Effects of High- and Low-proteindiets on Inflammatory Profiles in People With Morbid Obesity: A 3-week Intervention Study

Liselot Koelman
Senior Scientist Group Nutrition, Immunity and Metabolism,Department of Nutrition and Gerontology, German Institute of Human Nutrition Potsdam-Rehbruecke, Nuthetal, Germany

Mariya Markova
Department of Clinical Nutrition, German Institute of Human Nutrition Potsdam-Rehbrücke (DIfE), Nuthetal 14558, Germany;

Nicole Seebeck
Department of Clinical Nutrition, German Institute of Human Nutrition Potsdam-Rehbrücke (DIfE), Nuthetal 14558, Germany;

Silke Hornemann
Department of Clinical Nutrition, German Institute of Human Nutrition Potsdam-Rehbrücke (DIfE), Nuthetal 14558, Germany;

Anke Rosenthal
Clinic for Nutritional Medicine, Berlin 10625, Germany;

Volker Lange
Centre for Obesity and Metabolic Surgery, Vivantes Hospital, Berlin 13585, Germany;

Olga Pivovarova-Ramich
Department of Clinical Nutrition, German Institute of Human Nutrition Potsdam-Rehbrücke (DIfE), Nuthetal 14558, Germany;

Krasimira Aleksandrova (krasimira.aleksandrova@dife.de)
Senior Scientist Group Nutrition, Immunity and Metabolism,Department of Nutrition and Gerontology, German Institute of Human Nutrition Potsdam-Rehbruecke, Nuthetal, Germany 2University of Potsdam, Institute of Nutritional Science, Potsdam, Germany https://orcid.org/0000-0002-1275-1827

Research

Keywords: high-protein, low-protein,nutritional intervention, morbid obesity, inflammatory biomarkers

Posted Date: August 13th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-58016/v1

Abstract

Background: Nutritional interventions in morbidly obese individuals that effectively reverse pro-inflammatory state and prevent obesity-associated medical complications are highly warranted. Our aim was to evaluate the effect of high- (HP) or low- (LP) protein diets on circulating immune-inflammatory biomarkers, includingC-reactive protein (CRP), interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-a), interleukin-10 (IL-10), monocyte chemoattractant protein-1 (MCP-1),chemerin, omentin, leptin, total adiponectin, high molecular weight adiponectin and fetuin-A.

Methods:18 people with morbid obesity were matched into two hypocaloric diet groups: HP (30E% protein, n=8) and LP (10E% protein, n=10) for three weeks.Biomarkers were measured pre-post intervention.We used linear mixed-effects models to investigate differences of least squares means for biomarkers, adjusted for age, sex, BMI, and baseline value.

Results: Consuming HP or LP diets resulted in reduced CRP(HP: -2.2 ± 1.0 mg/l, LP: -2.3 ± 0.9 mg/l) and chemerin (HP: -17.9 ± 8.6 ng/ml, LP: -20.0 ± 7.4 ng/ml). People following the LP diet showed decreased leptin (-19.2± 6.0 ng/ml), IL-6 (-0.4 ± 0.1pg/ml) and increased total adiponectin (1.6 ± 0.6µg/ml). Changes were observed for remaining biomarkers yetto a smaller degree.

Conclusions: These data suggest LP dietsmodulatea wider range of immune-inflammatory biomarkers compared to HP diets in morbidly obese individuals.Larger trials are needed to allow firm conclusions on the suggested effects.

Trial registration:

DRKS00009509. Registered 25 January 2016 – Retrospectively registered, www.drks.de

Background

The worldwide prevalence of obesity has nearly tripled since 1975 (1). As the prevalence of obesity has increased globally, adverse health risks and healthcare expenditure have amplified at an accelerating rate (2). Especially worrying is the increasing proportion of people with morbid obesity characterized by body mass index (BMI) ≥ 35 kg/m^2 (3). These people are exposed to a higher risk of various chronic diseases, premature ageing and overall mortality. Along with metabolic complications such as hyperinsulinemia, hypertension and hyperlipidemia, obesity leads also to a disturbed immune balance and chronic low-grade inflammation [4].

Excess adipose tissue provides an environment for secretion of multiple cytokines and hormones that exert regulatory functions in energy metabolism, inflammation and insulin sensitivity [5]. In morbid obesity, the immune system is especially challenged and constantly struggling to cope with the flow of these proinflammatory triggers and preserve healthy functioning of all organs and systems [6, 7]. Finding approaches to lower obesity may also support the immune system in its battle with the systemic pro-

inflammatory response and favorably influence overall health. General lifestyle interventions such as low-calorie diets and physical activity regimens have shown low compliance and limited effectiveness in people with severe obesity (4). Bariatric surgery has gained increasing popularity as a treatment strategy in patients with morbid obesity (5). Patients that have undergone bariatric surgery experience lower inflammatory concentrations and improved insulin resistance that could be explained by reduced systemic and adipocyte inflammation and secretion of adipocyte derived cytokines (6, 7). However, both surgical treatment and weight loss interventions have not proven successful in the long run (4, 8, 9). The challenge remains to identify novel strategies that bear potential for obesity treatment and management in people with severe obesity targeted at specific pathophysiological pathways.

Emerging evidence shows dietary components can modulate key pathways to inflammation. For instance, omega-3 fatty acid intake can dampen NF-kB activation and modulate the magnitude of inflammatory responses to stressors (10). Dietary flavonoids have also been found capable of modulating cytokines and CRP production in intervention studies (11). However, for individuals with morbid obesity adapting to diets that consist of specific food components may be a challenge. In this vein, dietary plans balancing macronutrient composition may represent a promising and non-drastic intervention approach that can be adopted by people with morbid obesity in sustaining long-term health goals.

Over the recent years, evidence emerged to suggest that high-protein diets may have beneficial effects on postprandial and fasting glucose concentrations (12), postprandial satiety (13), as well as on blood pressure and blood lipids (14). High-protein diets were particularly suggested to modulate inflammatory concentrations in patients with obesity and diabetes (15), and in the ageing population (16, 17). On the other hand, a low-protein diet especially low-methionine diet was shown to beneficially influence glucose intolerance (18) and modulate immune-inflammatory state (19, 20).

Overall, evidence on the role of both high- and low-protein diets in modulating metabolic and inflammatory profile in individuals with obesity has been increasing over the recent years, hence it remains inconclusive. No studies have simultaneously assessed effects of high- and low-protein diets on inflammatory profiles captured by multiple biomarkers. This may be particularly important, because single biomarkers may not sufficiently capture the effect of diet on the complete inflammatory phenotype associated with obesity (21). Studies in people with morbid obesity that may most benefit from such interventions are particularly sparse (9).

To address these gaps, we evaluated the effect of a 3-week low-protein (LP) and a high-protein (HP) dietary intervention on immune-inflammatory profiles depicted by various serum biomarkers measured in individuals with morbid obesity.

Methods

Study design and dietary intervention

We used data collected from a dietary intervention study that included 20 patients with morbid obesity (n = 7 males and n = 13 females) aged 40–50 years old who were recruited from patient lists of the Vivantes Klinikum, Berlin, Germany in the period between January 2016 and June 2017. The primary objective of the original study was to investigate whether LP or HP diets exert greater effects on liver fat reduction (22). A secondary objective of the study was to assess the effect of high- and low-protein diets on changes in inflammatory biomarkers. Inclusion criteria were people with BMI > 40 kg/m^2 or BMI > 35 kg/m^2 and obesity related co-morbidities (type 2 diabetes, hypertension, dyslipidemia, obstructive sleep apnoe syndrome). Excluded were patients suffering from liver cirrhosis, infectious disease, cancer, or interfering chronic diseases. Participants were randomized into two intervention groups, but due to unsuccessful randomization they were matched for age, sex, and body mass index (BMI). Participants received either a hypocaloric (1500–1600 kcal/day) high-protein (HP: 30 E% protein, 25–30 E% fat, 35–45 E% carbohydrates, n = 10) or low-protein (LP: 10 E% protein, 25–35 E% fat, 55–65 E% carbohydrates, n = 10) diet for three weeks. N = 2 participants were excluded due to insufficient repeated biomarker measurements and non-compliance of high protein diet, measured by reduction of serum urea. This resulted in 18 participants who completed this study (n = 7 males, n = 11 females) (see Fig. 1 and Supplementary Fig. 1, Additional File 1).

The HP diet consisted of 3074.6 ± 105.4 mg methionine, whereas the LP diet included 483.7 ± 28.4 mg methionine. Participants received food plans with 10-d rotating menus including recipes. HP food plans consisted of low-fat dairy products, eggs, meat, fruits and vegetables, whereas LP food plans consisted mainly of bread, rice, potatoes, soy products, fruits and vegetables. Sweets, soft drinks and cookies were excluded from diets in both groups. There were two follow-up phone calls which took place after week 1 and week 2 of the intervention. Dietary composition of LP and HP diets can be found in Supplementary Table 1, Additional File 1. Part of the food was provided to the participants (e.g. protein shakes). Food protocols were made with the help of PRODI (Nutri-Science GmbH, Hausauch, Germany).

At the beginning (week 0) and at the end of the intervention (week 3), anthropometric measurements (weight, height, waist and hip circumference), fasting blood sample collection, and body composition determination via BOD POD (Cosmed, Rome, Italy) were performed.

Biomarker measurements

The following biomarkers were measured to assess inflammatory profiles in study participants: C-reactive protein (CRP), interleukin-6 (IL-6), tumor necrosis factor alpha (TNF-a), interleukin-10 (IL-10), monocyte chemoattractant protein 1 (MCP-1), chemerin, omentin, leptin, total adiponectin, high molecular weight (HMW) adiponectin and fetuin-A. Non-HMW adiponectin was estimated based on the difference between total and HMW adiponectin. Venous blood samples were immediately centrifuged and frozen at -80 °C until analysis. CRP concentrations were determined by a highly sensitive immunoturbidimetric assay using ABX Pentra 400 reagents on an ABX Pentra 400 (Horiba ABX, Montpellier, France). Commercially available ELISA kits were used for the measurements of serum leptin (R&D Systems, Minneapolis, USA), total adiponectin, chemerin, omentin, fetuin-A (all from Biovendor, Germany), high molecular weight

adiponectin (Merck Millipore KGaA, Darmstadt, Germany), and U-Plex assay was used to measure IL-6, TNF-a, MCP-1 and IL-10 (MSD, Rockville, USA).

Statistics

Descriptive characteristics presented as medians and interquartile ranges have been calculated for all study participants at study baseline. Associations among immune-inflammatory biomarker measurements at baseline were explored using Spearman partial correlation coefficients adjusted for age, sex, and BMI. Corresponding *P*-values and 95% confidence intervals were calculated using Fisher`s z transformation.

To evaluate the effect of low-protein (LP) and high-protein (HP) diets on serum concentrations of immune-inflammatory biomarkers, the differences in outcome variables between baseline and post-intervention were calculated using linear mixed-effects models with restricted maximum likelihood (REML) method. The fixed effects were modelled for intervention to get the between-subject effect, and for time to get the within-subject effect. The random effects were the individual participants. In order to make pairwise comparisons of biomarkers per diet group over time, we computed differences between least squares means where the obtained p-value was based on the t-test. The models were adjusted for age, sex, BMI, and baseline biomarker measurement in order to correct for changes over time and differences in baseline. Kenward-Roger correction was applied for analysis of mixed models (23); an approach based on estimated covariance parameters in formulas that assume these are known. This corrects for naive test statistics biased upward and standard errors biased downwards.

All statistical analyses were performed using SAS software package, release 14.2 (SAS Institute, Cary, NC, USA). Figure illustrating differences in least squares means of biomarkers was created in R Studio with ggplot2 package. P-values are obtained from t-test, and are two-sided.

Results

In total, 18 participants completed the intervention with repeated biomarker measurements. Table 1 shows the baseline characteristics of the study population. The LP group consisted of 10 participants (6 females/4 males) with a median age of 48.7 (38.1–56.0) years, median weight at baseline of 126.8 (117.0-157.1) kg, and median BMI at baseline of 43.5 (43.1–47.4) kg/m^2. The HP group consisted of 8 participants (5 females/3 males), with a median age of 48.4 (44.9–55.7) years, median weight at baseline of 154.2 (121.4–160.4) kg, and median BMI at baseline of 45.1 (42.3–47.9) kg/m^2. Baseline differences in age, weight, and BMI between LP and HP groups are non-significant.

Table 1
Descriptive characteristics of study population, according to diet

Characteristics	High-protein (n = 8)	Low-protein (n = 10)
Demographics		
Age [years]	48.4 (44.9–55.7)	48.7 (38.1–56.0)
Female – n (%)	5 (62.5)	6 (60.0)
Anthropometrics		
Weight [kg]	154.2 (121.4–160.4)	126.8 (117.0–157.1)
Waist circumference [cm]	135.0 (123.1–150.8)	134.5 (124.3–145.0)
Waist-to-hip ratio	0.9 (0.9–1.1)	0.9 (0.9–1.1)
Body mass index [kg/m²]	45.1 (42.3–47.9)	43.5 (43.1–47.4)
Fat mass [%]	55.4 (51.4–61.1)	54.4 (50.8–56.2)

Data are shown as median (interquartile range). Abbreviations: cm, centimeters; kg, kilograms; m, meters; n, number.

Supplementary Table 2, Additional File 1 presents the correlations among the evaluated biomarkers, adjusted for age, sex, and BMI. IL-6 correlated positively with CRP (ρ: 0.71; 95% CI: 0.28–0.89) and leptin (ρ: 0.64; 95% CI: 0.16–0.86), whereas inverse associations were seen for omentin with MCP1 (ρ: -0.54; 95% CI: -0.82 – -0.02) and fetuin-A with IL-10 (ρ: -0.57; 95% CI: -0.81-0.005).

Figure 2 shows the estimated differences of least squares means of biomarkers over time per diet group, adjusted for age, sex, BMI, and baseline value. Following either HP and LP diet resulted in reduced concentrations of CRP and chemerin in both intervention arms (CRP: estimate±SE in HP and LP: -2.2±1.0 mg/l; *P*-diff: 0.045 and − 2.3±0.9 mg/l; *P*-diff: 0.019 and chemerin: -17.9±8.6 ng/ml; *P*-diff: 0.051 and − 20.0±7.4 ng/ml; *P*-diff: 0.016, respectively). Further, following LP diet resulted in reduction in concentrations of IL-6 and leptin (IL-6: -0.4±0.1 pg/ml; *P*-diff: 0.018 and leptin: -19.2±6.0 ng/ml; *P*-diff: 0.006, respectively); whereas total adiponectin concentrations were increased (1.6±0.6 µg/ml; *P*-diff: 0.017). Changes in concentrations, albeit less pronounced, were further observed for the following biomarkers: omentin, HP and LP: -20.2±27.3 ng/ml; *P*-diff: 0.469 and − 41.2±23.6 ng/ml; *P*-diff: 0.099; fetuin A, HP and LP: -10.6±16.5 µg/ml; *P*-diff: 0.528 and − 13.9±14.3 µg/ml; *P*-diff: 0.345; TNF-a, HP and LP: -0.3±0.2 pg/ml; *P*-diff: 0.083 and − 0.01±0.1 pg/ml; *P*-diff: 0.940; and leptin, HP: -12.0±6.9 ng/ml; *P*-diff: 0.098.

Table 2 presents the baseline and post-intervention median biomarker concentrations, and the differences by intervention and time*intervention interactions. No substantial differences between intervention groups or interaction between intervention arm and time could be detected for the measured biomarkers.

Table 2
High- and low-protein diet intervention effects on circulating immune-inflammatory biomarkers

	Assessment period				**Group difference** ß (95% CI)[1]	**Group-by-time interaction** ß (95% CI)[1]
	Baseline		Week 3		Ref: LP	Ref: LP*Baseline
	Median (95% CI)	n	Median (95% CI)	n		
CRP (mg/l)						
HP	10.0 (3.9–16.2)	8	3.5 (2.5–11.8)	7	0.1 (-1.7–1.9)	0.08 (-2.7–2.9)
LP	10.5 (4.1–14.2)	10	6.4 (2.7–11.4)	10		
P-value					0.894	0.955
IL-6 (pg/ml)						
HP	1.7 (0.8–2.3)	8	1.4 (0.9–2.1)	7	-0.02 (-0.3–0.3)	0.4 (-0.1–0.8)
LP	2.3 (1.4–3.2)	10	1.8 (1.1–2.8)	10		
P-value					0.884	0.130
TNF-a (pg/ml)						
HP	2.5 (2.3–2.6)	8	2.3 (1.8–2.7)	7	-0.07 (-0.4–0.3)	-0.3 (-0.8–0.2)
LP	3.1 (2.4–3.4)	10	3.0 (2.6–3.1)	10		
P-value					0.665	0.191
IL-10 (pg/ml)						
HP	0.2 (0.1–0.4)	8	0.3 (0.2–0.4)	7	0.1 (-0.3–0.5)	-0.2 (-1.0–0.5)

[1]All models adjusted for age, sex, body mass index, and baseline biomarker value, and Kenward-Roger (KR) correction, [3]high molecular weight; Abbreviations: CI, confidence interval, CRP, C-reactive protein; HMW, high molecular weight; HP, high-protein diet; IL-6, interleukin-6; IL-10, interleukin-10; l, liter; LP, low-protein diet; MCP-1, monocyte chemoattractant protein 1; mg, milligram; ml, milliliter; n, number; ng, nanogram; pg, petagram; TNF-a, tumor necrosis factor alpha; µg, microgram.

Abbreviations: CRP, C-reactive protein; IL-6, interleukin-6; TNF-a, tumor necrosis factor alpha; IL-10, interleukin-10; MCP-1, monocyte chemoattractant protein 1; HMW, high molecular weight.

	Assessment period				Group difference ß (95% CI)[1]	Group-by-time interaction ß (95% CI)[1]
LP	0.4 (0.3–0.4)	10	0.3 (0.2–0.5)	10		
P-value					0.533	0.528
MCP-1 (pg/ml)						
HP	325.7 (265.0–347.5)	8	355.4 (305.0–362.8)	7	-2.0 (-37.2–33.2)	32.8 (-22.0–87.5)
LP	332.8 (239.0–409.2)	10	322.9 (232.7–371.4)	10		
P-value					0.907	0.223
Chemerin (ng/ml)						
HP	208.7 (178.5–219.7)	8	177.9 (155.2–225.9)	7	1.1 (-13.4–15.6)	2.1 (-21.6–25.8)
LP	187.8 (147.0–221.4)	10	163.0 (152.8–186.0)	10		
P-value					0.877	0.854
Omentin (ng/ml)						
HP	346.0 (301.0–416.4)	8	333.9 (296.9–405.5)	7	-3.0 (-52.4–46.4)	21.0 (-54.4–96.4)
LP	384.5 (248.9–491.4)	10	324.1 (221.6–491.3)	10		
P-value					0.902	0.564
Leptin (ng/ml)						

[1]All models adjusted for age, sex, body mass index, and baseline biomarker value, and Kenward-Roger (KR) correction, [3]high molecular weight; Abbreviations: CI, confidence interval, CRP, C-reactive protein; HMW, high molecular weight; HP, high-protein diet; IL-6, interleukin-6; IL-10, interleukin-10; l, liter; LP, low-protein diet; MCP-1, monocyte chemoattractant protein 1; mg, milligram; ml, milliliter; n, number; ng, nanogram; pg, petagram; TNF-a, tumor necrosis factor alpha; µg, microgram.

Abbreviations: CRP, C-reactive protein; IL-6, interleukin-6; TNF-a, tumor necrosis factor alpha; IL-10, interleukin-10; MCP-1, monocyte chemoattractant protein 1; HMW, high molecular weight.

	Assessment period				Group difference ß (95% CI)[1]	Group-by-time interaction ß (95% CI)[1]
HP	56.8 (43.1–77.9)	8	50.3 (33.0–53.4)	7	1.1 (-9.5–11.7)	7.2 (-12.0–26.4)
LP	54.9 (41.8–76.0)	10	36.4 (24.4–45.7)	10		
P-value					0.837	0.440
Total adiponectin (µg/ml)						
HP	5.3 (4.4–7.1)	8	6.4 (5.2–7.1)	7	0.2 (-1.2–1.5)	-1.3 (-3.3–0.6)
LP	4.4 (3.9–6.3)	10	5.6 (4.4–8.9)	10		
P-value					0.818	0.157
HMW[3] adiponectin (µg/ml)						
HP	1.3 (0.9–1.9)	5	2.5 (1.6–2.7)	5	0.2 (-0.3–0.6)	-0.2 (-0.8–0.3)
LP	0.7 (0.4–1.5)	7	1.0 (0.8–2.0)	9		
P-value					0.425	0.351
Non-HMW adiponectin (µg/ml)						
HP	4.7 (3.5–6.9)	8	4.4 (2.3–6.4)	7	0.002 (-1.9–1.9)	-1.8 (-4.4–0.9)
LP	4.3 (3.7–4.8)	10	4.3 (3.5–8.1)	10		
P-value					0.998	0.173
Fetuin-A (µg/ml)						

[1]All models adjusted for age, sex, body mass index, and baseline biomarker value, and Kenward-Roger (KR) correction, [3]high molecular weight; Abbreviations: CI, confidence interval, CRP, C-reactive protein; HMW, high molecular weight; HP, high-protein diet; IL-6, interleukin-6; IL-10, interleukin-10; l, liter; LP, low-protein diet; MCP-1, monocyte chemoattractant protein 1; mg, milligram; ml, milliliter; n, number; ng, nanogram; pg, petagram; TNF-a, tumor necrosis factor alpha; µg, microgram.

Abbreviations: CRP, C-reactive protein; IL-6, interleukin-6; TNF-a, tumor necrosis factor alpha; IL-10, interleukin-10; MCP-1, monocyte chemoattractant protein 1; HMW, high molecular weight.

	Assessment period				Group difference ß (95% CI)[1]	Group-by-time interaction ß (95% CI)[1]
HP	254.5 (230.0–300.0)	8	243.0 (194.0–302.0)	7	3.0 (-27.3-33.2)	3.2 (-42.3-48.8)
LP	253.0 (228.0–299.0)	10	236.5 (207.0–250.0)	10		
P-value					0.843	0.882

[1]All models adjusted for age, sex, body mass index, and baseline biomarker value, and Kenward-Roger (KR) correction, [3]high molecular weight; Abbreviations: CI, confidence interval, CRP, C-reactive protein; HMW, high molecular weight; HP, high-protein diet; IL-6, interleukin-6; IL-10, interleukin-10; l, liter; LP, low-protein diet; MCP-1, monocyte chemoattractant protein 1; mg, milligram; ml, milliliter; n, number; ng, nanogram; pg, petagram; TNF-a, tumor necrosis factor alpha; µg, microgram.

Abbreviations: CRP, C-reactive protein; IL-6, interleukin-6; TNF-a, tumor necrosis factor alpha; IL-10, interleukin-10; MCP-1, monocyte chemoattractant protein 1; HMW, high molecular weight.

Discussion

In this dietary intervention study, adherence to either HP or LP diet resulted in reduced concentrations of various inflammatory biomarkers in people with morbid obesity. Results were especially pronounced for CRP and chemerin, two biomarkers reflecting inflammation and cardiovascular risk. Following LP diet was also associated with decrease in leptin and IL-6 concentrations and an increase in adiponectin concentrations. Effects were less prominent for the remaining biomarkers. To our knowledge, this is the first intervention study that explored the effects of varying amounts of dietary protein on changes in various immune-inflammatory biomarkers in people with morbid obesity.

Our results suggested that LP diet was associated with a wider range of beneficial effects including reducing concentrations of CRP, IL-6, chemerin, and leptin and increasing total adiponectin concentration, whereas the effect of the HP diet was most pronounced for reduced CRP and chemerin concentrations. LP diet can be also characterized by reduced exposure to methionine and a number of animal studies have shown that methionine restriction modulates metabolism and improves health span (24, 25). Low-methionine diets have shown to decrease inflammation (24, 26, 27), reduce adiposity (28, 29), decrease oxidative stress (30), and increase insulin sensitivity (28, 31, 32). Compared to calorie restriction, responses to methionine restriction were found to be more robust over the long-run (27). Dietary methionine restriction has been especially associated with metabolic changes in adipose tissue and liver resulting in enhanced insulin sensitivity and energy expenditure (33). In animal studies, methionine restriction was shown to reduce concentrations of insulin, insulin-like growth factor-1, glucose, and leptin and increased adiponectin (33). However, evidence from human research has been sparse. In a large

cross-sectional study of US adults, methionine-rich diets were associated with a higher prevalence of cardiometabolic disease risk factors, i.e. higher levels of cholesterol, glucose, glycated hemoglobin, uric acid and insulin (33). The concentrations of CRP also showed to be higher with higher intake of methionine-rich diet, albeit the trend did not reach statistical significance. A randomized trial that evaluated the effect of a 16-week methionine restricted intervention (> 80% relative to controls), showed that people with obesity and metabolic syndrome had increased adiponectin concentrations (34). As our participants in the LP group received both calorie restricted diet with reduced methionine, a next step would be to reproduce the beneficial effects of the methionine restricted diet in people with morbid obesity without imposition of severe calorie restriction.

The beneficial effects of HP were restricted to reducing concentrations of CRP and chemerin. These results are in line with our previous work, where we evaluated the effect of HP diet in a 6-week intervention study among diabetes patients with obesity (21). HP has a stronger effect on satiety compared to diets of LP content and with equivalent quantities of E from carbohydrate or fat (35). Although there is no formal definition of 'high-protein' as percentage of E in a diet, above 25% E can be seen as high based on a review on satiety and US dietary recommended intakes (36). The effects seen in HP diets may be explained by the high-protein content per se, however, they may also be confounded by other components in the diet. The HP diet in this study and in our previous study contained dairy components. In particular, fermented dairy products (i.e. yoghurt) have been associated to lower levels of inflammation in observational and intervention studies (37, 38). These anti-inflammatory effects could be possibly accounted for by beneficial properties of bacteria species (39) and bioactive peptides that interact with gut microbes and immune cells (40). Further work would be warranted to explore the influence of dietary interventions on gut microbiota composition and immune status in people with morbid obesity.

Up to date, there is still no consensus as to which biomarkers may best represent low-grade inflammation (41). Most dietary intervention studies have been limited in the range of evaluated inflammatory biomarkers (11). CRP is the most established biomarker of inflammation, often used as proxy, sometimes together with IL-6 that stimulates production of CRP. However, CRP alone may not sufficiently capture the effect of diet on the complete inflammatory phenotype associated with obesity. We therefore assessed additional circulating molecules that have been suggested as biomarkers of increased risk and contributing to the pathophysiology of comorbidities of obesity. We were especially interested to evaluate established adipokines such as adiponectin and leptin, as well as novel proinflammatory adipokines, i.e. omentin, chemerin and MCP-1 shown to induce insulin resistance, endothelial dysfunction, and systemic inflammation (42). We were further interested in specific immune-related biomarkers, i.e. chemokines and cytokines that mediate both immune cell recruitment and complex intracellular signaling control mechanisms in obesity, inflammation and chronic disease development (43). Finally, we focused on fetuin-A as biomarker of fatty liver and inflammation, known to exert important roles in in the pathophysiology of insulin resistance and atherosclerosis (44).

This study also has several limitations. We used data from a clinical trial that was designed and powered to study the effects of LP and HP diets on changes in liver fat, whereas the outcome of our study was changes in inflammatory biomarkers. The sample size was relatively small which could have influenced the precision of the observed results. In addition, the duration of the intervention was short, so how long the effects of the intervention will last and whether similar effects will be seen on the long run is to be elucidated. Furthermore, the intervention consisted of a hypocaloric diet, so participants lost weight. The caloric restriction of these patients may have acted as an activator of protective metabolic pathways, in addition to protein intake or methionine restriction. In the analysis we adjusted for BMI change pre-post intervention, however, the molecular mechanisms underlying the effects of dietary protein or the metabolic effects of weight change may not have been captured sufficiently by the adjustment of BMI. We conducted this study to see whether a change in protein content or methionine restriction in terms of the hypocaloric diet could improve inflammation. If the participants maintained their usual caloric intake, the effects of protein or methionine per se would have been captured better. As there are a number of modifying factors that affect the concentration of an inflammatory marker at a given time (45), including age, diet and body fatness, among others, we controlled (diet) or corrected (age, sex, BMI) for these in our analyses.

Conclusions

In this intervention study, adherence to either HP or LP diet effectively modulated concentrations of inflammatory biomarkers in individuals with morbid obesity. These effects were more pronounced for LP diet which lead to modulation of a wider range of inflammatory targets, including the adipokines leptin and adiponectin. Further studies with larger size and duration, as well as encompassing wider range of obesity categories, would be warranted to evaluate the role of high- and low-protein diets in modulating inflammatory profiles in obesity.

List Of Abbreviations

BMI Body mass index

CI Confidence Interval

CRP C-reactive protein

E Energy

HMW High molecular weight

HP High-protein

IL-6 Interleukin-6

IL-10 Interleukin-10

LP Low-protein

MCP-1 Monocyte chemoattractant protein-1

TNF-a Tumor necrosis factor alpha

Declarations

Ethics approval and consent to participate

The trial was approved by the Ethics Committee of the Charité University Medicine in Berlin (Application No. EA4/006/15), conducted in accordance with the Declaration of Helsinki. All participants provided written informed consent before entering the study.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analyzed during this study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

Funding

The study was supported by an European Association for the Study of Diabetes (EASD) grant “Identification of individual response criteria for reduction of hepatic fat by nutritional approaches and subsequent maintenance by dietary strategies”, by the German Center for Diabetes Research (DZD) grant “Is FGF21 as novel bidirectional regulator of protein preference and aversion: regulatory mechanisms and clinical implications”, and by the DZD grant “Role of Wnt-inducible signaling pathway protein-1 (WISP-1) in adipose tissue and liver fibrosis in mice and men”.

Authors’ contributions

KA and OPR designed research; MM, NS, SH, AR, and VL conducted research; LK analyzed data; KA and LK wrote the paper; KA and OPR had primary responsibility for final content; All authors read and approved the final manuscript.

Acknowledgements

We acknowledge all study participants for their cooperation. We gratefully thank Melanie Hannemann, Dominique Zschau, Erik Wehrstedt, Katja Treu, Katrin Ritter, and Andrea Borchert for technical assistance in the clinical trial.

References

1. WHO. Obesity and Overweight 2020 [updated 1.04.2020. Available from: https://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight.
2. Andreyeva T, Sturm R, Ringel JS. Moderate and severe obesity have large differences in health care costs. Obes Res. 2004;12(12):1936–43.
3. Sturm R, Hattori A. Morbid obesity rates continue to rise rapidly in the United States. International journal of obesity (2005). 2013;37(6):889 – 91.
4. Fildes A, Charlton J, Rudisill C, Littlejohns P, Prevost AT, Gulliford MC. Probability of an Obese Person Attaining Normal Body Weight: Cohort Study Using Electronic Health Records. Am J Public Health. 2015;105(9):e54-e9.
5. Gulliford MC, Charlton J, Prevost T, Booth H, Fildes A, Ashworth M, et al. Costs and Outcomes of Increasing Access to Bariatric Surgery: Cohort Study and Cost-Effectiveness Analysis Using Electronic Health Records. Value Health. 2017;20(1):85–92.
6. Jouan Y, Blasco H, Bongrani A, Couet C, Dupont J, Maillot F. Preoperative Chemerin Level Is Predictive of Inflammatory Status 1 Year After Bariatric Surgery. Obes Surg. 2020.
7. Courcoulas AP, Christian NJ, Belle SH, Berk PD, Flum DR, Garcia L, et al. Weight change and health outcomes at 3 years after bariatric surgery among individuals with severe obesity. Jama. 2013;310(22):2416–25.
8. You T, Arsenis NC, Disanzo BL, Lamonte MJ. Effects of exercise training on chronic inflammation in obesity: current evidence and potential mechanisms. Sports Med. 2013;43(4):243–56.
9. Williamson K, Nimegeer A, Lean M. Rising prevalence of BMI ≥ 40 kg/m2: A high-demand epidemic needing better documentation. Obes Rev. 2020;21(4):e12986.
10. Kiecolt-Glaser JK. Stress, food, and inflammation: psychoneuroimmunology and nutrition at the cutting edge. Psychosom Med. 2010;72(4):365–9.
11. Minihane AM, Vinoy S, Russell WR, Baka A, Roche HM, Tuohy KM, et al. Low-grade inflammation, diet composition and health: current research evidence and its translation. Br J Nutr. 2015;114(7):999–1012.
12. Clifton PM, Keogh J. Metabolic effects of high-protein diets. Curr Atheroscler Rep. 2007;9(6):472–8.
13. Batterham RL, Cowley MA, Small CJ, Herzog H, Cohen MA, Dakin CL, et al. Gut hormone PYY3-36 physiologically inhibits food intake. Nature. 2002;418(6898):650–4.
14. Dong JY, Zhang ZL, Wang PY, Qin LQ. Effects of high-protein diets on body weight, glycaemic control, blood lipids and blood pressure in type 2 diabetes: meta-analysis of randomised controlled trials. Br J Nutr. 2013;110(5):781–9.

15. Pivovarova-Ramich O, Markova M, Weber D, Sucher S, Hornemann S, Rudovich N, et al. Effects of diets high in animal or plant protein on oxidative stress in individuals with type 2 diabetes: A randomized clinical trial. Redox Biology. 2020;29:101397.
16. Draganidis D, Karagounis LG, Athanailidis I, Chatzinikolaou A, Jamurtas AZ, Fatouros IG. Inflammaging and Skeletal Muscle: Can Protein Intake Make a Difference? J Nutr. 2016;146(10):1940–52.
17. Hruby A, Jacques PF. Dietary Protein and Changes in Biomarkers of Inflammation and Oxidative Stress in the Framingham Heart Study Offspring Cohort. Curr Dev Nutr. 2019;3(5):nzz019.
18. Castaño-Martinez T, Schumacher F, Schumacher S, Kochlik B, Weber D, Grune T, et al. Methionine restriction prevents onset of type 2 diabetes in NZO mice. FASEB J. 2019;33(6):7092–102.
19. Roy DG, Chen J, Mamane V, Ma EH, Muhire BM, Sheldon RD, et al. Methionine Metabolism Shapes T Helper Cell Responses through Regulation of Epigenetic Reprogramming. Cell Metab. 2020;31(2):250. :– 66.e9..
20. Gögebakan O, Kohl A, Osterhoff MA, van Baak MA, Jebb SA, Papadaki A, et al. 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. Circulation. 2011;124(25):2829–38.
21. Markova M, Koelman L, Hornemann S, Pivovarova O, Sucher S, Machann J, et al. Effects of plant and animal high protein diets on immune-inflammatory biomarkers: A 6-week intervention trial. Clin Nutr. 2020;39(3):862–9.
22. Xu C, Markova M, Seebeck N, Loft A, Hornemann S, Gantert T, et al. High-protein diet more effectively reduces hepatic fat than low-protein diet despite lower autophagy and FGF21 levels. Liver International. 2020; In Press.
23. Roy J. SAS for Mixed Models, Second Edition. C.Littell R, Milliken GA, Stroup WW, Wolfinger RD, Schabenberger O. Journal of Biopharmaceutical Statistics. 2007;17(2):188.
24. Bárcena C, Quirós PM, Durand S, Mayoral P, Rodríguez F, Caravia XM, et al. Methionine Restriction Extends Lifespan in Progeroid Mice and Alters Lipid and Bile Acid Metabolism. Cell Rep. 2018;24(9):2392–403.
25. Mladenović D, Radosavljević T, Hrnčić D, Rasic-Markovic A, Stanojlović O. The effects of dietary methionine restriction on the function and metabolic reprogramming in the liver and brain - implications for longevity. Rev Neurosci. 2019;30(6):581–93.
26. Sharma S, Dixon T, Jung S, Graff EC, Forney LA, Gettys TW, et al. Dietary Methionine Restriction Reduces Inflammation Independent of FGF21 Action. Obesity (Silver Spring). 2019;27(8):1305–13.
27. Wanders D, Ghosh S, Stone KP, Van NT, Gettys TW. Transcriptional impact of dietary methionine restriction on systemic inflammation: Relevance to biomarkers of metabolic disease during aging. BioFactors. 2014;40(1):13–26.
28. Malloy VL, Krajcik RA, Bailey SJ, Hristopoulos G, Plummer JD, Orentreich N. Methionine restriction decreases visceral fat mass and preserves insulin action in aging male Fischer 344 rats independent

of energy restriction. Aging Cell. 2006;5(4):305–14.

29. Hasek BE, Stewart LK, Henagan TM, Boudreau A, Lenard NR, Black C, et al. Dietary methionine restriction enhances metabolic flexibility and increases uncoupled respiration in both fed and fasted states. Am J Physiol Regul Integr Comp Physiol. 2010;299(3):R728-39.
30. Martínez Y, Li X, Liu G, Bin P, Yan W, Más D, et al. The role of methionine on metabolism, oxidative stress, and diseases. Amino Acids. 2017;49(12):2091–8.
31. Ables GP, Perrone CE, Orentreich D, Orentreich N. Methionine-restricted C57BL/6J mice are resistant to diet-induced obesity and insulin resistance but have low bone density. PLoS One. 2012;7(12):e51357.
32. Miller RA, Buehner G, Chang Y, Harper JM, Sigler R, Smith-Wheelock M. Methionine-deficient diet extends mouse lifespan, slows immune and lens aging, alters glucose, T4, IGF-I and insulin levels, and increases hepatocyte MIF levels and stress resistance. Aging Cell. 2005;4(3):119–25.
33. Dong Z, Sinha R, Richie JP. Jr. Disease prevention and delayed aging by dietary sulfur amino acid restriction: translational implications. Ann N Y Acad Sci. 2018;1418(1):44–55.
34. Plaisance EP, Greenway FL, Boudreau A, Hill KL, Johnson WD, Krajcik RA, et al. Dietary methionine restriction increases fat oxidation in obese adults with metabolic syndrome. J Clin Endocrinol Metab. 2011;96(5):E836-40.
35. Halton TL, Hu FB. The effects of high protein diets on thermogenesis, satiety and weight loss: a critical review. J Am Coll Nutr. 2004;23(5):373–85.
36. Eisenstein J, Roberts SB, Dallal G, Saltzman E. High-protein weight-loss diets: are they safe and do they work? A review of the experimental and epidemiologic data. Nutr Rev. 2002;60(7 Pt 1):189–200.
37. Gonzalez S, Fernandez-Navarro T, Arboleya S, de Los Reyes-Gavilan CG, Salazar N, Gueimonde M. Fermented Dairy Foods: Impact on Intestinal Microbiota and Health-Linked Biomarkers. Front Microbiol. 2019;10:1046.
38. Bordoni A, Danesi F, Dardevet D, Dupont D, Fernandez AS, Gille D, et al. Dairy products and inflammation: A review of the clinical evidence. Crit Rev Food Sci Nutr. 2017;57(12):2497–525.
39. Lomax AR, Calder PC. Probiotics, immune function, infection and inflammation: a review of the evidence from studies conducted in humans. Curr Pharm Des. 2009;15(13):1428–518.
40. Ceapa C, Wopereis H, Rezaïki L, Kleerebezem M, Knol J, Oozeer R. Influence of fermented milk products, prebiotics and probiotics on microbiota composition and health. Best Pract Res Clin Gastroenterol. 2013;27(1):139–55.
41. Calder PC, Ahluwalia N, Brouns F, Buetler T, Clement K, Cunningham K, et al. Dietary factors and low-grade inflammation in relation to overweight and obesity. Br J Nutr. 2011;106(Suppl 3):5–78.
42. Rocha VZ, Libby P. Obesity, inflammation, and atherosclerosis. Nature Reviews Cardiology. 2009;6(6):399–409.
43. Forrester JS, Libby P. The Inflammation Hypothesis and Its Potential Relevance to Statin Therapy. The American Journal of Cardiology. 2007;99(5):732–8.

44. Trepanowski JF, Mey J, Varady KA. Fetuin-A: a novel link between obesity and related complications. International Journal of Obesity. 2015;39(5):734–41.
45. Calder PC, Ahluwalia N, Albers R, Bosco N, Bourdet-Sicard R, Haller D, et al. A consideration of biomarkers to be used for evaluation of inflammation in human nutritional studies. Br J Nutr. 2013;109(Suppl 1):1–34.

Figures

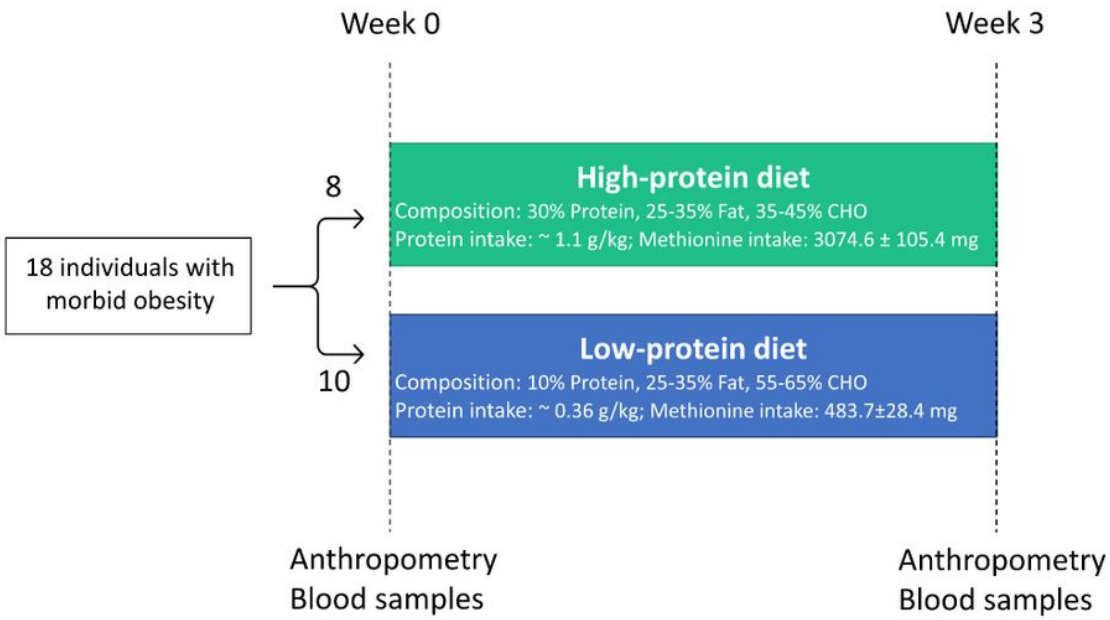

Figure 1

Study design of the intervention study. In total, n=18 participants completed the intervention study and were included for final analysis. Participants were matched according to age, sex, and body mass index into high-protein and low-protein diet groups. Single blood samples and anthropometric measurements were collected on two occasions: before the intervention and after 3 weeks.

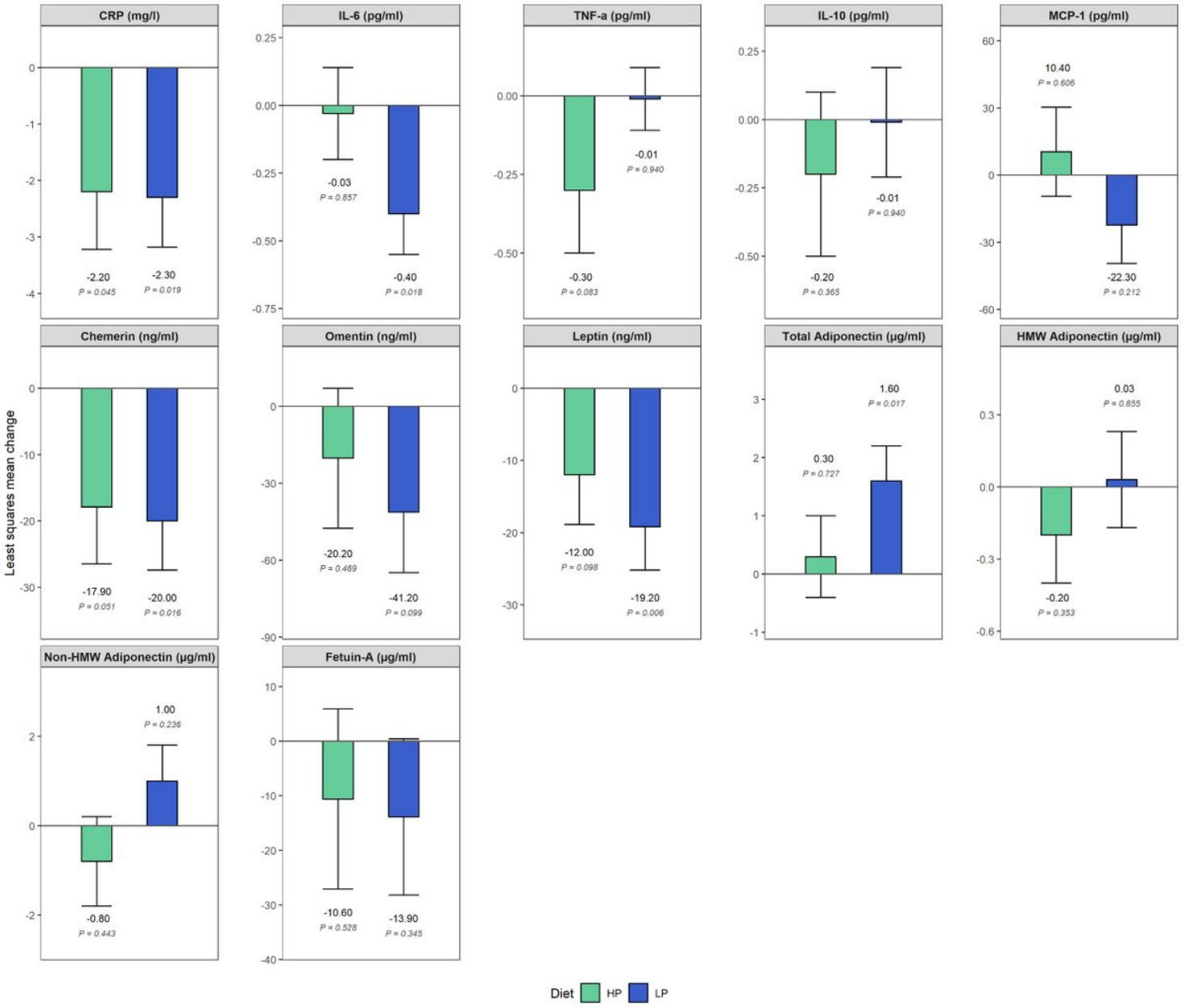

Figure 2

Differences of least squares means of biomarkers over time, grouped by diet, adjusted for age, sex, body mass index, and baseline value. Participants receiving high-protein (HP) diet are represented in green; participants receiving low-protein (LP) diet are represented in blue. P-values are obtained from t-test. Abbreviations: CRP, C-reactive protein; IL-6, interleukin-6; TNF-a, tumor necrosis factor alpha; IL-10, interleukin-10; MCP-1, monocyte chemoattractant protein 1; HMW, high molecular weight.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Consortchecklist.docx

- AdditionalFile1.docx