RESEARCH ARTICLE

Mechanisms of weight maintenance under high- and low-protein, low-glycaemic index diets

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Scope: Weight maintenance after intended weight loss is a challenge in an obesogenic environment. In a large multicentre dietary intervention study (DiOGenes), it has recently been demonstrated that a high-protein/low-glycaemic index (HP/LGI) diet was slightly more efficient in maintaining weight loss than low-protein/LGI or high-GI (LP/LGI or HGI) diets. Here, we use a proteomic approach to assess the molecular mechanisms behind this positive effect.
Methods and results: A subset of the most successful (weight loser, $n = 12$) and unsuccessful (weight re-gainer, $n = 12$) individuals consuming the LGI diets with either high- or low-protein content (HP or LP/LGI), following an initial calorie deficit run-in weight loss phase, were analyzed at the plasma protein level. Proteomic analysis revealed 18 proteins regulated after 6 months of the dietary weight maintenance phase. Furthermore, 12 proteins were significantly regulated as a function of success rate under an HP diet, arising as candidate biomarkers of mechanisms of successful weight maintenance under an HP/LGI diet. Pregnancy-zone protein (PZP) and protein S (PROS1) were revealed as novel biomarkers of weight maintenance showing opposite effects.
Conclusion: Semantic network analysis of the 12 regulated proteins revealed that under an HP/LGI an anti-atherogenic effect and alterations of fat metabolism were associated with the success of maintaining the initial weight loss.

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1 Introduction

Obesity is a major public health problem. Currently, two-third of the adult US population and more than half of the adults in Europe have a BMI $\geq 25\,kg/m^2$ [1, 2] (http://www.euro.who.int/__data/assets/pdf_file/0009/87462/E89567.pdf). Moreover, not only weight maintenance per se but also the avoidance of diseases concomitant to obesity, like cardiovascular disease, is a major public concern. Several health-promoting campaigns have been undertaken to inform the population about the risks associated with an overweight condition, intending to promote

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Abbreviations: CID, clinical investigation day; **LGI,** low-glycaemic index; **HGI,** high-glycaemic index; **HP,** high-protein; **LP,** low-protein; **PROS,** protein S; **PZP,** pregnancy-zone protein

*In memory of our colleague and friend Andreas Fuerholz.
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maintenance or achievement of a normal body weight. Apart from the initial challenge of losing excess weight, an additional burden is to keep it off once it is lost, given the current obesogenic environment. Only approximately 20% of the individuals that intentionally lost weight maintained it successfully after one year [3, 4]. Intensive efforts focus on improving the success rate of losing and maintaining weight. Treatments generally include both an increment of physical activity and a change of eating behaviours. One reason of poor weight maintenance is psychological due to the weight loss-accompanying changes in behaviour. Another main reason is of physiological nature, because during weight loss several metabolic adaptations occur that render weight maintenance increasingly difficult [5]. An extensive debate is still ongoing concerning how changes in macronutrient content and types can contribute to an increased success in body weight maintenance as these lead to different physiological adaptations [6, 7]. The importance to lower fat intake to prevent excess calorie intake is well accepted [7]. Carbohydrates have been classified based on the postprandial glucose profiles generated after ingestion, i.e. the GI [8]. However, no conclusive results have yet been obtained as to whether low-GI diets are a promising intervention for prevention of regain after weight loss [6]. Another suggested treatment is to modulate the amount of proteins. It is well established that HP diets induce satiety and preserve muscle mass, but the impact of the protein content on weight maintenance is still controversial [5, 9].

A European multicentre, randomized, controlled dietary intervention study was conducted to investigate the effects of protein intake and GI on weight maintenance, metabolic and cardiovascular risk factors in obese and overweight families, within the DiOGenes project (DiOGenes: 'Diet, Obesity and Genes' is a project supported by the European Community, Contract no. FOOD-CT-2005-513946, Clinical-Trials.gov number, NCT00390637) [10, 11]. After 8 wk with a low-calorie dietary intervention adults ($n = 773$) were randomized and instructed to eat ad libitum either a control diet or 4 different diets with low-protein/low-GI (LP/LGI), low-protein/high-GI (HP/HGI), high-protein/low-GI (HP/LGI) and high-protein/high-GI (HP/HGI) content all with a reduction of fat content during 6–12 months. After the caloric restriction phase, a moderate decrease in GI and a moderate increase in the protein content in the diet resulted in a slight improvement of weight maintenance. Moreover, the completion rate of the study was best with individuals consuming an HP/LGI diet [12].

Since the emergence of functional genomics, *omics* technologies have demonstrated their power in holistically understanding physiological and disease states. Proteomics has emerged as a potent technique in understanding the molecular basis of cellular and physiological processes, based on the composition and stoichiometry of protein complexes and their functional organisation within pathways [13]. Moreover, proteomics has been extensively deployed in the elucidation of (candidate) biomarkers for health and disease [14]. Nevertheless, to date only a limited number of nutritional intervention studies have applied *omics* technologies to understand the mechanisms behind the physiological effects, to assess the impact of the intervention or to identify biomarkers for mechanisms or efficacy [15]. Here, we investigate the mechanisms underlying success in weight maintenance (after initial calorie deficit-induced weight loss) following a low-GI diet with different protein contents during the 6 months weight maintenance phase using a proteomic approach.

2 Materials and methods

2.1 Study design

The cohort under investigation was part of a randomized and controlled dietary intervention study, the DioOGenes study [10, 11]. Briefly, 891 families with at least one-overweight parent underwent screening in eight European centres. After 8 wk with a low-caloric dietary intervention (3.3–4.2 MJ/d) those families with minimum one parent attaining a weight loss $\geq 8\%$ ($n = 773$) were randomized to one of five different diets during 6 months [11]. The diets included a control diet and 4 moderate-fat (25–30 energy%) ad libitum diets with different proteins and GI contents: Diet 1 low-protein and low-GI (LP/LGI), Diet 2 low-protein and high-GI (LP/HGI), Diet 3 high-protein and low-GI (HP/LGI) and Diet 4 high-protein and high-GI (HP/HGI). The volunteers were instructed to maintain their weight loss during the intervention periods, but further weight reductions were also permitted. The study protocol was approved by each local ethical committee and all participants signed an informed consent. Anthropometrical, food records, clinical chemistry parameters in plasma and urine and hormones in plasma were measured to follow-up the dietary intervention at three different stages: CID1 (clinical investigation day 1) before weight loss, CID2 after weight loss and CID3 after the 6-month weight maintenance phase [10]. Moreover, subcutaneous fat biopsies were sampled.

2.2 Selection of the study sub-cohort for proteomic assessment

Among the 548 adults who completed the entire dietary program a subset of 236 women were selected meeting following the criteria described in [16]. Applying the so-called *S* factor, subjects were classified in accordance to their weight maintenance considering their initial weight and the weight loss achieved during the low-caloric intervention phase:

$$S = \Delta\text{bodyweight}_{(CID3-CID2)}\ \Delta\text{bodyweight}_{(CID1-CID2)}.$$

After exclusion of subjects with an *S* factor below the 10th percentile and above the 90th percentile within both LGI diet groups, 12 subjects with the lowest *S* factor

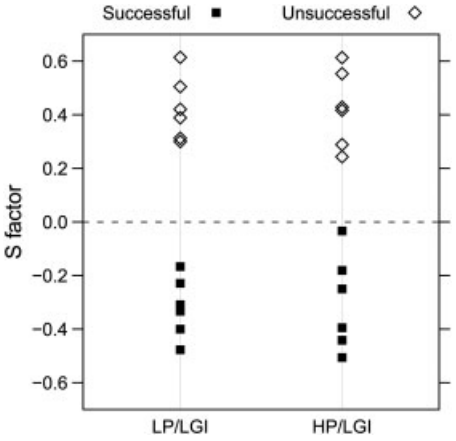

Figure 1. Classification of selected individuals into successful (weight losers) and unsuccessful weight maintainers (weight re-gainers) under the two different diets administered over 6 months (HP/LGI: high-protein/low-glycaemic index; LP/LGI: low-protein/low-glycaemic index), based on the *S* factor = Δbody weight$_{(CID3\text{-}CID2)}$/Δbody weight$_{(CID1\text{-}CID2)}$.

($S = -0.31 \pm 0.14$, $n = 6$ for each diet) classified as the most successful individuals (weight loser) and 12 with the highest *S* factor ($S = 0.42 \pm 0.13$, $n = 6$ for each diet) classified as the most unsuccessful individuals (weight re-gainer) were investigated (Fig. 1). These subjects did not differ in age or weight at the beginning of the intervention study (at CID1, age: successful 39.75 ± 6.66 y versus unsuccessful 39.42 ± 4.74 y, *p-value* = 0.89; weight: successful 100.10 ± 17.70 kg versus unsuccessful 96.48 ± 12.43 kg, *p-value* = 0.57). Here, we analyzed fasting plasma obtained after the low-caloric phase (CID2) and after consuming whether an LP/LGI or HP/LGI diet for 6 months (CID3).

2.3 Clinical chemistry and hormone measurements

Blood samples and anthropometric measurements were handled and analyzed in the different centres following standard procedure protocols (details reported in [10]). Blood collection and anthropometric measurements were performed in the fasting state. Individuals were asked not to consume any food or liquids (except for 350–500 mL water) at least 10 h before blood sampling. After the drawing of fasting blood sample an oral glucose tolerance test (75 g glucose diluted in 250 mL tepid water) followed for 120 min. Blood samples were centrifuged and plasma and serum samples were aliquoted, frozen and shipped to a central laboratory for clinical chemistry and hormone measurements.

2.4 Targeted proteomics

Three steroids and 29 blood proteins were analyzed in plasma as described previously [17]. Cortisol, progesterone, testosterone, luteinizing hormone, follicle stimulation hormone, prolactin, cortisol, angiotensin I converting enyme 1, angiotensinogen, leptin, resistin, acylation stimulation protein, adiponectin, insulin, glucagon, insulin-like growth factor 1, glucagon-like peptide-1 (total) plasminogen activator inhibitor-1 (active), retinol binding protein 4, macrophage migration inhibiting factor, growth hormone, vascular endothelial growth factor-D, pigment epithelium-derived factor, insulin-like growth factor binding protein 1,insulin-like growth factor binding protein 3, interleukin (IL) 6, IL-8, tumor necrosis factor α, amylin, matrix metalloproteinase 9, haptoglobin and serum C-reactive protein were determined.

2.5 Discovery proteomics

The proteomic approach applied here has been reported in detail elsewhere [18]. Briefly, 25 μL of the plasma samples in 75 μL of the manufacturer's equilibration buffer were injected onto the HPLC column-based Multiple Affinity Removal System (MARS, Agilent Technologies, Basel, Switzerland) for depletion of the seven most abundant proteins: albumin, transferin, IgG, IgA, haptoglobin, anti-trypsin and fibrinogen. Samples were further analyzed at the level of relative protein abundance quantification between conditions, by an in-house developed stabled-isotope labelling technique, AniBAL [19]. 100 μg protein aliquots of the depleted samples were derivatized with aniline and benzoic acid (either ^{12}C or ^{13}C). The labelled proteins were mixed, precipitated and digested with trypsin before being submitted to peptide Off-Gel electrophoresis with the 3100 Off-Gel Fractionator (Agilent Technologies). Immobiline dry strips (GE Healthcare, Munich, Germany), 3–10 pH for benzoic acid derivatized samples or 7–11 pH for aniline-derivatized samples were used with 12-well separation. The recovered fractions were analyzed by a nanoLC-ESI-MS/MS system, HCT ultra ion-trap mass spectrometer (Bruker Daltonics, Bremen, Germany) coupled on-line to an Ultimate 3000 HPLC system (Dionex, Olten, Switzerland) equipped with an analytical Magic C reversed-phase column (100×0.075 mm, 5 μm).

2.6 Data processing and interpretation

Statistical analysis was performed with R (version 2.11.1 [20], http://www. R-project.org) and *Partek Genomic Suite* software (Partek Incorporated, St. Louis, USA). The clinical and anthropometric parameters, hormones and targeted proteomic data from the sub-study cohort were analyzed and compared with the data obtained from the proteomic analysis with the same plasma samples, i.e. 24 individuals analyzed at time-points CID2 and CID3. Data are presented as mean ± SD unless otherwise indicated.

Peptide and protein identification and quantification was performed with the Mascot Search (Matrix Science, London, UK) and Trans-Proteomic Pipeline v.3.4 (Inst. Systems Biol., Seattle, USA). The Xpress and ASAP ratios obtained for each protein were averaged (arithmetic mean) and transformed to obtain a normal distribution by calculating the natural logarithm. For statistical analysis proteins showing a probability cut-off >0.9 and a false-positive rate $<5\%$ were selected. Nested 4-factors ANOVA analysis was applied. The factors were diet (HP/LGI and LP/LGI), success rate (weight re-gainers and weight loser), subject nested in diet and success and time point (CID). Analysis was followed by pair-wise comparisons. The fold-change for the second order interaction diet and success rate was calculated with following formula:

$$FC_{Int} = e^{(R1-R2)-(R3-R4)} \quad (1)$$

with *R1*: $x_{t=\mathrm{CID3}}/x_{t=\mathrm{CID2}}$ in weight loser consuming HP/LGI diet; *R2*: $x_{t=\mathrm{CID3}}/x_{t=\mathrm{CID2}}$ in weight loser consuming LP/LGI diet; *R3*: $x_{t=\mathrm{CID3}}/x_{t=\mathrm{CID2}}$ in weight re-gainers consuming HP/LGI diet and *R4*: $x_{t=\mathrm{CID3}}/x_{t=\mathrm{CID2}}$ in weight re-gainers consuming LP/LGI diet. Being x the arithmetic mean from Xpress and ASAP ratios.

The software Ingenuity Pathways Analysis (IPA) (Ingenuity Systems, Redwood City, CA, USA; http://www.ingenuity.com) was used for data interpretation.

3 Results and discussion

3.1 Selection of DiOGenes sub-cohort for proteomics

In the DiOGenes study, after an 8 wk caloric restriction phase, those individuals with at least $\geq 8\%$ body weight loss were randomly assigned to one of five different diets with different amounts of proteins and GI for 6 months to assess their efficacy in weight maintenance. A modest reduction of GI and a modest increment of protein content improved intervention completion rate and maintenance of body weight [12]. In order to understand the mechanisms behind successful weight maintenance, *omics* approaches were applied to DiOGenes sub-cohorts.

Márquez-Quiñones et al. shown using transcriptomics on subcutaneous adipose tissue biopsies, that 60 genes were differentially expressed independently of the diet consumed during the intervention [16]. In weight losers compared to the weight re-gainers, genes associated with inflammatory response and cell proliferation were down-regulated, whereas genes associated with mitochondrial function were up-regulated. In the present study, proteomics was applied to understand the mechanisms behind successful weight maintenance in individuals that consumed an LGI diet differing in protein content. Individuals were classified into weight losers or weight re-gainers by applying the so-called *S* factor (see Section 2). Within a subset of 236 women the 6 most successful (weight loser) and most unsuccessful (weight re-gainer) individuals, for each selected LP/LGI and HP/LGI diet, were selected (Fig. 1). Proteomic analysis was performed on blood samples obtained from these 24 individuals before (CID2) and after (CID3) the 6-month weight maintenance. The phenotypic and clinical data of the selected subjects for this sub-cohort at both time points (i.e. before and after the weight maintenance phase) are listed in Table 1. Importantly, no statistically significant difference was detected in the clinical parameters at time point CID2 (after the caloric restriction phase and before starting the dietary weight maintenance) between weight losers and weight re-gainers.

3.2 Clinical chemistry and hormone levels as candidate biomarkers for weight loss

The CID3/CID2 ratio of clinical and phenotypic values was calculated for each subgroup (Table 1). Besides weight and BMI ratios, which were by definition significantly lower in the weight losers than the weight re-gainers, hip, waist and sagittal CID3/CID2 ratios were significantly different between weight losers and weight re-gainers. Within diets (Table 1), the hip CID3/CID2 ratio was lower in weight losers versus weight re-gainers for both diets. Waist and sagittal CID3/CID2 ratios were only lower within the LP/LGI diet. C-reactive protein CID3/CID2 ratios were lower in weight losers versus weight re-gainers among the individuals consuming an HP/LGI diet.

3.3 Identification of candidate biomarkers of weight loss by discovery proteomics

We applied a relative quantification proteomics workflow to blood samples from the cohort defined above in order to identify candidate biomarkers of successful weight loss under an HP/LGI and LP/LGI [18]. Seventy-five proteins were identified and quantified across all technical and biological replicates and are listed in Table 2. Using the DAVID gene enrichment tool [21], most proteins were associated with protein activation, immune response, complement and coagulation cascades or lipid metabolism.

Nested ANOVA analysis revealed 18 proteins differentially expressed as a function of success rate and protein content in the LGI diet (Table 2). Different proteins were identified as candidate biomarkers to distinguish between weight losers and weight re-gainers consuming an LP/LGI (APOE, ITIH1, ORM1, PON, PROS1, PZPZ) or consuming an HP/LGI (C8A, C9, KNG1, PROS1, PZP, RBP4, SERPINF1) diet. Moreover, as indicated in Tables 2, 6 proteins are candidate biomarkers to distinguish the two diets consumed within the weight loser group, and 3 within the weight re-gainer group.

PROS1 levels and an increase in PZP were found indicative of successful body weight maintenance and also of the

Table 1. Clinical and anthropometric features of the study sub-cohort (extreme weight losers and re-gainers) selected for the present proteomic analysis

	LP/LGI						HP/LGI					
	Weigh loser			Weigh re-gainer			Weight loser			Weight re-gainer		
	CID2	CID3	CID3/CID2	CID2	CID3	CID3/CID2	CID2	CID3	CID3/CID2	CID2	CID3	CID3/CID2
Weight (kg)	90.95±22.72	87.2±21.81	0.96±0.02	88.78±6.94	93.17±8.43	1.05±0.02[a)]	86.55±11.63	83.25±11.32	0.96±0.02	85.4±15.78	89.1±16.71	1.04±0.01[a)]
BMI (kg/m^2)	31.85±5.79	30.54±5.55	0.96±0.02	30.96±3.74	32.47±4.04	1.05±0.02[a)]	31.2±3.88	30.04±4.02	0.96±0.02	29.45±4.5	30.71±4.74	1.04±0.01[a)]
Waist (cm)	99.22±9.63	95.58±11.2	0.96±0.06	96.16±9.29	100.28±7.39	1.05±0.04[b)]	95.59±8.34	88.9±7.53	0.94±0.07	97.71±21.14	95.65±14.07	0.99±0.08
Hip (cm)	114.59±8.46	106.09±8.93	0.93±0.07	110.26±7.81	116.96±8.9	1.06±0.08[b)]	113.23±7.18	108.85±7.23	0.97±0.05	113.47±14.35	118.3±13.74	1.04±0.04[b)]
Waist/Hip ratio	0.87±0.06	0.90±0.07	1.04±0.07	0.87±0.04	0.86±0.05	0.99±0.08	0.84±0.06	0.82±0.05	0.97±0.03	0.85±0.09	0.81±0.05	0.95±0.07
Sagittal (cm)	25.11±5.79	23.56±5.04	0.94±0.03	21.2±1.88	22.62±2.27	1.07±0.02[a)]	20.44±1.82	18.35±3.74	0.91±0.16	21.73±4.58	22.18±3.88	1.03±0.09
SBP (mm Hg)	130.33±9.37	121.92±13.79	0.94±0.11	114.67±13.23	124.08±11.29	1.09±0.14	112.25±9.83	114.42±17.55	1.03±0.2	116±13.63	121.42±23.67	1.04±0.12
DBP (mm Hg)	71.42±8.27	73.17±10.7	1.03±0.14	77.33±11.72	76±8.96	1±0.19	67.75±7.87	68.33±5.09	1.02±0.09	72.42±14.5	72.58±15.51	1.01±0.1
Cholesterol (mmol/L)	4.38±0.79	4.66±0.52	1.08±0.09	4.31±0.82	4.8±0.86	1.13±0.19	4.06±0.36	4.66±0.7	1.15±0.18	4.43±0.91	4.55±0.76	1.05±0.1
Triglycerides (mmol/L)	1.17±0.36	1±0.41	0.85±0.21	1.3±0.8	1.31±0.76	1.38±1.42	1.13±0.33	1.15±0.63	0.98±0.27	1.02±0.35	1.04±0.48	1.01±0.33
HDL (mmol/L)	1.18±0.17	1.44±0.2	1.23±0.14	1.22±0.3	1.44±0.35	1.2±0.22	1.21±0.27	1.46±0.24	1.24±0.17	1.48±0.34	1.58±0.32	1.09±0.08
Fructosamin (μmol/L)	211.33±11.78	210.83±9.6	1±0.06	196±31.35	208.17±16.3	1.08±0.15	209.83±26.85	216.33±16.84	1.04±0.09	217±29.41	215.8±17.14	1.02±0.10
Adiponectin (μg/L)	9.84±3.85	10.95±4.23	1.13±0.25	7.6±3.04	8.76±1.3	1.29±0.42	11.68±2.69	10.45±2.88	0.94±0.32	14.75±7.31	12.33±4.6	0.88±0.22
LDL (mmol/L)	2.68±0.8	2.78±0.64	1.06±0.11	2.5±0.62	2.77±0.35	1.15±0.23	2.35±0.33	2.68±0.59	1.15±0.21	2.49±0.87	2.49±0.71	1.06±0.2
Glucose (mmol/L)	5.33±1.3	4.93±0.34	0.96±0.17	5.12±0.57	4.98±0.61	0.97±0.03	4.68±0.73	4.65±0.54	1±0.06	4.73±0.38	4.65±0.51	0.98±0.04
Insulin (μIU/mL)	7.42±2.8	8.34±4.42	1.22±0.47	11.31±5.13	10.89±5.15	1.06±0.4	7.74±4.47	5.17±4.12	0.66±0.25	5.54±2.25	6.39±3.31	1.02±0.31
AUC glucose (mol/L × min)	0.4±0.1	0.3±0.22	0.83±0.76	0.32±0.19	0.31±0.22	1.03±0.52	0.4±0.11	0.35±0.13	0.93±0.39	0.3±0.14	0.23±0.15	0.97±0.73
AUC insulin (mIU/mL × min)	4.76±2.53	5.07±1.72	1.17±0.43	9.55±8.53	11.16±10.3	1.2±0.43	3.36±1.3	4.8±3.99	1.42±0.76	4.1±1.46	3.56±1.31	0.87±0.14

All parameter measured after overnight fasting.

a) p adjusted <0.001 t-test between the CID3/CID2 ratio of weight loser and weight re-gainers within a diet.

b) p adjusted <0.05.

Table 2. Proteins and candidate biomarkers semi-quantified in human plasma (across all technical and biological replicates) before and after the 6-month dietary intervention with LP/LGI and HP/LGI diets

IPI ID	Symbol	Entrez gene name	Biomarker[a)]				
			Su/USu LP/LGI[b)]	Su/Usu HP/LGI[c)]	HP/LP Su[d)]	HP/LP USu[e)]	Int[f)]
IPI00022895	A1BG	α-1-B Glycoprotein					
IPI00478003	A2M	α-2-Macroglobulin					
IPI00019943	AFM	Afamin					
IPI00032220	AGT	Angiotensinogen (serpin peptidase inhibitor, clade A, member 8)					
IPI00022431	AHSG	α-2-HS-Glycoprotein			↑ (1.2)	↓ (0.2)	
IPI00022426	AMBP	α-1-Microglobulin/bikunin precursor					
IPI00022391	APCS	Amyloid P component, serum					↓ (0.5)
IPI00021841	APOA1	Apolipoprotein A-I					
IPI00304273	APOA4	Apolipoprotein A-IV					
IPI00022229	APOB	Apolipoprotein B (including Ag(x) antigen)					
IPI00021856	APOC2	Apolipoprotein C-II					
IPI00021857	APOC3	Apolipoprotein C-III					
IPI00021842	APOE	Apolipoprotein E	↑ (1.4)				
IPI00299435	APOF	Apolipoprotein F					
IPI00298828	APOH	Apolipoprotein H (β-2-glycoprotein I)					
IPI00186903	APOL1	Apolipoprotein L, 1					↑ (2.2)
IPI00030739	APOM	Apolipoprotein M					↑ (1.7)
IPI00166729	AZGP1	α-2-Glycoprotein 1, zinc-binding					
IPI00296165	C1R	Complement component 1, r subcomponent					
IPI00017696	C1S	Complement component 1, s subcomponent					
IPI00303963	C2	Complement component 2					
IPI00783987	C3	Complement component 3					
IPI00032258	C4A	Complement component 4A					
IPI00021727	C4BPA	Complement component 4 binding protein, α					
IPI00654875	C4B	Complement component 4B					
IPI00032291	C5	Complement component 5					
IPI00879709	C6	Complement component 6					
IPI00011252	C8A	Complement component 8, α Polypeptide		↑ (1.6)	↑ (1.9)		
IPI00294395	C8B	Complement component 8, β polypeptide					
IPI00022395	C9	Complement component 9		↑ (1.5)			↓ (0.7)
IPI00019591	CFB	Complement factor B					
IPI00029739	CFH	Isoform of complement factor H					
IPI00515041	CFH	Putative uncharacterized protein CFH			↓ (0.5)		
IPI00291867	CFI	Complement factor I				↓(0.3)	
IPI00011264	CFHR1	Complement factor H-related protein 1					
IPI00291262	CLU	Clusterin					
IPI00017601	CP	Ceruloplasmin (ferroxidase)					
IPI00019581	F12	Coagulation factor XII (Hageman factor)					
IPI00019568	F2	Coagulation factor II (thrombin)					
IPI00022418	FN1	Fibronectin 1					
IPI00339224	FN1	Fibronectin 1 isoform 4 preprotein					
IPI00555812	GC	Vitamin D binding protein					
IPI00742696	GC	Vitamin D binding protein precursor					
IPI00026314	GSN	Gelsolin					
IPI00022488	HPX	Hemopexin					
IPI00022371	HRG	Histidine-rich glycoprotein					
IPI00020996	IGFALS	Insulin-like growth factor binding protein, acid labile subunit					↑ (1.4)
IPI00383164	IGHA1	Immunoglobulin heavy constant α 1					
IPI00896380	IGHM	Ig mu heavy chain disease protein					

Table 2. Continued

IPI ID	Symbol	Entrez gene name	Biomarker[a)]				
			Su/USu LP/LGI[b)]	Su/Usu HP/LGI[c)]	HP/LP Su[d)]	HP/LP USu[e)]	Int[f)]
IPI00292530	ITIH1	Inter-α (globulin) inhibitor H1	↑ (1.5)				
IPI00305461	ITIH2	Inter-α (globulin) inhibitor H2					
IPI00218192	ITIH4	Inter-α (globulin) inhibitor H4					
IPI00654888	KLKB1	Kallikrein B, plasma (Fletcher factor) 1					
IPI00032328	KNG1	Isoform HMW of kininogen 1					
IPI00215894	KNG1	Isoform LMW of kininogen 1		↓ (0.7)			
IPI00022429	ORM1	α-1-Acid glycoprotein 1	↑ (1.5)				↑ (1.5)
IPI00884926	ORM1	Orosomucoid 1					
IPI00020091	ORM2	α-1-Acid glycoprotein 2					
IPI00163207	PGLYRP2	Peptidoglycan recognition protein 2					
IPI00019580	PLG	Plasminogen					
IPI00218732	PON1	Paraoxonase 1	↑ (1.5)				↑ (1.5)
IPI00294004	PROS1	Protein S (α)	↑ (1.4)	↓ (0.6)	↓ (0.7)	↓ (0.4)	↑ (2.3)
IPI00025426	PZP	Pregnancy-zone protein	↓ (0.6)	↑ (2.0)	↑ (2.3)		↓ (0.3)
IPI00022420	RBP4	Retinol binding protein 4, plasma		↓ (0.8)			↑ (1.4)
IPI00019399	SAA4	Serum amyloid A4, constitutive					
IPI00550991	SERPINA3	Serpin peptidase inhibitor, clade A, member 3					
IPI00032179	SERPINC1	Serpin peptidase inhibitor, clade C (antithrombin), member 1					
IPI00844156	SERPINC1	Serpin peptidase inhibitor, clade C (antithrombin), member 1					
IPI00292950	SERPIND1	Serpin peptidase inhibitor, clade D (heparin cofactor), member 1					
IPI00006114	SERPINF1	Pigment epithelium derived factor		↑ (1.6)	↑ (1.9)		
IPI00291866	SERPING1	Serpin peptidase inhibitor, clade G (C1 inhibitor), member 1					
IPI00022432	TTR	Transthyretin					
IPI00646384	TTR	13 kDa protein					
IPI00029863	Unknown	55 kDa protein					
IPI00298971	VTN	Vitronectin					

a) Proteins showing significantly (*p-value* < 0.1) upregulated (↑) or downregulated (↓) levels under different comparisons. In brackets the fold-change is represented.
b) Su/USu LP/LGI: weight loser versus weight re-gainer within LP/LGI diet.
c) Su/USu HP/LGI: weight loser versus weight re-gainer with HP/LGI diet.
d) HP/LP Su within weight loser differences between HP/LGI versus LP/LGI diets.
e) HP/LP USu: within weight re-gainer differences between HP/LGI versus LP/LGI diets.
f) Int: proteins with different levels due to the second-order interaction of the factors diet and success rate.

consumption of an HP versus LP diet among the successful individuals. Protein S (PROS1), a vitamin K-dependent protein that inhibits blood clotting, was identified as altered depending on both success and diet. PROS1 is a non-enzymatic cofactor of activated protein C (PROC) and thereby involved in the inactivation of pro-coagulant factors V and VII. Totally, 40% of PROS1 is the active form and circulates in free form in plasma, whereas 60% binds to complement component 4 binding protein (C4BP) [22]. The α chain of C4BP (C4BPA) did not show altered plasma levels in any condition. Pregnancy-zone protein (PZP), an endopeptidase inhibitor, was significantly different in abundance between most conditions and showed the opposite plasma profile compared with PROS1. PZP levels in blood are usually low, but increase drastically during gestation and decrease during parturition [23]. Nevertheless, its physiological function remains unknown. Recently, a genome-wide association study correlated the levels of aspartate aminotransferase in individuals with non-alcoholic liver fibrosis, a liver disease concomitant to obesity, with variants in the PZP gene [24]. Additional studies are necessary to elucidate the mechanisms how PZP could contribute to the regulation of body weight.

3.4 Identification of weight maintenance mechanisms by protein network analysis

In order to better understand the mechanisms underlying successful weight maintenance after consumption of an HP/LGI diet, we performed a semantic network analysis

with the 10 proteins identified to be significantly regulated in an analysis of the interaction of the two factors: dietary protein content and weight maintenance success rate (Table 2). Moreover, we included the proteins found by targeted proteomics that were also identified to be significantly regulated due to this interaction using a two-way ANOVA analysis, CRP (FC = 0.31, p-value = 0.024) and adiponectin (FC = 1.33, p-value = 0.040).

Using IPA, 11 of the 12 proteins regulated as a function of dietary protein content and weight maintenance success rate were mapped onto the networks of antigen presentation; cell-to-cell interaction and signalling; and haematological system development. Success rate and HP dietary content are indicated by 4 down-regulated (PZP, C9, CRP and APCS) and 7 up-regulated proteins (APOM, APOL1, RBP4, IGFALS, ORM1, ADIPOQ and PROS1) (Fig. 2).

It was surprising to find both the up-regulation of RBP4 and the down-regulation of C9, because, according to the literature, these proteins are expected to show the opposite direction of regulation in successful individuals: RBP4 is the major carrier of vitamin A in plasma and has been described as promoting insulin resistance during obesity [25, 26]. C9 is one of the late complement components of plasma that is known to play a major role in the immune response, with the individual roles of these different complement components known to be multifaceted and complex

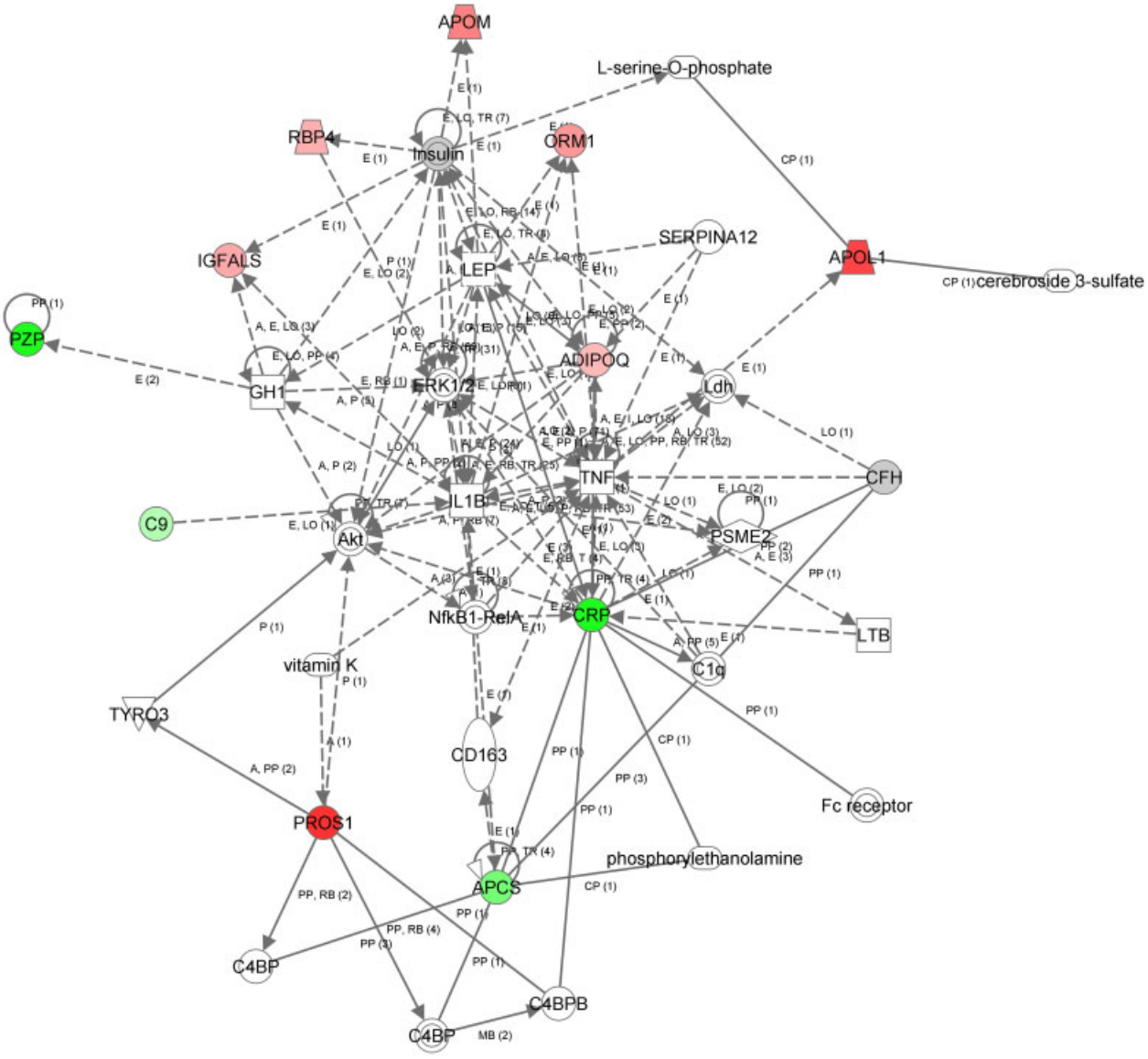

Figure 2. Semantic network analysis of the proteins significantly regulated by diet and success rate as identified by both untargeted (MS) and targeted (ELISA) proteomic analysis. Proteins in red were found up-regulated and those in green down-regulated. The intensity of the colours reflects the extent of the regulation. The fold-changes were calculated as explained in Section 2. Network analysis was performed with IPA (Ingenuity Systems; www.ingenuity.com).

[25, 27]. The most probable explanation why the regulation of C9 and RBP-4 was found to be in the opposite direction compared with previous studies is that the consumption of a high-protein diet may alter the direction of their regulation. In any case, this study also found evidence supporting their role as candidate biomarkers in weight maintenance.

All other regulated proteins identified by analysis of the interaction of diet and success rate indicate that success of weight maintenance under a low-GI diet is related to a reduced risk of atherosclerosis and altered fat metabolism. This is in accordance with previous findings, where a low-GI diet did not only lead to improved weight maintenance but also reduced the risk of concomitant diseases, like diabetes and heart disease [28].

C-reactive protein, an inflammatory marker associated with cardiovascular disease, has been previously shown to decrease continually during weight loss dietary interventions [29, 30]. APOM is an apolipoprotein that binds to a heterogeneous population of HDL and protects against oxidation and stimulates cholesterol efflux [31]. PON1, not displayed in the network, was up-regulated (Table 2) and is also known to have anti-atherogenic effects. PON1 is an enzyme anchored to HDL particles and an important mediator of the antioxidant effects of HDL [31, 32]. Recently, attention has been drawn to APOL1, one of the proteins found also increased. Genetic variants of APOL1 are associated with hypertension-attributed end-stage kidney disease but these variants play also a role in resistance to infectious disease caused by *Trypanosoma brucei* [33, 34]. Otherwise, the function of APOL1 has been associated with lipid export and cholesterol transport because it binds to apolipoprotein A-1, a major apoprotein of HDL. Adiponectin is also elevated. Adiponectin is secreted by adipose tissue and plays a major role in lipid metabolism and insulin sensitivity and as an anti-diabetic, anti-atherogenic and anti-inflammatory hormone [35]. ORM1, also called α-1 acid glycoprotein, is an acute-phase response protein as it is increased under inflammation, but its function is still not well elucidated. Recently, increased expression of ORM proteins have been found in plasma and adipose tissue of obese and *db/db* mice, thereby improving systemic insulin resistance. It has been suggested that ORM expression is induced to maintain metabolic homeostasis by suppressing local systemic inflammation [36]. Serum amyloid P (APCS) levels are correlated with the severity of atherosclerosis lesions [25]. Finally, over-expression of IGFALS in mice has been associated with significant weight loss when compared with wild-type mice [37].

4 Concluding remarks

We elucidated anti-atherogenic effects and the alteration of fat metabolism as the major mechanisms behind the probability of successful weight maintenance (after a calorie deficit-induced weight loss) upon consumption of an HP/LGI diet. In order to compensate for the limited number of individuals analyzed by proteomics and to deliver a proof-of-concept with extreme cases of study participants, we selected the most successful (weight losers) and unsuccessful individuals (weight re-gainers) in terms of weight maintenance. This approach resulted in the identification of candidate biomarkers for weight management and delivered insights into its mechanisms.

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