1.66; $P=0.267$) (Table 3).

Sensitivity Analysis

In the sensitivity analysis, the decrease in hsCRP was −0.33 mg/L (95% CI, −0.65 to −0.02) greater in the low-glycemic-index versus the high-glycemic-index groups ($P<0.001$) and, as well, was −0.13 mg/L (95% CI, −0.45 to 0.19) greater in the low-protein versus the high-protein groups ($P=0.001$) and was therefore similar to that in the intention-to-treat analysis (Table 3).

Completion Analysis

In the completion analysis, only participants randomized to the low-glycemic-index diet groups had significantly reduced hsCRP concentrations after the 26-week intervention period (low glycemic index/low protein: −0.59 mg/L; 95% CI, −1.04 to −0.15; low glycemic index/high protein: −0.43; 95% CI, −0.89 to 0.03) (Table 2). ANCOVA confirmed the significantly different influences on hsCRP among the diets ($P<0.001$). The isolated effect on lowering hsCRP was more pronounced in the low-glycemic-index groups compared with the high-glycemic-index groups than in the low-protein groups compared with the high-protein groups (low glycemic index versus high glycemic index: −0.51 mg/L; 95% CI, −0.98 to −0.04; $P<0.001$; low protein versus high protein: −0.15 mg/L; 95% CI, −0.62 to 0.32; $P=0.037$) (Table 3).

Lipid Profile

Triglycerides, total cholesterol, high-density lipoprotein cholesterol, and low-density lipoprotein cholesterol decreased significantly in all groups after the low-calorie diet period ($P<0.001$; Figures 3 and 4 and Table I in the online-only Data Supplement) and increased again significantly during the 26-week dietary intervention period ($P<0.001$; Table 2 and Figures 3 and 4). Triglycerides tended to remain lower than at baseline (Figure 3A and Table 2). High-density lipoprotein cholesterol also decreased substantially during the low-calorie diet but then increased to significantly higher levels than before the low-calorie diet in all dietary groups ($P<0.001$) (Figure 4A and Table 2). Despite lower body weight at the end of the intervention, total cholesterol and low-density lipoprotein cholesterol returned to the baseline level after the intervention (Figures 3B and 4B and Table 2). The total cholesterol/high-density lipoprotein cholesterol ratio decreased significantly during the low-calorie diet period

Table 3. Lowering of hsCRP and Odds Ratios for Achievement of Additional Loss of >15% of hsCRP at Randomization During the 26-Week Diet Intervention Period*

Variable	Change in hsCRP mg/L, (95% CI)		Comparison of Glycemic-Index Groups Low Glycemic Index-High Glycemic Index, mg/L (95% CI)	P	Change in hsCRP, mg/L (95% CI)		Comparison of Protein Groups Low Protein-High Protein, mg/L (95% CI)	P	Control Change in hsCRP, mg/L (95% CI)
	Low Glycemic Index	High Glycemic Index			Low Protein	High Protein			
hsCRP decrease									
Intention-to-treat analysis	−0.64 (−0.88 to −0.41)	−0.18 (−0.41 to 0.05)	−0.46 (−0.79 to −0.13)	<0.001	−0.55 (−0.81 to −0.28)	−0.29 (−0.49 to −0.09)	−0.25 (−0.59 to −0.17)	<0.001	−0.16 (−0.45 to 0.12)
Sensitivity analysis	−0.41 (−0.63 to −0.18)	−0.07 (−0.29 to 0.15)	−0.33 (−0.65 to −0.02)	<0.001	−0.31 (−0.56 to −0.06)	−0.18 (−0.37 to 0.02)	−0.13 (−0.45 to 0.19)	0.001	−0.07 (−0.35 to 0.21)
Completion analysis	−0.59 (−0.92 to −0.27)	−0.08 (−0.42 to 0.26)	−0.51 (−0.98 to −0.04)	<0.001	−0.43 (−0.82 to −0.04)	−0.28 (−0.57 to 0.00)	−0.15 (−0.62 to 0.32)	0.037	−0.10 (−0.49 to 0.29)
	Odds (95% CI)		Odds Ratio for Low Glycemic Index vs High Glycemic Index (95% CI)		Odds (95% CI)		Odds Ratio for Low Protein vs High Protein (95% CI)		Odds (95% CI)
Achievement of additional loss of >15% of hsCRP at randomization									
Intention-to-treat analysis	1.21 (0.91 to 1.61)	0.77 (0.58 to 1.03)	1.57 (1.13 to 2.17)	0.007	1.05 (0.79 to 1.39)	0.87 (0.66 to 1.15)	1.20 (0.87 to 1.66)	0.267	0.77 (0.51 to 1.15)
Sensitivity analysis	0.60 (0.45 to 0.79)	0.39 (0.29 to 0.53)	1.52 (1.07 to 2.15)	0.019	0.46 (0.34 to 0.63)	0.51 (0.38 to 0.68)	0.91 (0.64 to 1.27)	0.593	0.54 (0.36 to 0.82)
Completion analysis	1.13 (0.81 to 1.59)	0.82 (0.57 to 1.17)	1.39 (0.93 to 2.08)	0.109	0.99 (0.69 to 1.42)	0.96 (0.69 to 1.35)	1.03 (0.69 to 1.54)	0.891	0.96 (0.60 to 1.54)

hsCRP indicates high-sensitivity C-reactive protein; CI, confidence interval.

*All 773 participants who passed the randomization were included into the intention-to-treat analysis and the sensitivity analysis. For the sensitivity analysis, we assumed that the hsCRP of participants who dropped out of the diet intervention period remained on the level at randomization. The completion analysis included 487 participants who had complete records for hsCRP at randomization and the end of the diet intervention period.

but showed no significant change during the 26-week dietary intervention (data not shown). Furthermore, changes during the dietary intervention showed no differences between groups in regard to the lipid profile. Similar to the analysis of hsCRP, we fitted a linear mixed model for triglycerides, total cholesterol, high-density lipoprotein cholesterol, and low-density lipoprotein cholesterol, which showed no significant diet term ($P>0.7$ for all parameters). A summary of all fitted models is shown in Table II in the online-only Data Supplement.

Blood Pressure

Both systolic and diastolic blood pressure decreased significantly during the low-calorie diet period and increased again in all dietary groups during the 26-week intervention period (Figure 5, Table 2, and Table I in the online-only Data Supplement).

Although there was no obvious difference between groups in increase of systolic blood pressure during the 26-week intervention period, the diastolic blood pressure tended to increase less in the low-protein, low-glycemic-index group compared with the low-protein/high-glycemic-index group (1.01 ± 7.89 versus 3.55 ± 7.68 mm Hg). Again, a linear mixed model failed to reach significance level for the diet term for both systolic blood pressure ($P=0.556$) and diastolic blood pressure ($P=0.098$). A summary of all fitted models is shown in Table II in the online-only Data Supplement.

Discussion

This dietary intervention study compared the subsequent effects of low or high glycemic index and protein content diets on cardiovascular risk factors in healthy overweight adults, after a substantial weight loss exceeding 8% of body weight. The energy-restricted period resulted in an expected and marked improvement of blood lipids, blood pressure, and hsCRP as a marker for inflammation.[23] Caloric restriction is known to be a strong activator of protective metabolic pathways, thereby leading to lower blood pressure, improved blood lipids, and reduced inflammatory markers, including hsCRP.[24–28] However, little is known about the effects of subsequent non–energy-restricted diets varying in protein content and glycemic index on these end points. Previous studies comparing different diets under ad libitum conditions were small and of short duration[29,30] or included patients with other comorbidities such as diabetes mellitus.[8] Moreover, the design of the DiOGenes study allowed a clear separation of the effects of caloric restriction from those of dietary composition.

The main outcome of this study was a significant further decrease of hsCRP after the initial weight loss, which was observed with the low-glycemic-index diets only and independent of protein content and small weight changes (Table 2 and Figure 1A). This might be related to expected reductions of postprandial glucose levels with low-glycemic-index diets, with glucose known to stimulate the expression of inflammatory genes by epigenetic mechanisms.[27,31,32] Furthermore, transient increases in glucose induce persistent changes in histone methylation patterns at promoters of inflammatory genes,[31–34] which is related to glucose-induced mitochondrial generation of oxygen radicals. Long-term increases in basal glucose concentrations are associated with both high fasting

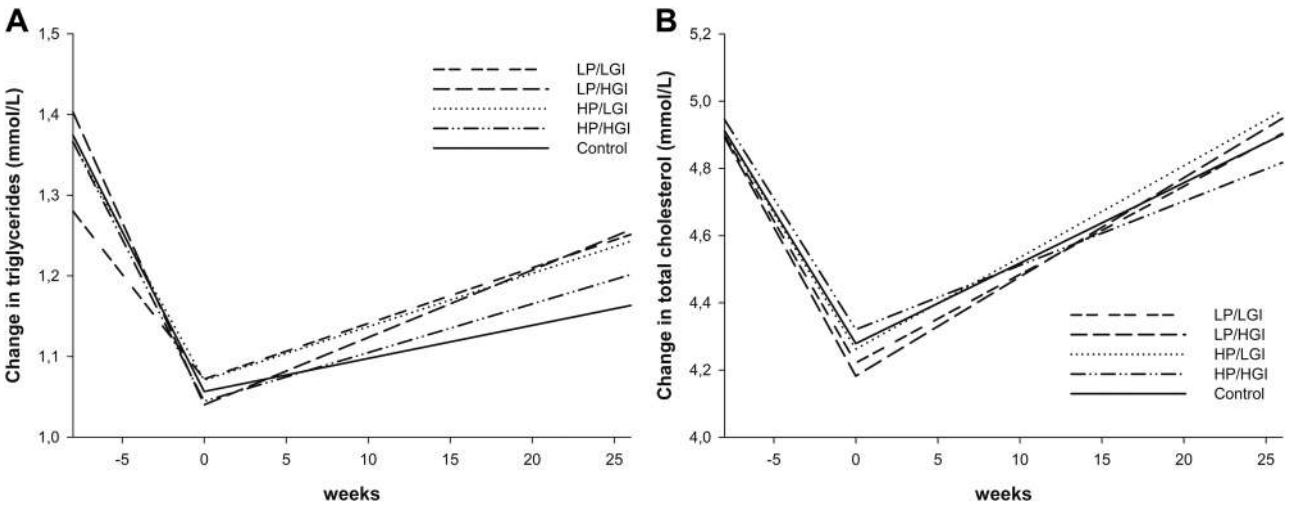

Figure 3. Changes of triglycerides (**A**) and total cholesterol (**B**) on the different diets between pre–low-calorie diet (week 8), post–low-calorie diet (week 0), and postintervention (week 26). LP indicates low protein; LGI, low glycemic index; HGI, high glycemic index; and HP, high protein.

insulin concentrations and insulin resistance, which is known to promote inflammatory processes and an increment of hsCRP.[35]

A low-glycemic-index diet was also associated with reduced levels of hsCRP in a prospective Canadian study in subjects with type 2 diabetes mellitus.[8] Moreover, a cross-sectional study in a Dutch population associated a low glycemic index with lower values of hsCRP.[36] Furthermore, in randomized controlled trials in overweight subjects, it was shown that diets based on low-glycemic-index carbohydrates produced better cardiovascular-related outcomes than conventional low-fat diets.[37] Otherwise, no significant effects on hsCRP and other cardiovascular risk factors were observed in shorter and smaller ad libitum studies combining low glycemic index and weight loss.[38]

The DiOGenes study goes beyond these observations: All diets supported the maintenance of reduced hsCRP concentrations as achieved by weight loss, with a background of a healthy, low-fat food pattern that was rich in vegetables and fruits in all groups. However, a further decrease in hsCRP was associated with a low glycemic index and, to a lesser extent, a lower protein intake only. Thus, repeated increases in postprandial blood glucose related to high-glycemic-index food components appear to play an important role in modulating hsCRP. The observation that the dietary protein content influences hsCRP values has not been reported previously. The effect was small but significant in all analyses, showing the robustness of this result. The higher protein content appeared to interfere with a further decrease of hsCRP compared with the low-protein diet. Since we have also shown recently that a high-protein diet in comparison with an isoenergetic carbohydrate-rich diet high in cereal fibers unfavorably influences whole-body insulin sensitivity in overweight and obese participants,[16] evidence is accumulating that high-protein diets may have less favorable metabolic effects in comparison to high-fiber and/or low-glycemic-index diets,[39] despite their known beneficial effects on weight loss and blood lipids.

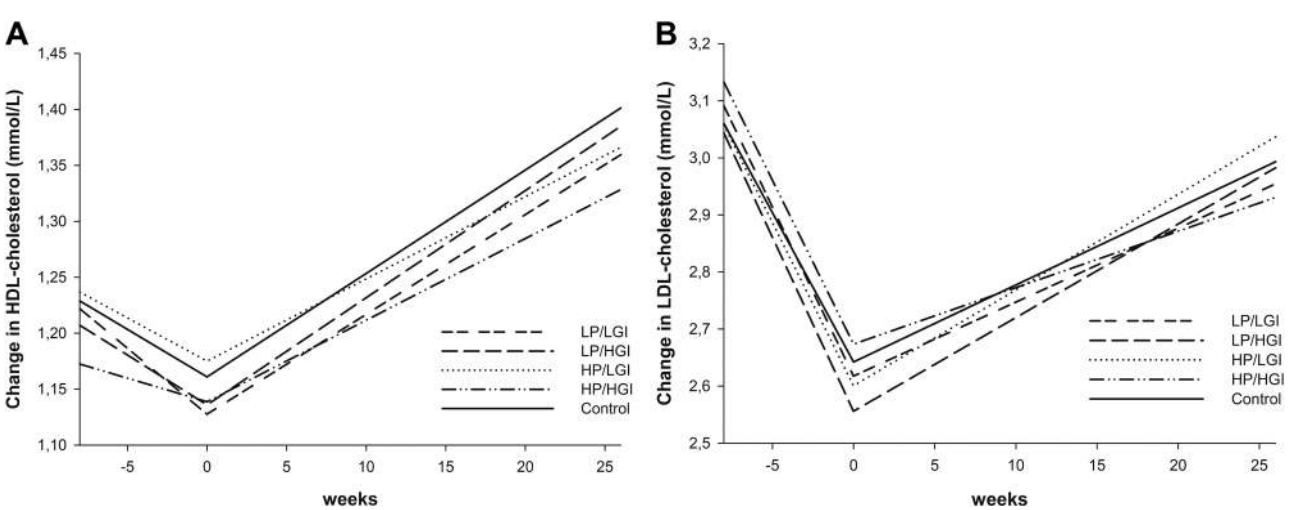

Figure 4. Changes of high-density lipoprotein (HDL) cholesterol (**A**) and low-density lipoprotein (LDL) cholesterol (**B**) on the different diets between pre–low-calorie diet (week 8), post–low-calorie diet (week 0), and postintervention (week 26). LP indicates low protein; LGI, low glycemic index; HGI, high glycemic index; and HP, high protein.

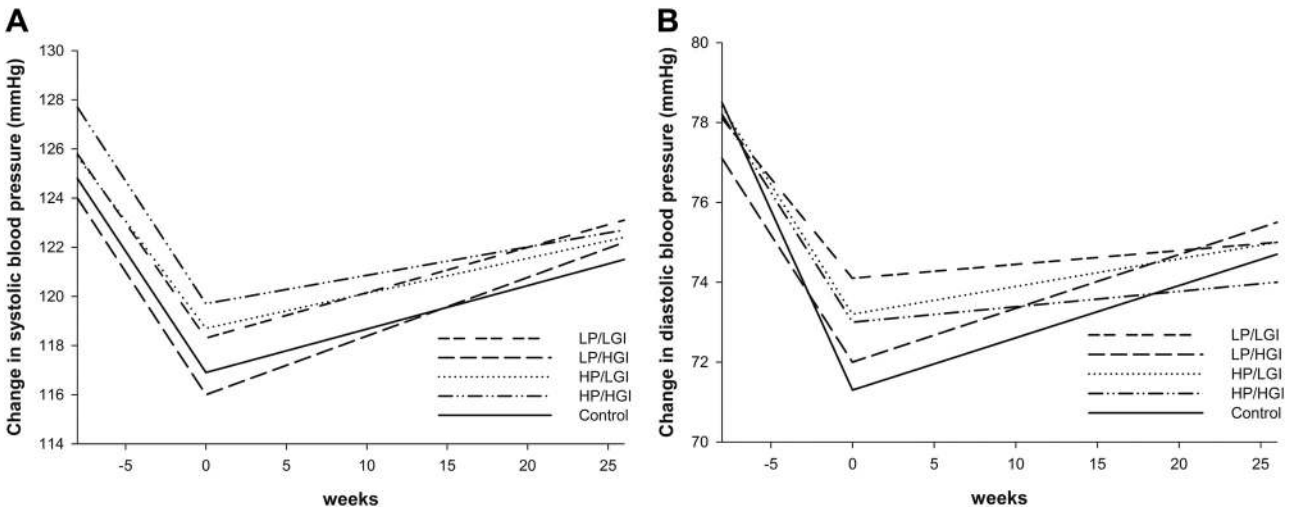

Figure 5. Changes of systolic (**A**) and diastolic (**B**) blood pressure on the different diets between pre–low-calorie diet (week 8), post–low-calorie diet (week 0), and postintervention (week 26). LP indicates low protein; LGI, low glycemic index; HGI, high glycemic index; and HP, high protein.

The initial low-calorie diets resulted in decreases of triglycerides, as expected.[40–43] The subsequent increase of triglycerides during the ad libitum food intake did not differ between the diets and remained below the initial levels. Thus, neither the glycemic index nor the protein content significantly influenced triglyceride levels under these conditions.

Changes in lipids were described in shorter-term studies that usually were associated with weight loss.[21] Remarkably, a 1-year study of diabetic patients treated with a low-glycemic-index diet similarly did not observe changes in triglyceride or cholesterol levels or hemoglobin A_{1c},[8] whereas cross-sectional studies typically observe an increase of triglycerides with higher glycemic index.[44] These associations thus appear to be related to the overall pattern of nutrition rather than the glycemic index only. However, improvements in blood lipids may also be expected because of the weight loss and the associated metabolic improvements of insulin resistance. Total and low-density lipoprotein cholesterol decreased markedly during the energy-restricted phase and then increased back to baseline levels and comparably in all dietary groups, which is in agreement with results from another long-term study in patients with type 2 diabetes mellitus.[8] By contrast, high-density lipoprotein cholesterol decreased slightly during the energy-restricted phase of the study but then increased comparably between groups and significantly during the diet phase to levels markedly above those before the low-calorie diet period at the start of the study, indicating an overall metabolic improvement due to weight loss and the healthy pattern of nutrition in all dietary groups. Cross-sectional studies reported higher levels of high-density lipoprotein cholesterol on low-glycemic-index diets, which again may be related to the general nutritional pattern rather than specifically to the low glycemic index.[36,42,44,45]

Systolic blood pressure decreased from normal values during weight loss but then increased to initial levels during the diet phases with no differences between diets. By contrast, diastolic blood pressure decreased during weight loss but did not return to the initial levels on all diets. Differences between diets were subtle, and the changes were not associated with protein content but may have been moderately influenced by the glycemic index.

Conclusions

In summary, our findings in this large and multinational cohort further confirm and substantially extend our knowledge regarding an overall benefit of a low-fat, low-glycemic-index food pattern. High-protein intake did not elicit relevant unfavorable effects on cardiovascular risk markers. However, a low-glycemic-index diet supported by a low-protein diet appears to further reduce hsCRP, and as such low-grade inflammation, even after a substantial reduction due to weight loss. These data therefore provide an important argument in favor of low-glycemic-index diets in obese healthy individuals.

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Disclosures

The European Union commission that decided on funding of this study has had no role in designing the study or in analyzing and interpreting the data. The authors report no conflicts.

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CLINICAL PERSPECTIVE

Food components are well known to affect cardiovascular risk, for which blood pressure, triglycerides, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, and the inflammatory marker C-reactive protein (CRP) are established biomarkers. In the present randomized, multicenter study, the separate effects of 11 kg weight loss achieved during an 8-week low-calorie diet as well as a subsequent 26-week intake of diets varying in protein and glycemic index on these biomarkers were studied. The choice of food was ad libitum but was strictly controlled by nutritional advice concerning the targeted fat and protein content as well as glycemic index. Expectedly, the initial weight loss significantly reduced systolic and diastolic blood pressure, triglycerides, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, and CRP. The subsequent consumption of different low-fat isocaloric diets resulted in moderate increases of blood lipids and blood pressure, which, however, were independent of the protein content and glycemic index of the diet. This clearly indicated that the beneficial effects on blood lipids and blood pressure were driven by the weight reduction itself but not by the dietary composition. In explicit contrast to the other biomarkers, consumption of low-glycemic-index diets led to a further decrease of CRP compared with high-glycemic-index diets. A low protein content enhanced the CRP-lowering effect, whereas a high protein content diminished it. Thus, the combination of low glycemic index and low protein intake appears to be most effective to reduce CRP, an established marker of low-grade inflammation and cardiovascular risk.

SUPPLEMENTAL MATERIAL

Supplemental Methods

Anthropometric measurements and blood pressure

Height was measured by using a stadiometer to the nearest 0.5cm and body weight by using a calibrated digital balance to the nearest 0.1kg. BMI was calculated by dividing the subject's weight (in kg) by the square of height (in meters). Waist circumference was taken midway between the lower rib and iliac crest at the umbilicus and hip circumference was taken at the largest circumference in the area around the buttocks (both measurements to the nearest 0.5 cm with the subject standing and breathing out). Systolic and diastolic blood pressure was measured three times by the same trained personnel always between 7:35 and 7:45 AM with an automatic device after at least 5 min while resting in a supine position according to WHO criteria. The mean value of the last two measurements was used. All measurements were performed according to the same standardized operating procedures in all participating centres.

Blood parameters

Blood was collected in SST vacutainers (glucose, lipids, hsCRP, insulin). After centrifugation (15 min at 2500 x g, at 22 ºC for gel tubes), plasma and serum were stored at -80 ºC until analysis. Analysis of all samples was performed at the Department of Clinical Biochemistry, Gentofte University Hospital, Denmark. Serum hsCRP was measured via an immunoturbidimetric assay (Roche Diagnostics), with use of monoclonal antibodies to hsCRP and a colorimetric assay (Roche Diagnostics), for the COBAS Integra 400 analyzer. Fasting insulin concentrations were measured by a solid-phase, two-site chemiluminescent immunometric assay (Siemens Medical Solutions Diagnostics, Ballerup, Denmark) for the IMMULITE 2500 analyzer. Fasting and OGTT serum glucose were analyzed by colorimetric assays (Ortho-Clinical Diagnostics, Johnson & Johnson, Birkerød, Denmark).

Fasting serum total cholesterol, HDL cholesterol and triglycerides were measured by routine enzymatic assays (Roche Diagnostics, Hvidovre, Denmark) in the COBAS Integra 400 analyzer. Serum low-density lipoprotein (LDL) cholesterol was calculated from total cholesterol, HDL cholesterol and triglycerides by the Friedewald equation.[1] Further details on methods have been reported elsewhere.[2]

Power calculation

The power calculation of the study estimated a sample size under the assumption that after the 26-week intervention, the smallest significant ($P < 0.05$) difference in weight change (estimated to 1.0 kg, standard deviation 2.01 kg) would be found between the low GI and the high GI diet groups with a power of 97%. It was estimated that a sample of 918 adults would be needed to detect a significant difference between the LGI and the HGI groups, assuming a dropout rate of 20% during the low calorie diet phase and a subsequent dropout rate of 15% during the 26-week diet intervention.[2]

Statistical analysis

Results are presented as means ± SD. Statistical significance was defined as $P<0.05$.

The dropout rates in the different diet groups were calculated by contingency tables and Fisher's exact test, the corresponding odds ratios by logistic regression.

Statistical significance of changes in hsCRP, parameters of lipid profile and blood pressure was tested by applying two-tailed Student's t-tests for unpaired samples. In case of hsCRP the matching of the diet groups during the LCD phase was tested by ANOVA without adjustment.

For the intention-to-treat analysis, data from all participants who underwent randomization were included. With respect to possible bias from different dropout rates among the diet groups, linear mixed models were fitted by top-down strategy and use of restricted maximum likelihood (REML) estimation to evaluate changes in hsCRP, triglyceride, total-, HDL- and LDL-cholesterol concentration as well as blood pressure. All outcome variables were

reasonably well behaved and showed a continuous and slightly skewed normal distribution. The untransformed variables were used for fitting the model since transformation of the variables did not result in a change of the respective model or its statistical significance. The intention-to-treat analysis gives unbiased results assuming missing data as missing-at-random.[3] This assumption seemed reasonable since missing data resulted both from participants dropping out before finishing as well as those finishing the study. In the latter case, i.e. missing blood samples, too small blood sample volume to measure all parameters or failed measurements were the reason. For the mixed model all available recordings were used from participants who were randomized to the intervention under the assumption that data of participants who dropped out during intervention followed the same course. Initially, the analyses were adjusted for the following covariates: age, BMI and body weight at pre-LCD, randomization and post-intervention, weight- and BMI-change during LCD and intervention, the outcome variable at randomization as well as their interaction terms. Furthermore, for the following factors was adjusted: diet group, sex, center type ("shop center" or "instruction center") and center (number of study-center) as well as their interactions terms. Statistically not relevant terms were successively excluded by calculation of the restricted -2 log likelihood and comparison by χ^2-test.

In the final model for the main outcome hsCRP the interactions between age and hsCRP at randomization as well as weight and hsCRP at randomization were included as covariates. As factors the diet group and the interactions between diet group and center as well as the interaction between diet group and sex were included. For missing values predictors were calculated by the model in an iteration process. The fitted models for all outcome variables regarding included factors, covariates and P-values for the diet-terms are summarized in Supplemental Table 2.

For a sensitivity analysis on hsCRP data, records from all participants who underwent randomization were included. The missing data from those participants who withdrew during the diet intervention period was filled in by carrying forward the baseline data at the time of randomization. The same model as in the intention-to-treat analysis was calculated.

A completion analysis was performed including all participants for whom data were available at randomization and at the end of the intervention. Effects on hsCRP during intervention were calculated by analysis of covariance (ANCOVA) adjusting for the same factors and covariates as in the intention-to-treat analysis.

In case of hsCRP, where the diet term was significant for the predicted model (intention-to-treat, sensitivity and completion analysis), significance between the main effects (GI and protein) was tested by applying two-tailed Student's t-tests for unpaired samples on the already adjusted model predictors.

The statistical analysis was performed using IBM SPSS Statistics version 18.0 (IBM, Somers, NY, USA).

Supplemental Results

Dropout rates

Between November 2005 and September 2006, 1209 adults (mean age 41 years, BMI 34 kg/m^2) were screened. A total of 932 adults initiated the LCD period, and 773 adults from 634 families were randomized to the 5 different diets. 546 adults (71%) remained at the end of the intervention period. After removal of cases lacking one or more hsCRP values, a data set of 487 adult subjects remained and will be referred to in the completion analysis.

The dropout rates in the LGI- and HP-groups were slightly lower than in the HGI/LP-group (29.8% and 30.6%, respectively, vs. 42.6%; P=0.007 and P=0.013 for both comparisons, respectively). The risk of dropout was lower in the LGI-group compared to HGI (odds ratio, 0.70; 95% CI, 0.50 to 0.98; P=0.036) and there was a trend to a lower risk for dropout in the HP- vs. LP-group (odds ratio, 0.75; 95% CI, 0.54 to 1.05; P=0.089).

Dietary intake

Detailed information on the distribution of energy intake from protein, carbohydrates, saturated fatty acids, monounsaturated fatty acids and polyunsaturated fatty acids for the different diet groups is given in Supplemental Table 3 and Supplemental Figure 5A-B. In the HP groups the amount of total energy consumed from protein was 5.4 percentage points higher and the amount of total energy consumed from carbohydrates was 6.7 percentage points lower than in the LP groups (P<0.001, respectively). In the HGI groups the mean GI was 5.1 units higher than in the LGI groups (P<0.001).

The participants adhered to the diet as was shown in detail previously.[4]

Supplemental Table 1. Characteristics of participants at beginning of the diet intervention period and changes between start of the low-calorie-diet (LCD) phase and randomization for the diet intervention.*

Variable	No. of Participants	Low Protein (N=179)		High Protein (N=204)		Control (N=104)	P-Value (Diet)	Total (N=487)
		Low Glycemic Index (N = 95)	High Glycemic Index (N = 84)	Low Glycemic Index (N = 108)	High Glycemic Index (N = 96)			
Age (yr)	487	42.1 ± 5.8	41.6 ± 5.9	42.6 ± 6.3	42.2 ± 5.6	42.8 ± 6.7	0.670	42.3±6.1
Height (cm)	487	170.5 ± 10.0	169.6 ± 7.8	170.5 ± 9.9	171.5 ± 9.8	169.9 ± 9.0	0.896	170.4±0.1
Body weight (kg)								
Mean	487	89.0 ± 15.8	86.1 ± 13.1	88.1 ± 14.6	88.7 ± 17.3	87.8 ± 16.4	0.598	88.0±15.5
Mean change during LCD phase	487	-11.5 ± 3.4	-10.6 ± 3.5	-11.2 ± 3.4	-11.2 ± 3.0	-11.5 ± 4.1	0.578	-11.2±3.5
Waist circumference (cm)								
Mean	477	96.7 ± 11.7	95.8 ± 10.5	96.5 ± 10.9	97.4 ± 13.6	96.8 ± 12.3	0.905	96.7±11.9
Mean change during LCD phase	477	-10.5 ± 4.9	-9.6 ± 4.7	-9.8 ± 4.7	-10.0 ± 4.8	-9.8 ± 5.3	0.977	-9.9±4.9
hsCRP (mg/L)								
Mean	487	2.96 ± 3.11	2.96 ± 2.84	2.93 ± 2.73	2.29 ± 1.94	2.50 ± 2.17	0.800	2.78±2.63
Mean change during LCD phase	487	-1.41 ± 5.55	-0.87 ± 3.55	-1.29 ± 2.84	-1.23 ± 2.90	-0.92 ± 1.76	0.703	-1.15±3.49
Cholesterol (mmol/L)								
Mean	487	4.14 ± 0.91	4.12 ± 0.92	4.17 ± 0.87	4.21 ± 0.98	4.13 ± 0.88	0.987	4.16±0.91
Mean change during LCD phase	480	-0.64 ± 0.85	-0.74 ± 0.68	-0.67 ± 0.76	-0.64 ± 0.73	-0.76 ± 0.64	0.785	-0.69±0.74
HDL (mmol/L)								
Mean	487	1.13 ± 0.28	1.17 ± 0.25	1.16 ± 0.29	1.14 ± 0.27	1.21 ± 0.26	0.105	1.16±0.27
Mean change during LCD phase	482	-0.09 ± 0.25	-0.08 ± 0.21	-0.07 ± 0.23	-0.04 ± 0.20	-0.08 ± 0.23	0.253	-0.07±0.23
LDL (mmol/L)								
Mean	485	2.54 ± 0.76	2.42 ± 0.79	2.54 ± 0.81	2.56 ± 0.81	2.49 ± 0.80	0.853	2.51±0.78
Mean change during LCD phase	478	-0.45 ± 0.69	-0.57 ± 0.60	-0.46 ± 0.65	-0.46 ± 0.64	-0.53 ± 0.56	0.611	-0.49±0.63
Triglycerides (mmol/L)								
Mean	484	1.06 ± 0.48	1.08 ± 0.55	1.03 ± 0.40	1.05 ± 0.42	0.95 ± 0.34	0.234	1.03±0.44
Mean change during LCD phase	482	-0.64 ± 0.85	-0.74 ± 0.68	-0.67 ± 0.76	-0.65 ± 0.73	-0.76 ± 0.64	0.785	-0.69±0.74
Systolic Blood Pressure (mmHg)								
Mean	477	118.6 ± 13.7	115.5 ± 13.9	118.8 ± 13.3	120.1 ± 15.0	116.8 ± 14.1	0.716	118.0±14.0
Mean change during LCD phase	469	-7.0 ± 10.1	-8.0 ± 10.1	-7.6 ± 12.0	-8.3 ± 13.1	-7.4 ± 11.4	0.918	-7.7±11.5
Diastolic Blood Pressure (mmHg)								
Mean	477	73.9 ± 9.4	71.4 ± 9.2	73.5 ± 9.6	73.4 ± 10.3	70.9 ± 10.1	0.428	72.6±9.8
Mean change during LCD phase	469	-4.3 ± 8.0	-5.1 ± 7.6	-5.5 ± 8.1	-5.7 ± 8.7	-7.1 ± 11.4	0.340	-5.6±8.9
Fasting Insulin (mU/L)								
Mean	422	10.17 ± 15.95	7.15 ± 4.10	6.51 ± 3.56	7.97 ± 6.12	8.04 ± 9.35	0.273	7.95±9.05
Mean change during LCD phase	407	-3.39 ± 7.33	-3.35 ± 3.99	-4.15 ± 5.02	-5.66 ± 13.04	-3.99 ± 6.65	0.393	-4.14±7.93
Insulin 120 min OGTT (mU/L)								
Mean	473	56.74 ± 42.81	50.78 ± 27.73	46.55 ± 24.80	52.11 ± 39.57	50.07 ± 31.19	0.643	51.09±33.78
Mean change during LCD phase	465	-15.32 ± 45.25	-10.86 ± 40.67	-20.87 ± 36.10	-16.73 ± 37.37	-16.01 ± 44.45	0.431	-16.23±40.84
Fasting Glucose (mmol/L)								
Mean	478	4.88 ± 0.63	4.69 ± 0.44	4.84 ± 0.55	4.83 ± 0.73	4.80 ± 0.53	0.252	4.81±0.59
Mean change during LCD phase	463	-0.33 ± 0.65	-0.18 ± 0.57	-0.27 ± 0.67	-0.22 ± 0.84	-0.27 ± 0.51	0.119	-0.26±0.65
Glucose 120 min OGTT (mmol/L)								
Mean	467	6.84 ± 2.07	6.75 ± 2.06	6.94 ± 2.10	6.70 ± 2.02	6.62 ± 1.76	0.310	6.77±2.00
Mean change during LCD phase	451	0.17 ± 2.14	0.37 ± 1.90	0.14 ± 1.98	0.14 ± 0.84	0.05 ± 1.65	0.891	0.17±1.92

* Values shown are means ± SD. Those participants of the study were included into this analysis, who completed the diet intervention period and had complete records for hsCRP at beginning of the low-calorie-diet phase and at the time of randomization.

Supplemental Table 2. Linear mixed models, fixed factors, random variables and P-values for all endpoints.

Endpoint	Regression-Model	Fixed	Random	P-Value for diet-term
Common	$Y_{ti} = \beta_1 \times X_{ti}^{(1)} + \beta_2 \times X_{ti}^{(2)} + \beta_3 \times X_{ti}^{(3)} + \cdots + \beta_p \times X_{ti}^{(p)} \Big\} \boldsymbol{fixed}$ $+u_{1i} \times Z_{ti}^{(1)} + \cdots + u_{qi} \times Z_{ti}^{(q)} + \varepsilon_{ti} \Big\} \boldsymbol{random}$			
hsCRP	$Y_{ti} = \beta_{diet} \times X_{ti}^{(diet)} + \beta_{diet*center} \times X_{ti}^{(diet*center)} + \beta_{diet*gender} \times X_{ti}^{(diet*gender)} \Big\} \boldsymbol{fixed}$ $+u_{crp2*age,i} \times Z_{ti}^{(crp2*age)} + u_{crp2*weight2,i} \times Z_{ti}^{(crp2*weight2)} + \varepsilon_{ti} \Big\} \boldsymbol{random}$	diet diet*center[a)] diet*gender	crp2*age[b)] crp2*weight2	GI: <0.001 Prot: <0.001 Total: <0.001
Cholesterol	$Y_{ti} = \beta_{diet} \times X_{ti}^{(diet)} + \beta_{center*gender} \times X_{ti}^{(center*gender)} \Big\} \boldsymbol{fixed}$ $+u_{age,i} \times Z_{ti}^{(age)} + u_{weight2,i} \times Z_{ti}^{(weight2)} + \varepsilon_{ti} \Big\} \boldsymbol{random}$	diet center*gender	age weight2[c)]	GI: 0.725 Prot: 0.828 Total: 0.703
HDL-Cholesterol	$Y_{ti} = \beta_{diet} \times X_{ti}^{(diet)} + \beta_{gender} \times X_{ti}^{(gender)} \Big\} \boldsymbol{fixed}$ $+u_{age,i} \times Z_{ti}^{(age)} + \varepsilon_{ti} \Big\} \boldsymbol{random}$	diet gender	age	GI: 0.900 Prot: 0.685 Total: 0.593
LDL-Cholesterol	$Y_{ti} = \beta_{diet} \times X_{ti}^{(diet)} + \beta_{gender} \times X_{ti}^{(gender)} \Big\} \boldsymbol{fixed}$ $+u_{age,i} \times Z_{ti}^{(age)} + \varepsilon_{ti} \Big\} \boldsymbol{random}$	diet gender	age	GI: 0.926 Prot: 0.898 Total: 0.730
Triglycerides	$Y_{ti} = \beta_{diet} \times X_{ti}^{(diet)} + \beta_{partner} \times X_{ti}^{(center)} + \beta_{gender} \times X_{ti}^{(gender)} \Big\} \boldsymbol{fixed}$ $+u_{weight2,i} \times Z_{ti}^{(weight2)} + \varepsilon_{ti} \Big\} \boldsymbol{random}$	diet center gender	weight2	GI: 0.966 Prot: 0.691 Total: 0.832
Systolic Blood Pressure	$Y_{ti} = \beta_{diet} \times X_{ti}^{(diet)} + \beta_{center*gender} \times X_{ti}^{(center*gender)} \Big\} \boldsymbol{fixed}$ $+u_{age,i} \times Z_{ti}^{(age)} + \varepsilon_{ti} \Big\} \boldsymbol{random}$	diet center*gender	age	GI: 0.668 Prot: 0.159 Total: 0.556
Diastolic Blood Pressure	$Y_{ti} = \beta_{diet} \times X_{ti}^{(diet)} + \beta_{center*gender} \times X_{ti}^{(center*gender)} \Big\} \boldsymbol{fixed}$ $+u_{age,i} \times Z_{ti}^{(age)} + \varepsilon_{ti} \Big\} \boldsymbol{random}$	diet center*gender	age	GI: 0.492 Prot: 0.363 Total: 0.098

a) Center: index of center where participants took part in the study; b) crp2: hsCRP at randomization; c) weight2: body weight at randomization
GI: high or low glycemic index; Prot: high or low protein; Total: LGI/LP, LGI/HP, HGI/LP, HGI/HP or control diet;
Y_{ti}: predicted value of outcome variable at time-point t for the i-th participant; X_{ti}: measured value of a fixed factor at time-point t for the i-th participant; β_p: regression term of the p-th fixed factor; Z_{ti}: measured value of a random effect at time-point-t for the i-th participant; u_{qi}: regression term of the q-th random effect for the i-th participant; ε_{ti}: residual at time-point t for the i-th participant.

Supplemental Table 3. Energy and nutrient intake between screening and week 26 of the diet intervention period.*

Variable	Low Protein				High Protein				Control	
	Low Glycemic Index		High Glycemic Index		Low Glycemic Index		High Glycemic Index			
	no. of participants	*intake*	*no. of participants*	*intake*	*no. of participants*	*intake*	*no. of participants*	*intake*	*no. of participants*	*intake*
Energy and nutrient intake										
Energy (kj/day)										
At screening	90	9075 ± 3388	75	9752 ± 3529	102	9657 ± 2868	90	9492 ± 3311	95	9843 ± 2924
Change from screening to wk 26	67	-2218 ± 3734	52	-2046 ± 3210	82	-2259 ± 2759	67	-2609 ± 2603	72	-2350 ± 3401
Carbohydrates (% of total energy intake)										
At screening	90	42.2 ± 9.0	75	44.7 ± 8.6	102	43.7 ± 8.8	90	45.2 ± 7.3	95	43.7 ± 8.4
Change from screening to wk 26	67	9.0 ± 8.6	52	6.0 ± 10.1	82	1.4 ± 10.7	67	0.5 ± 7.4	72	2.8 ± 9.1
Total fat (% of total energy intake)										
At screening	90	37.4 ± 7.8	75	36.3 ± 7.4	102	36.1 ± 7.5	90	36.3 ± 6.7	95	36.5 ± 6.8
Change from screening to wk 26	67	-7.7 ± 8.8	52	-5.5 ± 10.4	82	-4.9 ± 9.6	67	-5.9 ± 8.1	72	-3.4 ± 9.1
Saturated fat (% of total energy intake)										
At screening	80	13.2 ± 4.2	70	12.6 ± 3.8	89	12.7 ± 3.9	80	12.8 ± 3.9	84	12.6 ± 4.2
Change from screening to wk 26	57	-5.1 ± 5.5	47	-3.9 ± 6.0	70	-4.5 ± 6.2	58	-6.0 ± 5.0	62	-4.0 ± 6.0
Monounsaturated fat (% of total energy intake)										
At screening	80	12.4 ± 5.5	70	10.8 ± 4.6	89	11.0 ± 4.7	80	11.6 ± 5.2	83	11.1 ± 4.3
Change from screening to wk 26	57	-5.0 ± 6.7	47	-3.1 ± 5.5	70	-3.1 ± 5.3	58	-4.6 ± 5.6	62	-4.0 ± 5.5
Polyunsaturated fat (% of total energy intake)										
At screening	80	8.4 ± 5.5	70	8.0 ± 5.6	89	7.7 ± 4.7	80	7.8 ± 4.7	83	8.1 ± 4.8
Change from screening to wk 26	57	-2.7 ± 5.8	47	-2.1 ± 4.6	70	-2.2 ± 4.4	58	-2.7 ± 3.7	62	-2.7 ± 4.7
Protein (% of total energy intake)										
At screening	90	18.3 ± 5.2	75	17.0 ± 4.0	102	17.5 ± 4.0	90	16.0 ± 3.6	95	16.5 ± 4.2
Change from screening to wk 26	67	-0.3 ± 4.7	52	-0.7 ± 4.9	82	4.2 ± 4.5	67	6.4 ± 6.0	72	1.8 ± 5.1
Glycemic index										
At screening	90	61.0 ± 5.7	75	60.7 ± 4.7	102	61.1 ± 5.2	90	61.4 ± 4.4	95	61.3 ± 4.9
Change from screening to wk 26	67	-4.7 ± 6.8	52	0.3 ± 5.6	82	-4.9 ± 6.9	67	0.3 ± 6.0	72	-2.6 ± 6.2

* Values shown are means ± SD. Participants were included in this analysis if they had completed the diet intervention period and had complete records for hsCRP at the beginning (randomization) and the end of the diet intervention period.

Legends to Supplemental Figures

Supplemental Figure 1: Relative changes (%) of hsCRP between post-LCD (week 0) and post-intervention (week 26) (A). The values were normalized to post-LCD. For absolute values see Supplemental Table 1 and Table 2. Relative (%) changes of hsCRP between post-LCD (week 0) and post-intervention (week 26) (B) for the combined low GI diets (HP/LGI and LP/LGI) *vs.* high GI diets (HP/HGI and LP/HGI) and for the combined high protein (HP/HGI and HP/LGI) *vs.* the low protein diets (LP/LGI and LP/HGI).

Supplemental Figure 2: Relative changes (%) of triglycerides (A) and total cholesterol (B) on the different diets between pre-LCD (week 8), post-LCD (week 0) and post-intervention (week 26). The values were normalized to post-LCD.

Supplemental Figure 3: Relative changes (%) of HDL-cholesterol (A) and LDL-cholesterol (B) on the different diets between pre-LCD (week 8), post-LCD (week 0) and post-intervention (week 26). The values were normalized to post-LCD.

Supplemental Figure 4: Relative changes (%) of systolic (A) and diastolic (B) blood pressure on the different diets between pre-LCD (week 8), post-LCD (week 0) and post-intervention (week 26). The values were normalized to post-LCD.

Supplemental Figure 5: (A) Protein intake of the participants at week 4 of the intervention and post-intervention (week 26) shown for the combined low protein (LP/HGI and LP/LGI) *vs.* the combined high protein diets (HP/LGI and HP/HGI). (B) GI of consumed carbohydrates at week 4 of the intervention and post-intervention (week 26) shown for the combined low GI diets (HP/LGI and LP/LGI) *vs.* the combined high GI diets (HP/HGI and LP/HGI).

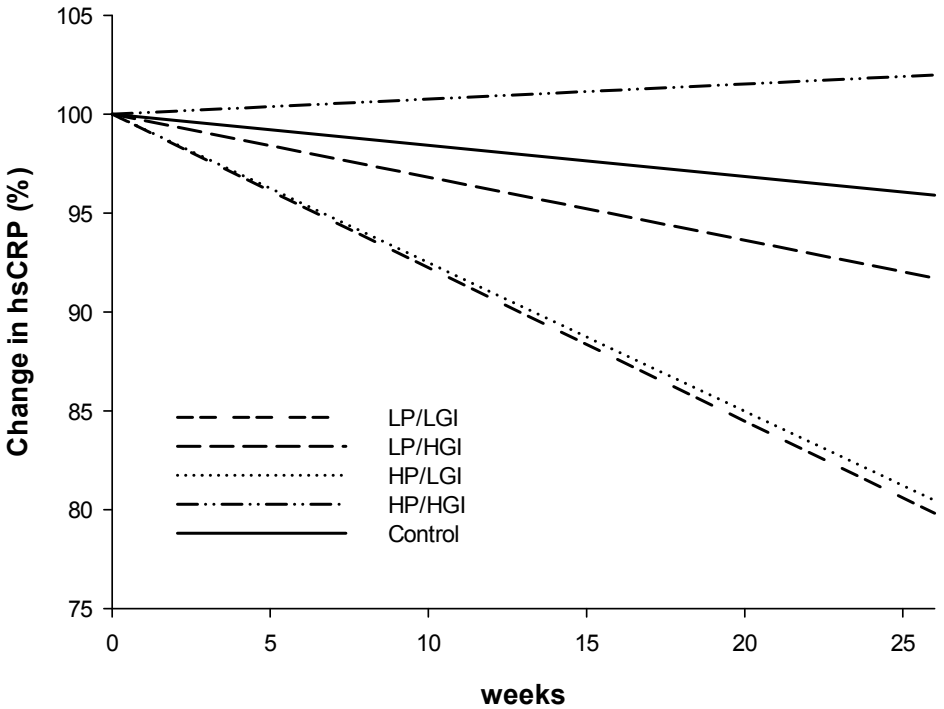
Supplemental Figure 6: Forest diagrams of triglycerides, LDL-cholesterol, HDL-cholesterol,

total cholesterol, hsCRP, fasting glucose, glucose at 120 min of oral glucose tolerance test (OGTT), fasting insulin, insulin at 120 min of OGTT, diastolic blood pressure, systolic blood pressure, sagittal diameter, hip circumference, waist circumference, fat mass, fat-free mass and body weigth. For absolute values refer to Table 2 of the main manuscript.

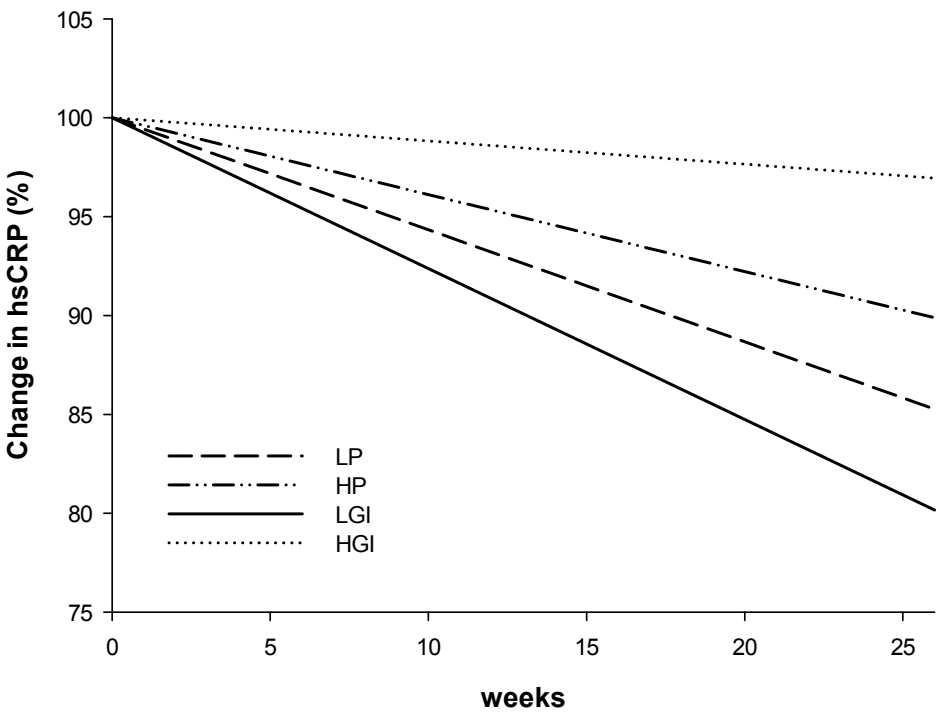
Supplemental Figure 7: Parameters of glucose metabolism pre-LCD (CID1), post-LCD (CID2) and post-intervention (CID3). (A) Fasting glucose, (B) glucose at 120 min of OGTT, (C) fasting insulin, and (D) insulin at 120 min of OGTT. None of these parameters indicates hyperglycemic states or impaired glucose tolerance of the participants.

Supplemental Figure 1

A)

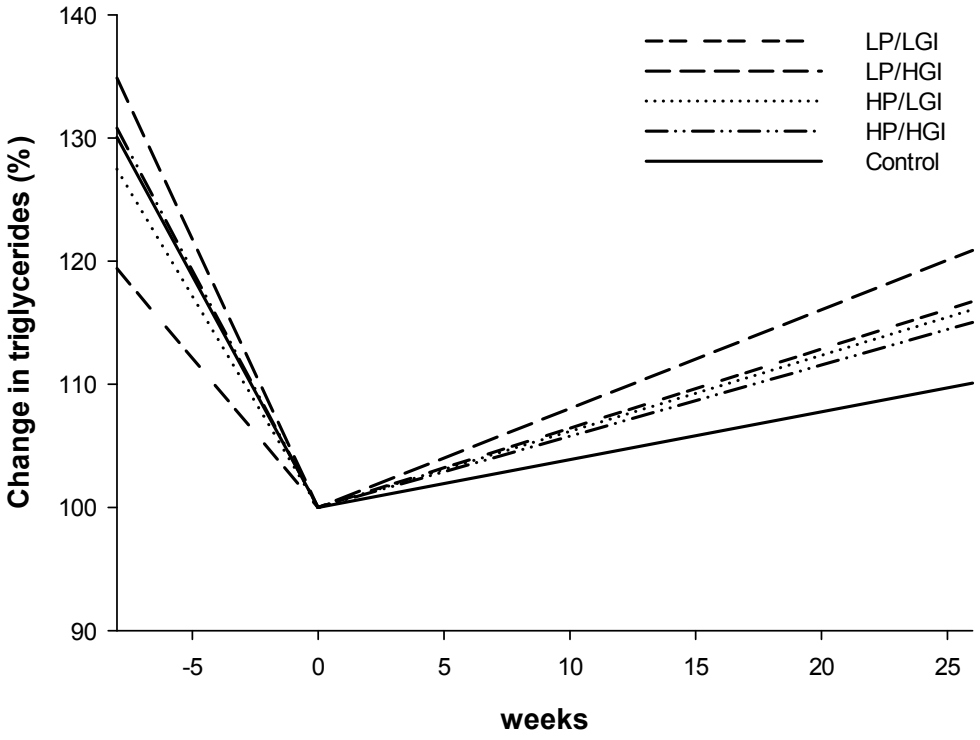


B)



Supplemental Figure 2

A)



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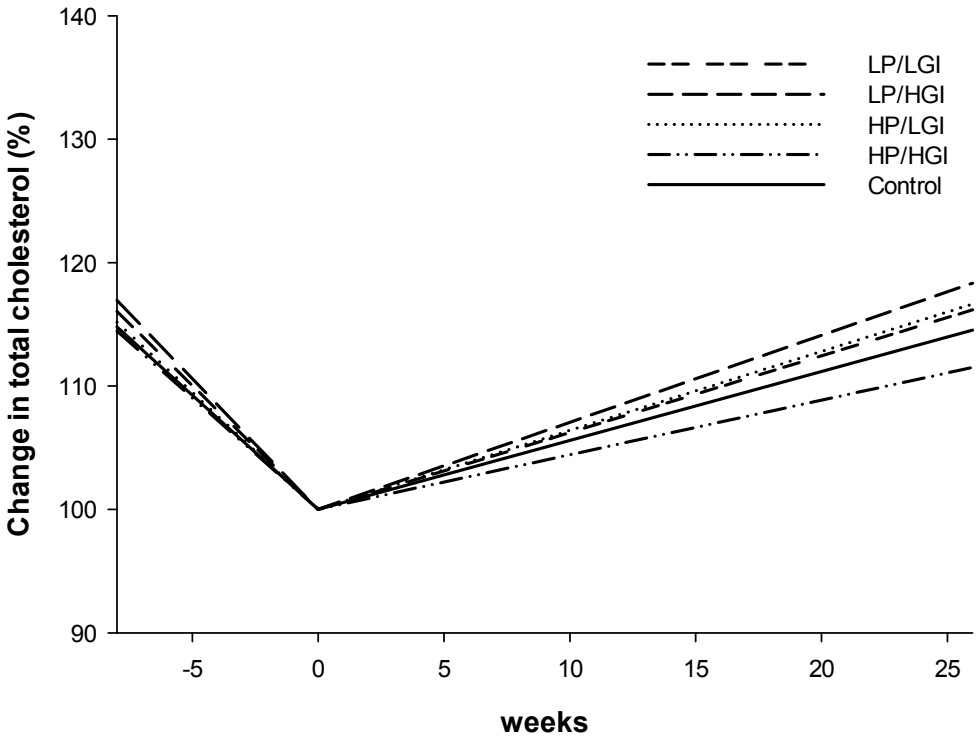
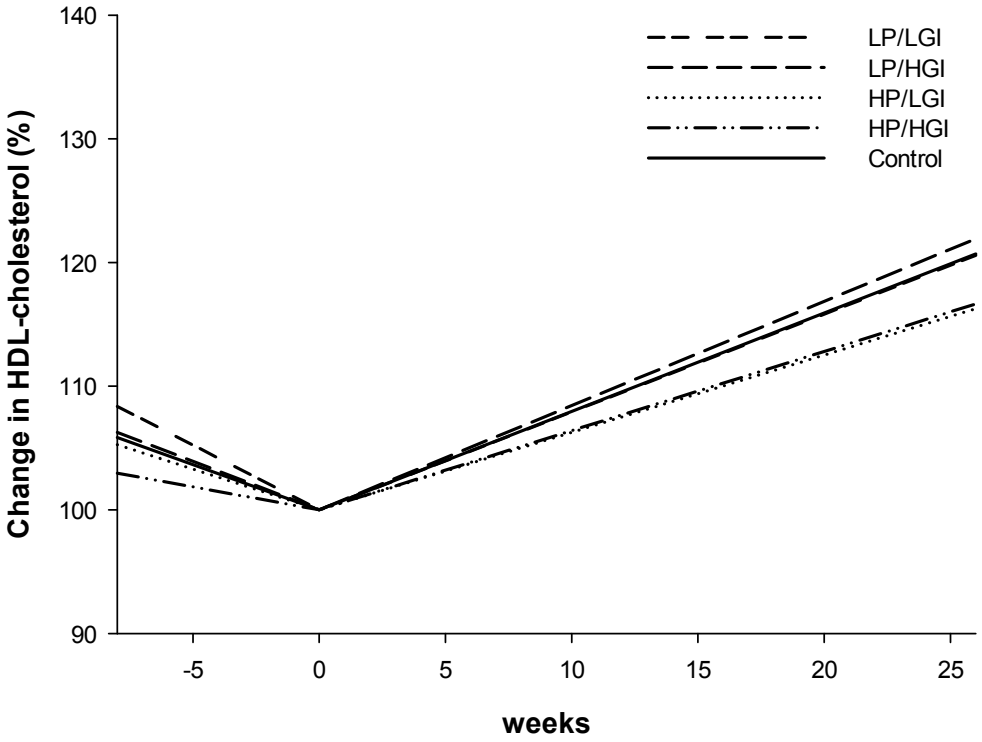
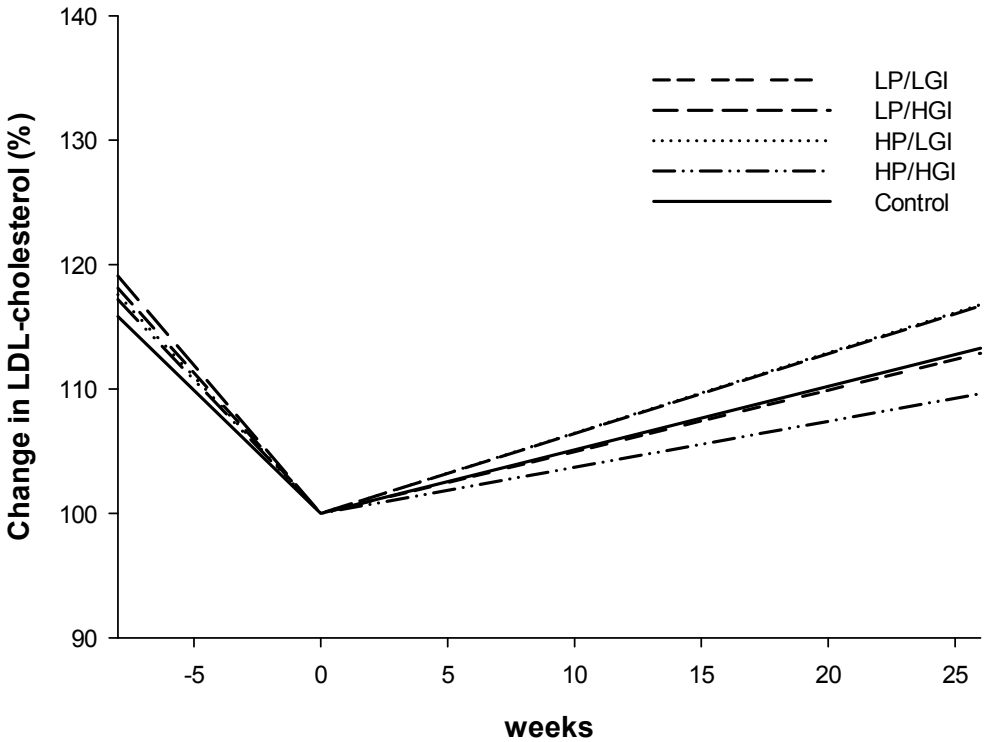


Supplemental Figure 3

A)

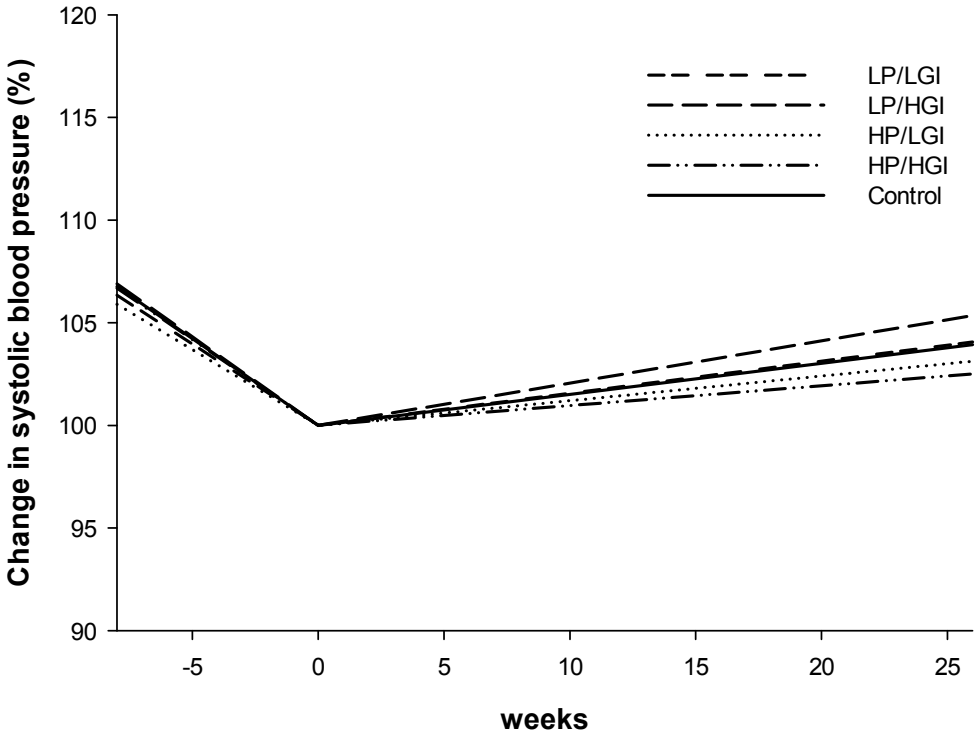


B)



Supplemental Figure 4

A)



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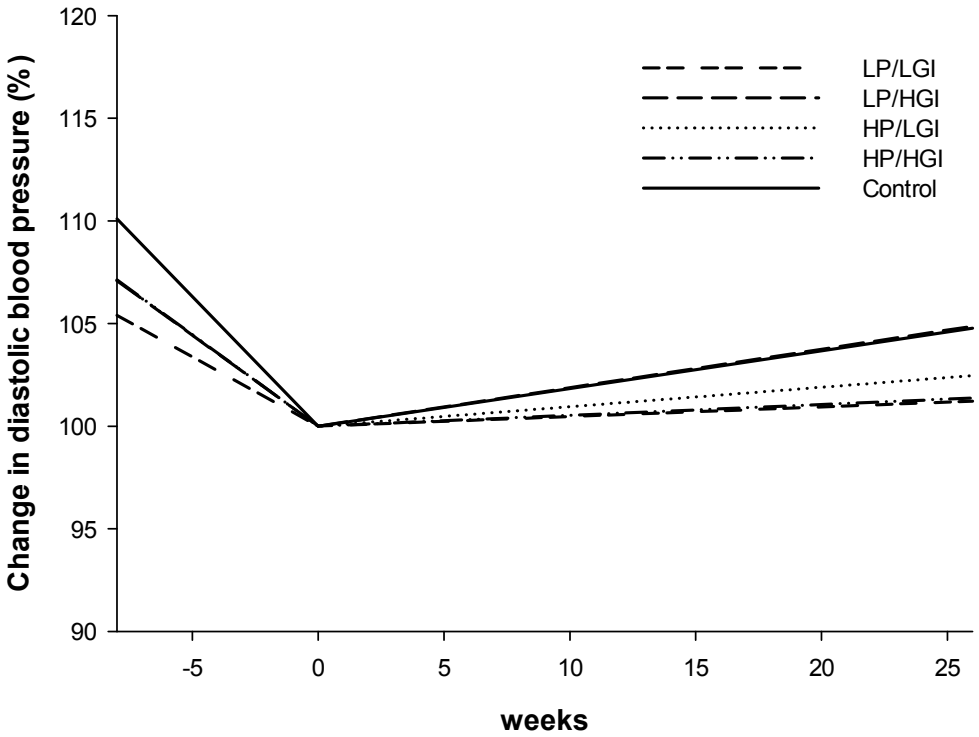
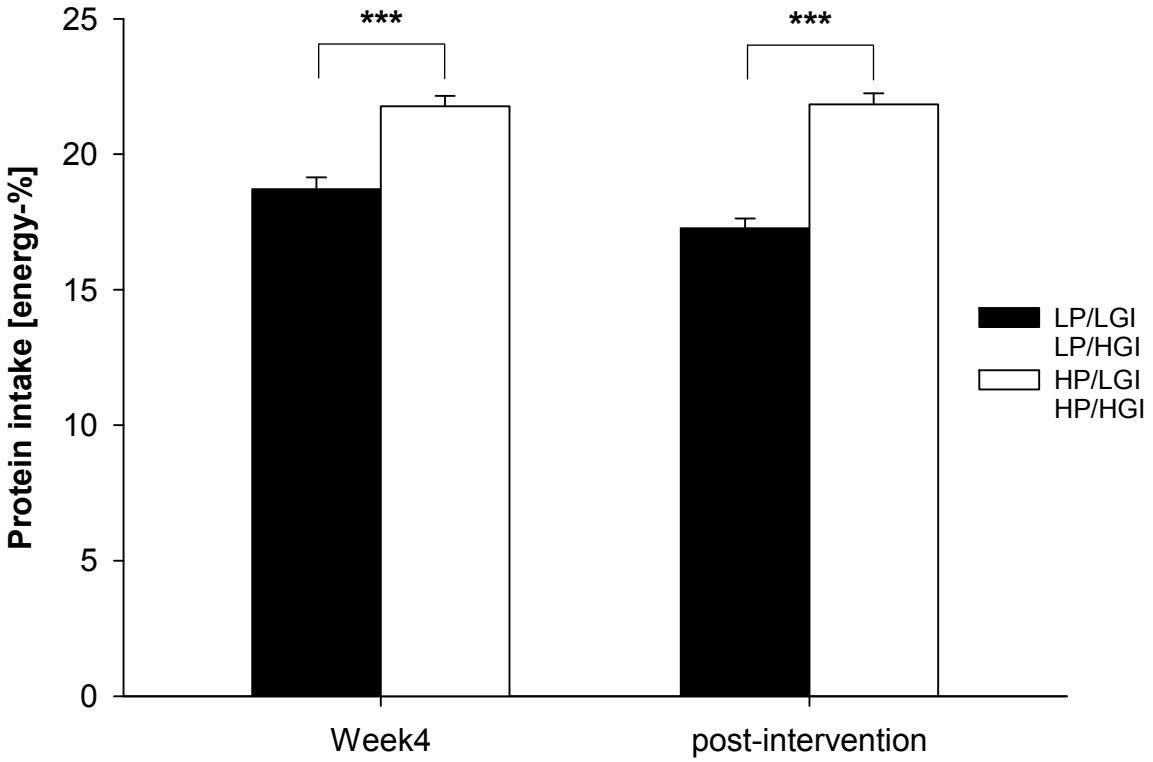
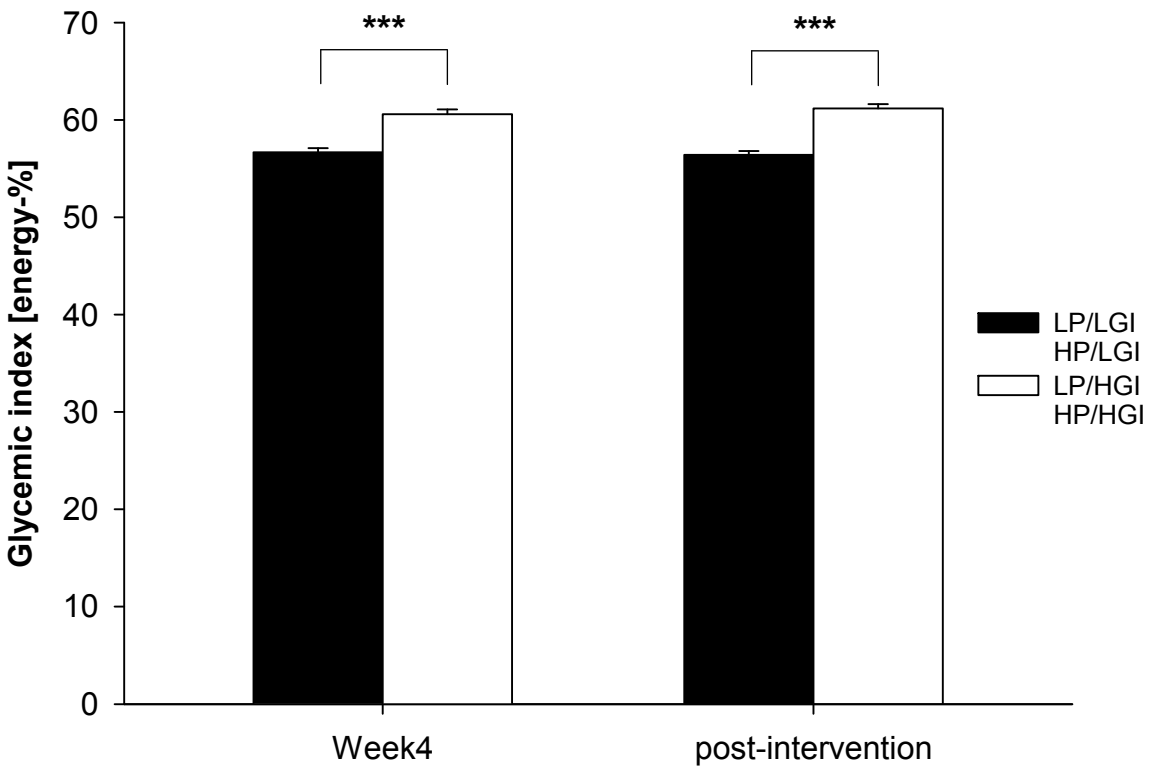


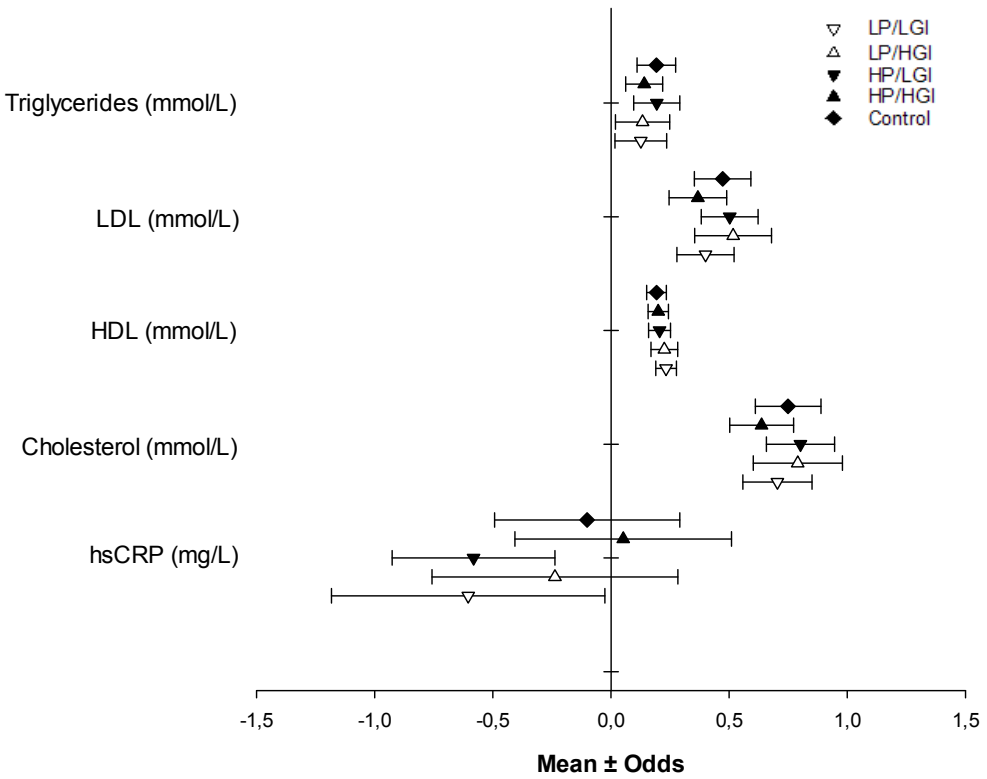
Supplemental Figure 5A. Protein intake of participants at week 4 and post-intervention

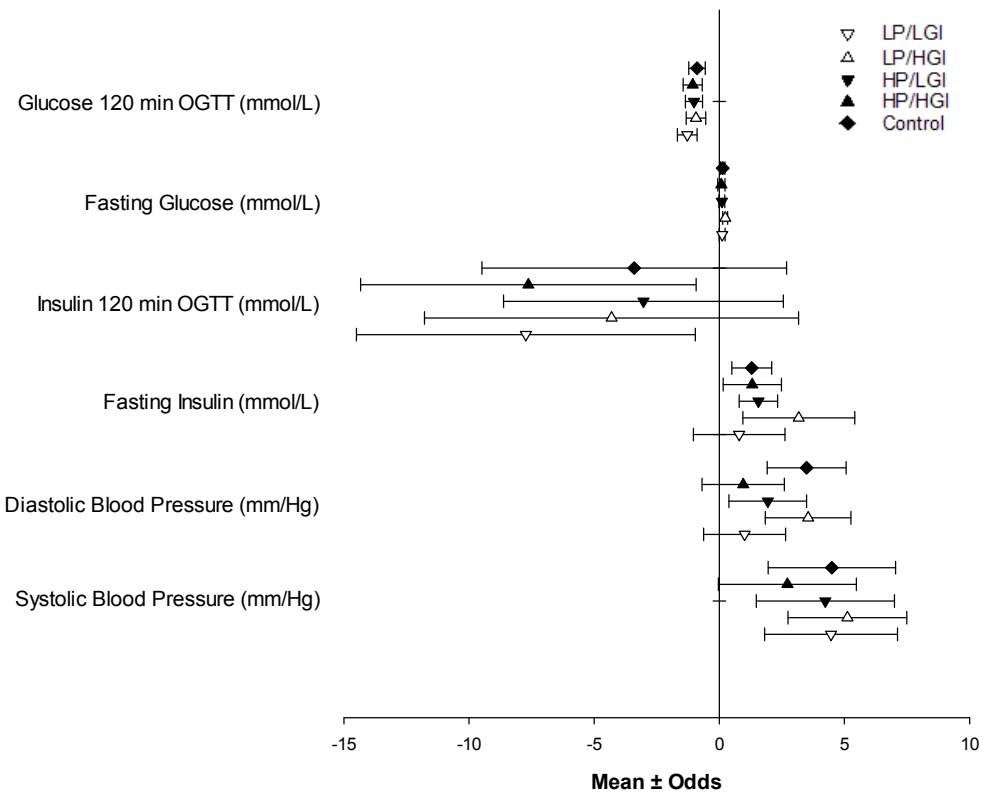


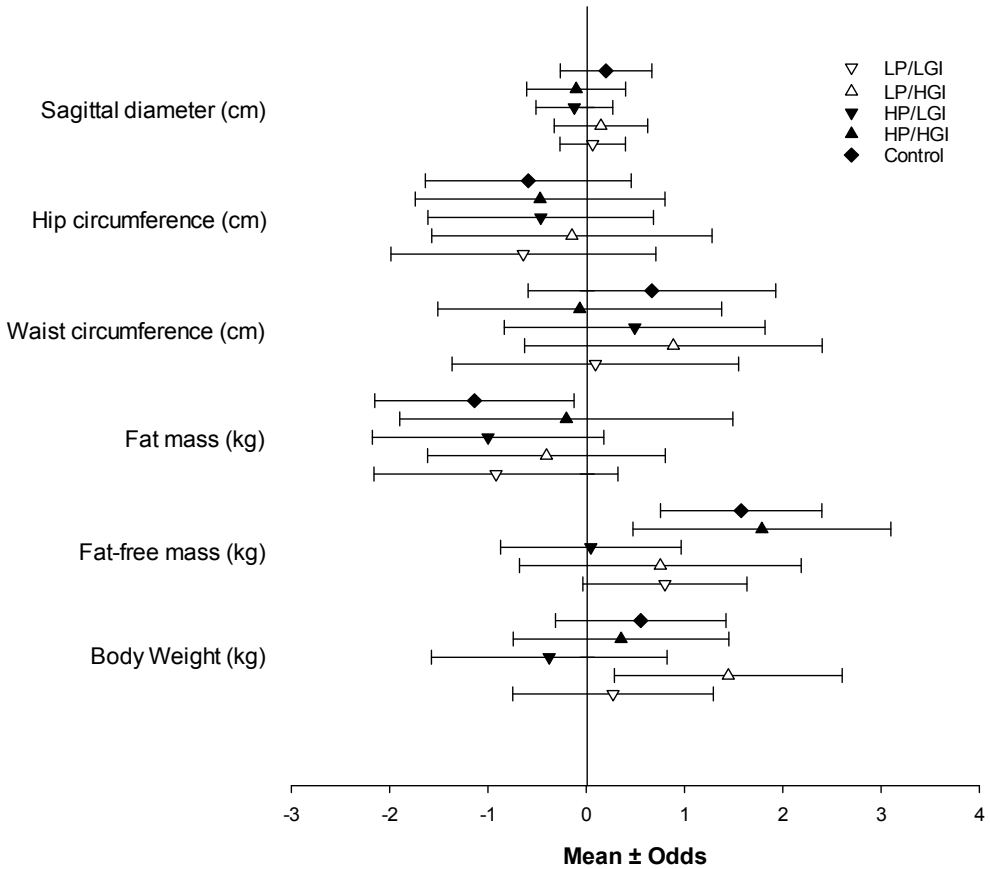
Supplemental Figure 5B. GI of consumed carbohydrates at week 4 and post-intervention



Supplemental Figure 6. Forest diagrams of parameters shown in Table 2.

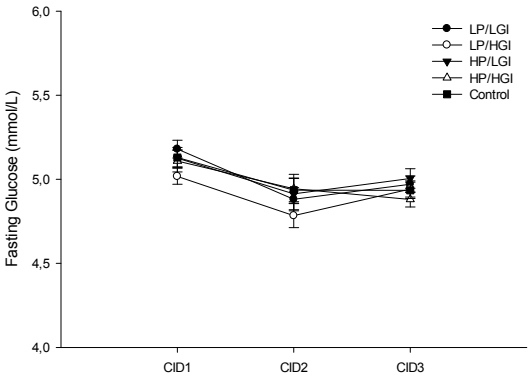




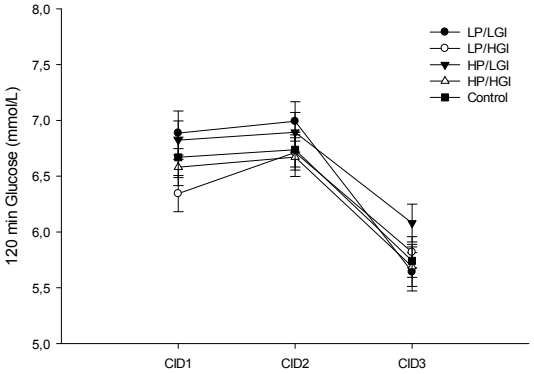

LP/LGI
LP/HGI
HP/LGI
HP/HGI
Control
Sagittal diameter (cm)
Hip circumference (cm)
Waist circumference (cm)
Fat mass (kg)
Fat-free mass (kg)
Body Weight (kg)
-3
-2
-1
0
1
2
3
4
Mean ± Odds

Supplemental Figure 7. Paramteres of glucose metabolism

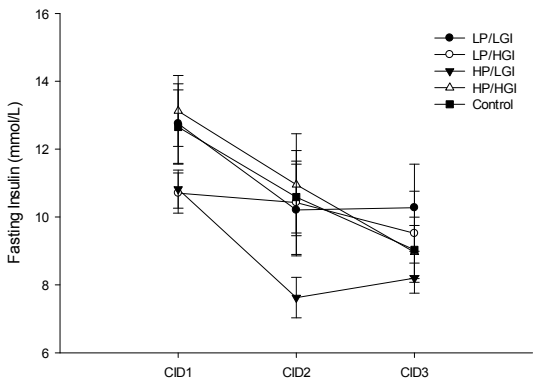
A. Fasting glucose



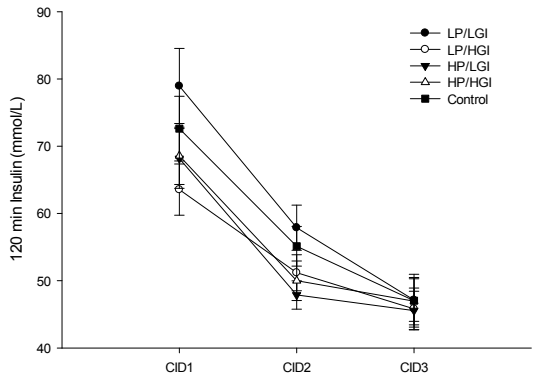
B. 120 min glucose



C. Fasting insulin



D. 120 min insulin



Supplemental References

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