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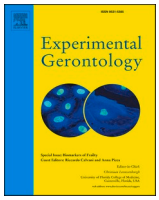

The effect of a combined lifestyle intervention with and without protein drink on inflammation in older adults with obesity and type 2 diabetes

Robert G. Memelink [a,b,c,*], Rose Njemini [d], Minse J.J. de Bos Kuil [a], Suzan Wopereis [e], Johan de Vogel-van den Bosch [f], Josje D. Schoufour [a], Michael Tieland [a,1], Peter J.M. Weijs [a,b,g], Ivan Bautmans [c,d,h,i]

[a] Faculty of Sports and Nutrition, Center of Expertise Urban Vitality, Amsterdam University of Applied Sciences (AUAS), 1067 SM Amsterdam, the Netherlands
[b] Amsterdam Movement Sciences research institute, Amsterdam UMC location Vrije Universiteit Amsterdam, 1081 HV Amsterdam, the Netherlands
[c] Gerontology Department, Faculty of Medicine and Pharmacy, Vrije Universiteit Brussel, 1090 Brussels, Belgium
[d] Frailty & Resilience in Ageing (FRIA) research department, Faculty of Medicine and Pharmacy, Vrije Universiteit Brussel, 1090 Brussels, Belgium
[e] Research group Microbiology & Systems Biology, Netherlands Organisation for Applied Scientific Research (TNO), 2333 BE Leiden, the Netherlands
[f] Danone Nutricia Research, 3584 CT Utrecht, the Netherlands
[g] Department of Nutrition and Dietetics, Amsterdam University Medical Centers, VU University, 1081 HV Amsterdam, the Netherlands
[h] Department of Geriatrics, Universitair Ziekenhuis Brussel, 1090 Brussels, Belgium
[i] SOMT University of Physiotherapy, 3821 BN Amersfoort, the Netherlands

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ABSTRACT

Background: Chronic low-grade inflammatory profile (CLIP) is one of the pathways involved in type 2 diabetes (T2D). Currently, there is limited evidence for ameliorating effects of combined lifestyle interventions on CLIP in type 2 diabetes. We investigated whether a 13-week combined lifestyle intervention, using hypocaloric diet and resistance exercise plus high-intensity interval training with or without consumption of a protein drink, affected CLIP in older adults with T2D.

Methods: In this post-hoc analysis of the PROBE study 114 adults (≥55 years) with obesity and type 2 (pre-) diabetes had measurements of C-reactive protein (CRP), pro-inflammatory cytokines interleukin (IL)-6, tumor-necrosis-factor (TNF)-α, and monocyte chemoattractant protein (MCP)-1, anti-inflammatory cytokines IL-10, IL-1 receptor antagonist (RA), and soluble tumor-necrosis-factor receptor (sTNFR)1, adipokines leptin and adiponectin, and glycation biomarkers carboxymethyl-lysine (CML) and soluble receptor for advanced glycation end products (sRAGE) from fasting blood samples. A linear mixed model was used to evaluate change in inflammatory biomarkers after lifestyle intervention and effect of the protein drink. Linear regression analysis was performed with parameters of body composition (by dual-energy X-ray absorptiometry) and parameters of insulin resistance (by oral glucose tolerance test).

Results: There were no significant differences in CLIP responses between the protein and the control groups. For all participants combined, IL-1RA, leptin and adiponectin decreased after 13 weeks ($p = 0.002$, $p < 0.001$ and $p < 0.001$), while ratios TNF-α/IL-10 and TNF-α/IL-1RA increased ($p = 0.003$ and $p = 0.035$). CRP increased by 12 % in participants with low to average CLIP (pre 1.91 ± 0.39 mg/L, post 2.13 ± 1.16 mg/L, $p = 0.006$) and decreased by 36 % in those with high CLIP (pre 5.14 mg/L ± 1.20, post 3.30 ± 2.29 mg/L, $p < 0.001$). Change in leptin and IL-1RA was positively associated with change in fat mass ($\beta = 0.133$, $p < 0.001$; $\beta = 0.017$, $p < 0.001$) and insulin resistance ($\beta = 0.095$, $p = 0.024$; $\beta = 0.020$, $p = 0.001$). Change in lean mass was not associated with any of the biomarkers.

Abbreviations: AGE, advanced glycation end product; Adipo-IR, adipose tissue insulin resistance index; ALM, appendicular lean mass; CLIP, Chronic low-grade inflammatory profile; CML, carboxymethyl-lysine; CRP, C-reactive protein; DI, disposition index; HOMA-IR, homeostatic model assessment for insulin resistance; IL-6, interleukin-6; IL-10, interleukin-10; IL-1RA, interleukin-1 receptor antagonist; LBM, lean body mass; MCP-1, monocyte chemoattractant protein-1; MIR, muscle insulin resistance; MISI, muscle insulin sensitivity index; OGTT, oral glucose tolerance test; T2D, type 2 diabetes; TNF-α, tumor necrosis factor α; sRAGE, soluble receptor for advanced glycation end products; sTNFR1, soluble tumor necrosis factor receptor 1; VAT, visceral adipose tissue.

* Corresponding author at: Faculty of Sports and Nutrition, Center of Expertise Urban Vitality, Amsterdam University of Applied Sciences (AUAS), 1067 SM Amsterdam, the Netherlands.
E-mail address: r.g.memelink@hva.nl (R.G. Memelink).
[1] Institute for Physical Activity and Nutrition, School of Exercise and Nutrition Sciences, Deakin University, Geelong, Victoria, Australia.

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Conclusion: 13 weeks of combined lifestyle intervention, either with or without protein drink, reduced circulating adipokines and anti-inflammatory cytokine IL-1RA, and increased inflammatory ratios TNF-α/IL-10 and TNF-α/IL-1RA in older adults with obesity and T2D. Effect on CLIP was inversely related to baseline inflammatory status.

1. Introduction

Ageing and obesity are well known risk factors for developing type 2 diabetes (Kolb and Martin, 2017; Yuan and Larsson, 2020) and contribute to the ongoing global rise in diabetes prevalence (International Diabetes Federation, 2021). Obesity management is beneficial in the treatment of type 2 diabetes (American Diabetes Association Professional Practice Committee, 2022), however, weight reduction is often accompanied by loss of muscle mass (Trouwborst et al., 2018). Additionally, type 2 diabetes is independently associated with excessive loss of muscle mass in older adults (Park et al., 2009), increasing the risk for the development of sarcopenia. To address these challenges, combined lifestyle interventions that include resistance exercise have the potential to preserve muscle mass during weight loss (Leitner et al., 2017; Memelink et al., 2020). This is particularly important in type 2 diabetes because skeletal muscle is the predominant site of insulin-mediated glucose uptake in the postprandial state (DeFronzo and Tripathy, 2009).

Ageing, obesity and type 2 diabetes are all associated with a chronic low-grade inflammatory profile (CLIP), characterised by increased levels of inflammatory biomarkers (Draganidis et al., 2016; Hotamisligil and Erbay, 2008; Liu et al., 2016). Moreover, elevated levels of C-reactive protein (CRP) and interleukin 6 (IL-6) predict the development of type 2 diabetes (Pradhan et al., 2001). Type 2 diabetes is also characterised by increased glycation, adding to the chronic inflammatory state (Singh et al., 2001). Advanced glycation end products (AGEs) induce their pro-inflammatory response by activating AGE receptors (Ramya et al., 2021), ultimately resulting in increased production of pro-inflammatory cytokine TNF-α. A soluble receptor for these AGEs (sRAGE) has the potential to counteract inflammation by binding to AGE, thereby preventing activation of AGE receptors (Steenbeke et al., 2021). Both chronic low-grade inflammation and glycation contribute to the development of microvascular complications in type 2 diabetes (Forbes and Cooper, 2013).

While there is ample evidence that combined lifestyle interventions can reduce glycation — often studied through HbA1c (Memelink et al., 2020; Franz et al., 2015; Terranova et al., 2015; Chen et al., 2015), there is limited evidence for the ameliorating effects of these interventions on chronic inflammation in individuals with type 2 diabetes (Giannopoulou et al., 2005; Johansen et al., 2020). A reduction in chronic inflammation can lead to improved muscle protein synthesis, as previous work suggested (Draganidis et al., 2016; Boirie, 2013), making inflammation a potential therapeutic target in the treatment of type 2 diabetes. The attenuating effect of exercise on inflammation in older adults has been well established. However its effect in older adults with chronic disease (e.g. type 2 diabetes) seems limited (Bautmans et al., 2021). Preliminary evidence suggests an anti-inflammatory role of protein supplementation in conditions with and without exercise (Draganidis et al., 2016). Furthermore, an attenuating effect on the pro-inflammatory marker IL-6 was observed in sarcopenic older adults upon consumption of a protein drink for 6 months without accompanying exercise program (Liberman et al., 2019).

We have previously observed reductions in body weight, fat mass and HbA1c after a 13-week combined lifestyle intervention in older adults with obesity and type 2 diabetes, both with and without the use of a protein drink enriched with leucine and vitamin D. The ingestion of the protein drink during this lifestyle intervention resulted in preservation of lean mass and improved insulin sensitivity (Memelink et al., 2020). We hypothesize that these changes could be mediated by a reduction in chronic low-grade inflammation. Though we did not observe significant changes in inflammatory biomarker CRP (Memelink et al., 2020), the inflammatory profile may still be related to the observed changes in muscle mass and insulin sensitivity and their differences between treatment groups. To test this hypothesis, we now evaluate in a post-hoc analysis whether the 13-week combined lifestyle intervention, with or without the addition of a protein drink, affected circulating levels of inflammatory biomarkers that included pro-inflammatory and anti-inflammatory cytokines, adipokines and glycation biomarkers in older adults with obesity and type 2 diabetes. In addition, we evaluate whether the inflammatory response depended on the baseline level of chronic low-grade inflammation. To gain a deeper understanding of how changes in the inflammatory profile relate to the earlier observed health outcomes, we evaluate associations between changes in inflammatory biomarkers and changes in body composition and insulin resistance.

2. Material and methods

2.1. Participants

We included participants from the PROBE study ($n = 123$). Briefly, these were older adults (≥ 55 years) with obesity (defined as having a body mass index (BMI) > 30 kg/m^2, or a BMI > 27 kg/m^2 with waist circumference > 88 cm for women or > 102 cm for men) and type 2 (pre-) diabetes. Potential participants were evaluated for in- and exclusion criteria during a screening visit as described in detail elsewhere (Memelink et al., 2020). Participants were included in a 13-week combined lifestyle intervention and were randomly allocated to either the protein group ($n = 62$) or the control group ($n = 61$). Randomization was stratified according to sex and the use of sulfonylurea derivatives at the start of the study. The PROBE study was pre-registered in the Netherlands Trial Register. Detailed information of the PROBE study is available in the International Clinical Trials Registry Platform (https://trialsearch.who.int; ID NTR4497). This post-hoc evaluation of the PROBE study is based on 114 participants with inflammatory biomarkers available at baseline or week 13 and without indication of acute inflammation (Fig. 1).

2.2. Combined lifestyle intervention

All participants were instructed to adhere to a hypocaloric diet of 600 kcal below estimated energy needs according to the Dutch guideline for treatment of obesity (Dutch Institute for Healthcare Improvement, 2008), that included the caloric content of the study products (see section Protein drink). Throughout the 13-week intervention, participants followed six individual dietary counselling sessions and six nutrition and lifestyle group sessions supervised by a dietician. In addition, all participants enrolled in an exercise program, which was conducted three times per week in 1 h group sessions under supervision of a qualified personal trainer. The exercise program consisted of progressive resistance exercise (starting at 60 % of one-repetition maximum and progressing to 80 %) and high intensity interval training (HIIT) on a cycle ergometer (using 30 s intervals starting at 70 % of maximal work capacity determined during a steep ramp test and progressing to 110 %). Details of the diet and exercise program are available in the main PROBE study publication (Memelink et al., 2020).

2.3. Protein drink

Participants either received a protein or isocaloric control drink for a

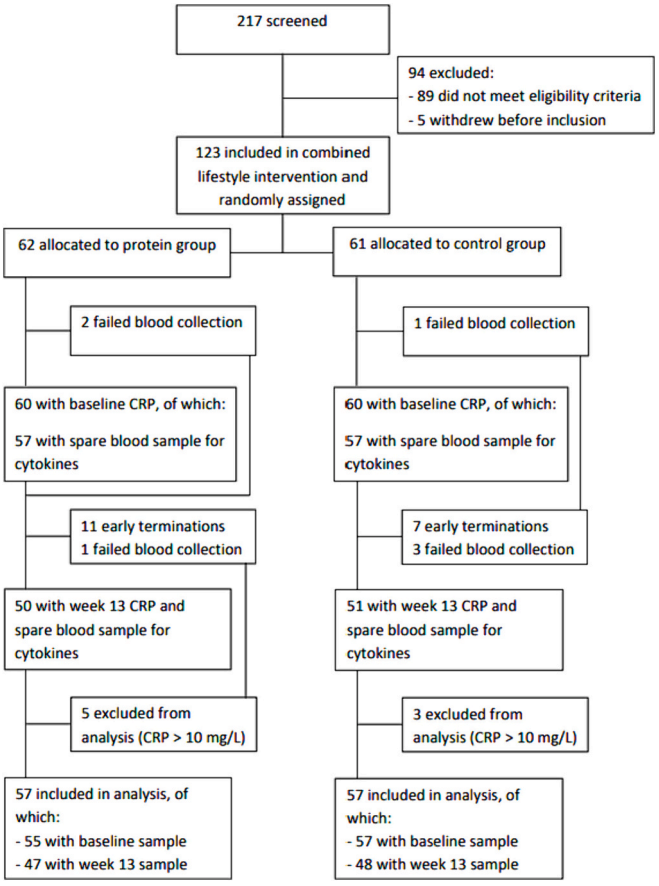

Fig. 1. Flow chart of the PROBE study participants for the post-hoc evaluation of inflammatory biomarker response.

double-blind period of 13 weeks. The study products were provided as powder in 40-g sachets and were dissolved in 125 mL of water, to be consumed during breakfast and after training on training days. Per serving, the protein drink contained 21 g of protein, of which 1 g free leucine, and a mixture of carbohydrates and fat providing 150 kcal per serving, 800 IU Vitamin D and a mixture of fibres, minerals and vitamins. The isocaloric control drink did not contain any protein, leucine or vitamin D; detailed compositions are shown in Appendix A of the main PROBE study publication (Memelink et al., 2020).

2.4. Participant characteristics and outcome measures

2.4.1. Participant characteristics, body composition, insulin resistance

Participant characteristics such as age, sex, BMI, duration of type 2 diabetes, and medication were recorded at the baseline visit. At baseline and after 13 weeks, participants underwent assessment of body composition, physical performance, dietary intake, and blood sampling as described previously (Memelink et al., 2020). Whole body and regional body composition was measured using dual-energy X-ray absorptiometry (DXA, Hologic Discovery A, Bedford, USA). We calculated the following body composition indices: lean body mass (LBM)/fat mass, LBM/fat mass percentage (fat mass %), appendicular lean mass (ALM)/BMI, and muscle quality indices (Nilsson et al., 2020): leg press strength/leg lean mass (LLM) and knee extension power/LLM. Parameters of insulin resistance (HOMA-IR, disposition index [DI], adipose tissue insulin resistance index [Adipo-IR], Matsuda index, muscle insulin sensitivity index [MISI]) were calculated from plasma glucose and insulin levels assessed during 2 h oral glucose tolerance test (OGTT) as described previously (Pasman et al., 2020).

2.4.2. Inflammatory biomarkers

Fasting blood samples were collected and sent to the VU University Medical Center Amsterdam for analysis of plasma C-reactive protein (CRP). Circulating levels of pro-inflammatory cytokines interleukin 6 (IL-6), tumor-necrosis-factor α (TNF-α) and monocyte chemoattractant protein 1 (MCP-1), anti-inflammatory cytokines IL-10, IL-1 receptor antagonist (IL-1RA) and soluble tumor-necrosis-factor receptor 1 (sTNFR1), adipokines leptin and adiponectin, and glycation biomarkers carboxymethyl-lysine (CML; a predominant AGE in type 2 diabetes (Ramya et al., 2021)) and soluble receptor for advanced glycation end products (sRAGE) were assayed at the University Hospital Brussels in spare plasma and serum samples stored at −80 °C for 4 to 6 years. IL-6, IL-10, TNF-α, MCP-1 and leptin were analysed in plasma. IL-1RA, sTNFR1, adiponectin, CML and sRAGE were analysed in serum. IL-6, IL-10, and TNF-α were analysed using a commercially available triplex kit (Quanterix, Billerica, USA). IL-1RA, sTNFR1, adiponectin and sRAGE (Lifetech, Carlsbad, CA), MCP-1 and leptin (Quanterix, Billerica, USA), and CML (Gentaur, Kampenhout, Belgium) were analysed separately using commercially available ELISA kits. For each participant, the samples at baseline and 13 weeks were analysed on the same plate to limit inter-assay variability. Sensitivity levels, intra-assay and inter-essay coefficients of variation are reported in Supplemental Table 1. Cytokines were available from 114 participants at baseline and 101 participants at 13 weeks (Fig. 1).

2.5. Statistical analyses

Before analysis, values below the lower limit of detection (LOD) were replaced by LOD/$\sqrt{2}$ and values above upper LOD were replaced by upper LOD. Normality assumption was checked using Q-Q plots. Because of non-normal distribution, all inflammatory biomarkers were log(10)-transformed to improve the distribution. Ratios of pro- and anti-inflammatory cytokines IL-6/IL-10, IL-6/IL-1RA, TNF-α/IL-10, TNF-α/IL-1RA and TNF-α/sTNFR1, and adipokine ratio leptin/adiponectin were calculated based on untransformed data, as indices of pro-/anti-inflammatory balance. Extreme outliers in these ratios, based on Q-Q plots, were removed from statistical analysis. Statistical analyses were performed excluding participants with CRP > 10 mg/L at any of the two timepoints, to eliminate potential bias due to acute inflammatory conditions (Martínez and González-Juanatey, 2009). Participants with missing values on one of the two timepoints were included in all mixed model analyses described below. Baseline characteristics were reported descriptively for both treatment groups.

As our main analysis, we evaluated the effect of the protein drink on the change in inflammatory biomarkers after 13 weeks of combined lifestyle intervention. A linear mixed model was applied with fixed factors for treatment (protein/control), time, time by treatment interaction, and stratification factors sex and SU-derivatives at study start (yes/no). The model included the baseline value of the outcome in the outcome vector. A random intercept for each participant was included and the 'unstructured' variance covariance structure was used. The significance of the two-way interaction including time and treatment was checked for evaluation of the treatment effect. In addition, an explorative responder analysis was performed on participants that had preserved and participants that had lost appendicular lean mass within the protein group, for indication of a possible mediating role for inflammation in the previously observed preservation of lean mass during weight loss upon consumption of the protein drink (Memelink et al., 2020).

We performed two sub-analyses. In sub-analysis 1, we evaluated the change in inflammatory biomarkers after 13 weeks of combined lifestyle intervention. Treatment (protein/control) and time by treatment interaction were removed from the linear mixed model. The significance of the change from baseline to week 13 was checked for evaluation of the change after 13 weeks of combined lifestyle intervention in the entire study population.

In sub-analyses 2, we evaluated potential interaction between the combined lifestyle intervention (change over time) and the level of chronic low-grade inflammation profile (low to average CLIP [baseline CRP $\leq$ 3 mg/L] vs. high CLIP [baseline CRP > 3 mg/L]) (Pearson et al., 2003). CLIP level and time by CLIP level were added to the linear mixed model as fixed factors. The significance of the two-way interaction including time and CLIP level was checked for evaluation of interaction. In case of interaction, stratified analysis for the subgroups was performed to evaluate the changes in inflammatory biomarkers after 13 weeks of combined lifestyle intervention, using the same model as in sub-analysis 1.

Finally, body composition indices, muscle quality indices, and parameters of regional fat mass that were not included in our RCT publication (Memelink et al., 2020) were evaluated for treatment effect and change after 13 weeks of combined lifestyle intervention as described above. And subsequently, correlations were evaluated using linear regression models with the change in inflammatory biomarker level after 13 weeks as independent, and change in parameters of body composition or insulin resistance as dependent variables, to further investigate the link between inflammation and body composition and insulin resistance. In addition, we performed linear regression models with the change in whole body fat mass or visceral fat mass after 13 weeks as independent, and change in inflammatory biomarker levels after 13 weeks as dependent variables. Inflammatory biomarker levels were not log-transformed because of incompatibility for negative change values, and normal distribution of regression residuals was checked post-hoc. Visual outliers were excluded from the regression analysis. Regression coefficient β is reported including 95 % confidence interval, for the crude model and corrected models if applicable. Potential confounders sex, age, duration of type 2 diabetes, energy balance (expressed as caloric deficit) at the end of intervention, number of training sessions attended, exercise protocol adherence (% of scheduled training sessions), relative training load, protein intake, zinc intake, vitamin D intake, plasma calcidiol, and use of anti-inflammatory medication (yes/no) were evaluated for correlation with the independent and dependent variables before step-by-step inclusion in the regression model, and were considered as a confounder when regression coefficient β changed > 10 %. However, close to zero beta values were considered irrelevant.

Statistical analyses were performed in IBM SPSS Statistics for Windows v28.0.1.0 (IBM Corp, Armonk, NY, USA). All test were conducted two-sided with a significance level of 5 %. All confidence intervals will be presented two-sided with a confidence level of 95 %.

3. Results

Baseline characteristics of participants included in the post-hoc analysis on the inflammatory biomarker profile of the PROBE study are shown in Table 1. Treatment groups were comparable at baseline. Participants excluded from the analysis (because of CRP > 10 mg/L) were comparable to participants included, but had longer duration of type to diabetes and a higher CRP level by definition (Supplemental Table 2).

3.1. Effect of protein drink

There were no significant differences after 13 weeks in any of the biomarkers between the protein group and the control group (Table 2). Il-10 tended to decrease ($p = 0.096$) and the ratio TNF-α/Il-10 tended to increase ($p = 0.088$) in the protein group compared to control.

Responder analysis for discrimination between participants that lost (non-responders, $n = 13$) and participants that preserved (responders, $n = 30$) appendicular lean mass within the protein group showed that leptin decreased in non-responders compared to responders ($p < 0.001$, both with and without correction for change in fat mass) and adiponectin decreased in both responders and non-responders. CRP tended to increase in responders compared to non-responders ($p = 0.072$; $p = 0.070$ after correction for change in fat mass).

Table 1
Participant's baseline characteristics.

		Protein group		Control group
	n	Mean ± SD	n	Mean ± SD
Age (years)	57	66.7 ± 6.0	57	65.7 ± 6.3
Sex (F; M)	19; 38		21; 36	
Type 2 diabetes duration (months)	53	80 (IQR 85)[a]	53	74 (IQR 90)[a]
Body weight (kg)	57	98.7 ± 15.4	57	99.6 ± 16.0
BMI (kg/m^2)	57	33.0 ± 4.4	57	33.3 ± 4.6
Fat mass (%)	55	33.8 ± 7.1	57	34.1 ± 6.0
VAT (cm^2)	56	183 ± 57	57	182 ± 52
NSAID use (n)	17		15	
Smoking (n)	7		6	
Alcohol use (n)	41		35	
Handgrip strength (kg)	55	36.0 ± 10.8	56	36.8 ± 10.4
Legpress 10-RM strength (kg)	52	133 ± 59	50	132 ± 60
SPPB score (0–12)	57	11.0 ± 1.1	57	10.9 ± 1.7
VO_2peak (L/min)	57	1.68 ± 0.43	56	1.83 ± 0.46
PAL (–)	48	1.19 ± 0.07	54	1.20 ± 0.09
Plasma calcidiol (nmol/L)	53	62.2 ± 27.9	55	61.3 ± 18.0
CRP (mg/L)	55	1.77 (IQR 1.03)[a]	57	1.77 (IQR 2.23)[a]
HbA1c (mmol/mol)	55	50.9 ± 9.9	55	53.3 ± 10.9
Fasting plasma glucose (mmol/L)	53	8.14 ± 1.74	55	8.24 ± 1.92
HOMA-IR	53	6.05 ± 4.50	54	5.32 ± 2.44
Matsuda index	51	2.24 ± 1.12	52	2.26 ± 0.94
Dietary protein intake (g/kg body weight/day)	55	0.84 ± 0.21	57	0.89 ± 0.31

BMI: body mass index (weight/height^2), VAT: visceral adipose tissue (by DXA), NSAID: non-steroidal anti-inflammatory drugs, SPPB: Short Physical Performance Battery, VO_2peak: peak oxygen uptake capacity (during steep ramp test on cycle ergometer), PAL: Physical Activity Level (by accelerometry).
[a] Median and IQR (inter-quartile range).

3.2. Change after 13-week combined lifestyle intervention

After 13 weeks of intervention, IL-1RA, leptin and adiponectin decreased significantly and Il-10 tended to decrease (Table 3). Based on back-transformation of the reported 10-log values, IL-1RA decreased by 12 % (from an average of 130 pg/L at baseline to 115 pg/L at week 13), leptin decreased by 12 % (from 23.2 ng/L to 20.4 ng/L) and adiponectin decreased by 17 % (from 10.7 μg/L to 8.9 μg/L). The other biomarkers did not change significantly after 13 weeks, while pro-/anti-inflammatory ratios TNF-α/IL-10 and TNF-α/IL-1RA increased significantly and ratio Il-6/Il-10 tended to increase (Table 3).

3.3. Low to average CLIP vs. high CLIP

Change in inflammatory biomarkers was statistically different between participants with low to average CLIP ($n = 84$) and participants with high CLIP ($n = 28$) for CRP only ($p < 0.001$). Stratified analysis within these subgroups showed that participants with low to average CLIP had results similar to the results in the entire study population, except for a significant increase in CRP in this subgroup (+0.048, 95 % CI 0.014 to 0.081, $p = 0.006$) (Supplemental Table 3a). This corresponds to a 12 % increase in CRP (from an average of 1.91 mg/L at baseline to 2.13 mg/L at week 13, based on back-transformation).

In participants with high CLIP, CRP decreased after 13 weeks (−0.193, 95 % CI −0.282 to −0.104, p < 0.001) and so did IL1-RA (−0.078, 95 % CI −0.147 to −0.010, $p = 0.027$) (Supplemental Table 3b). The change in CRP corresponds to a 36 % decrease (from an average of 5.14 mg/L at baseline to 3.30 mg/L at week 13, based on back-transformation).

Table 2
Effects of protein versus control on CRP, pro- and anti-inflammatory cytokines, adipokines and glycation markers. Results from mixed model analysis excluding participants with CRP > 10 mg/L indicating acute inflammation. Data are 10-log values, except for the ratios which are based on untransformed data.

	Protein		Control		Treatment effect	95 % Confidence Interval	*p*-value
	n	EMM ± SE	n	EMM ± SE			
CRP (mg/L)							
Baseline	55	0.373 ± 0.027	57	0.429 ± 0.026			
13 weeks	46	0.364 ± 0.029	48	0.418 ± 0.028	0.001	−0.079; 0.082	0.978
Pro-inflammatory cytokines							
IL-6 (pg/mL)							
Baseline	53	0.421 ± 0.029	53	0.389 ± 0.028			
13 weeks	47	0.402 ± 0.030	48	0.388 ± 0.029	−0.018	−0.092; 0.056	0.628
TNF-α (pg/mL)							
Baseline	53	0.527 ± 0.021	53	0.520 ± 0.020			
13 weeks	47	0.515 ± 0.021	48	0.511 ± 0.021	−0.002	−0.037; 0.033	0.908
MCP-1 (pg/mL)							
Baseline	53	2.195 ± 0.031	52	2.186 ± 0.031			
13 weeks	46	2.195 ± 0.034	47	2.139 ± 0.032	0.048	−0.061; 0.156	0.385
Anti-inflammatory cytokines							
IL-10 (pg/mL)							
Baseline	53	0.356 ± 0.029	53	0.323 ± 0.028			
13 weeks	47	0.300 ± 0.030	48	0.321 ± 0.029	−0.054	−0.117; −0.010	0.096
IL-1RA (pg/mL)							
Baseline	53	2.105 ± 0.042	54	2.120 ± 0.041			
13 weeks	47	2.046 ± 0.043	48	2.070 ± 0.041	−0.010	−0.080; 0.059	0.769
sTNFR1 (ng/mL)							
Baseline	53	0.373 ± 0.015	54	0.380 ± 0.014			
13 weeks	47	0.368 ± 0.015	48	0.386 ± 0.014	−0.011	−0.031; 0.009	0.287
Inflammatory cytokine ratios							
IL-6/IL-10 (−)							
Baseline	53	1.319 ± 0.098	53	1.318 ± 0.096			
13 weeks	47	1.480 ± 0.103	48	1.364 ± 0.099	0.114	−0.130; 0.359	0.355
IL-6/IL-1RA (−)							
Baseline	53	0.028 ± 0.003	52	0.023 ± 0.003			
13 weeks	47	0.030 ± 0.003	47	0.025 ± 0.003	0.000	−0.005; 0.005	0.910
TNF-α/IL-10 (−)							
Baseline	53	1.641 ± 0.101	53	1.672 ± 0.098			
13 weeks	47	1.823 ± 0.103	48	1.723 ± 0.099	0.130	−0.020; 0.280	0.088
TNF-α/IL-1RA (−)							
Baseline	53	0.033 ± 0.003	52	0.029 ± 0.003			
13 weeks	47	0.038 ± 0.003	47	0.030 ± 0.003	0.003	−0.002; 0.009	0.248
TNF-α/sTNFR1 (−)							
Baseline	52	1.406 ± 0.058	52	1.443 ± 0.055			
13 weeks	47	1.381 ± 0.060	47	1.405 ± 0.056	0.013	−0.103; 0.129	0.828
Adipokines							
Leptin (ng/mL)							
Baseline	53	1.367 ± 0.036	54	1.364 ± 0.035			
13 weeks	47	1.296 ± 0.037	47	1.323 ± 0.036	−0.030	−0.093; 0.033	0.349
Adiponectin (μg/mL)							
Baseline	53	1.056 ± 0.038	54	1.003 ± 0.037			
13 weeks	47	0.974 ± 0.039	48	0.926 ± 0.038	−0.005	−0.065; 0.055	0.869
Leptin/Adiponectin ratio (−)							
Baseline	52	2.791 ± 0.339	54	3.122 ± 0.327			
13 weeks	46	2.969 ± 0.349	47	3.406 ± 0.335	−0.105	−0.710; 0.500	0.730
Glycation biomarkers							
CML (ng/mL)							
Baseline	53	3.515 ± 0.037	54	3.560 ± 0.036			
13 weeks	43	3.510 ± 0.038	46	3.588 ± 0.036	−0.033	−0.085; 0.019	0.212
sRAGE (pg/mL)							
Baseline	53	1.758 ± 0.030	54	1.717 ± 0.029			
13 weeks	43	1.750 ± 0.031	46	1.735 ± 0.030	−0.026	−0.073; 0.021	0.280

EMM: estimated marginal mean, SE: standard error.

3.4. Association between inflammatory biomarkers and body composition and insulin resistance

Body composition indices, muscle quality indices, and parameters of regional fat mass significantly improved over time (Supplemental Table 4), but improvements were not statistically different between the protein and control groups (Supplemental Table 5).

Non-parametric correlation analysis showed weak correlations between change in IL-1RA and leptin and change in body composition (Supplemental Table 6a) and indices of insulin resistance (Supplemental

Table 3
Change in CRP, pro- and anti-inflammatory cytokines, adipokines and glycation markers after 13-week combined lifestyle intervention. Results from mixed model analysis excluding participants with CRP > 10 mg/L indicating acute inflammation. Data are 10-log values, except for the ratios which are based on untransformed data.

	Baseline		13 weeks		Change	95 % Confidence Interval	p-value
	n	EMM ± SE	n	EMM ± SE			
CRP (mg/L)	112	0.402 ± 0.020	94	0.392 ± 0.021	−0.010	−0.050; 0.030	0.619
Pro-inflammatory cytokines							
IL-6 (pg/mL)	106	0.405 ± 0.021	95	0.395 ± 0.022	−0.010	−0.047; 0.027	0.598
TNF-α (pg/mL)	106	0.524 ± 0.015	95	0.513 ± 0.015	−0.011	−0.028; 0.006	0.217
MCP-1 (pg/mL)	105	2.190 ± 0.023	93	2.166 ± 0.024	−0.024	−0.078; 0.030	0.384
Anti-inflammatory cytokines							
IL-10 (pg/mL)	106	0.340 ± 0.021	95	0.311 ± 0.022	−0.028	−0.060; 0.004	0.081
IL-1RA (pg/mL)	107	2.113 ± 0.030	95	2.059 ± 0.031	−0.054	−0.089; −0.020	0.002
sTNFR1 (ng/mL)	107	0.377 ± 0.011	95	0.377 ± 0.011	0.000	−0.010; 0.010	0.972
Inflammatory cytokine ratios							
IL-6/IL-10 (−)	106	1.317 ± 0.071	95	1.420 ± 0.074	0.103	−0.019; 0.224	0.098
IL-6/IL-1RA (−)	105	0.025 ± 0.002	94	0.028 ± 0.002	0.002	−0.001; 0.005	0.113
TNF-α/IL-10 (−)	106	1.655 ± 0.073	95	1.771 ± 0.074	0.116	0.040; 0.192	0.003
TNF-α/IL-1RA (−)	105	0.031 ± 0.002	94	0.034 ± 0.002	0.003	0.000; 0.006	0.035
TNF-α/sTNFR1 (−)	104	1.425 ± 0.041	94	1.394 ± 0.042	−0.031	−0.089; 0.026	0.284
Adipokines							
Leptin (ng/mL)	107	1.366 ± 0.026	94	1.310 ± 0.027	−0.056	−0.087; −0.024	<0.001
Adiponectin (μg/mL)	107	1.028 ± 0.028	95	0.949 ± 0.028	−0.079	−0.109; −0.050	<0.001
Leptin/Adiponectin ratio (−)	106	2.964 ± 0.243	93	3.197 ± 0.250	0.232	−0.069; 0.533	0.129
Glycation biomarkers							
CML (ng/mL)	107	3.538 ± 0.027	95	3.551 ± 0.027	0.012	−0.014; 0.038	0.347
sRAGE (pg/mL)	107	1.737 ± 0.022	95	1.742 ± 0.022	0.005	−0.019; 0.029	0.674

EMM: estimated marginal mean, SE: standard error.

Table 6b). Subsequent linear regression analysis showed several significant associations though explained variance was often low. Change in IL-1RA was positively associated with change in fat mass and HOMA-IR, and was negatively associated with change in DI and Matsuda index. Change in leptin was positively associated with change in fat mass, VAT, ALM/BMI, and HOMA-IR, and was negatively associated with change in DI and Matsuda index. Highest levels of explained variance were found for the association between change in leptin and change in fat mass ($\beta = 0.133$; $R^2 = 19.5$ %), and for the association between change in leptin and change in visceral fat in men ($\beta = 2.557$; $R^2 = 29.6$ %) (Table 4, Supplemental Table 7).

4. Discussion

In this post-hoc analysis of the PROBE study we aimed to determine whether a 13-week combined lifestyle intervention, with or without the addition of a protein drink enriched with leucine and vitamin D, affected inflammatory profile in older adults with obesity and type 2 diabetes. Additionally, we evaluated whether the inflammatory response depended on the baseline level of chronic low-grade inflammation. Our results showed that circulating IL-1RA and adipokines were reduced and ratios of pro- and anti-inflammatory markers TNF-α/IL-10 and TNF-α/IL-1RA were increased after the 13-week intervention. The protein drink did not have a significant impact on the inflammatory biomarker response. We found a differential effect of the combined lifestyle intervention on CRP depending on the baseline level of chronic low-grade inflammation.

4.1. Effect of the protein drink on inflammation

Our study did not reveal any differences between treatment groups, suggesting that addition of the protein drink did not influence inflammatory profile. In contrast, a reduction in pro-inflammatory adipokines chemerin and progranulin was found upon both a high-animal protein and a high-plant protein diet (30 % of dietary energy from protein) after 6 weeks of intervention in adults with type 2 diabetes, although no statistically significant changes in circulating IL-6 or TNF-α were observed (Markova et al., 2020). In the DiOGenes study, groups on (ad libitum) high-protein diets during weight maintenance showed lower reduction in CRP than those on low-protein diets (+0.25 mg/L; 95 % CI: −0.59 to −0.17) (Gögebakan et al., 2011). In our study, we had anticipated an attenuating effect of the protein drink on pro-inflammatory markers like IL-6, based on previous results in sarcopenic older adults using the same study product (Liberman et al., 2019). However, such effect was found in a setting without the combined lifestyle intervention with caloric restriction, resistance exercise and HIIT. Therefore, one could question whether the combination of intensive exercise and weight loss (i.e. caloric restriction) as performed in our study is optimal for reducing systemic inflammation in older adults with obesity and type 2 diabetes. Caloric restriction may have led to low glycogen levels before training and limited carbohydrate replenishment after training. Next to its role as exercise signalling molecule, the myokine IL-6 also acts as a short-term energy allocator (Kistner et al., 2022). Expression of IL-6 after exercise is increased when a low amount of carbohydrates is available (Starkie et al., 2001; Knuiman et al., 2018). This might have led to increased IL-6 signalling on top of the existing chronic low-grade inflammation in our study population. Normal cytokine signalling shows a delicate balance with multiple cytokines involved, including cytokines like IL-6 that can exhibit both pro- and anti-inflammatory effects depending on the physiological context (de Oliveira Dos Santos et al., 2021; Llanos and Palomero, 2022). The tendency towards a reduced anti-inflammatory profile (IL-10, TNF-α/IL-10) in the protein group, that consumed less carbohydrates than the control group, supports the suggestion of a possible influence of carbohydrate availability on cytokine signalling in our study.

Table 4
Associations between change in cytokines that changed significantly after 13 weeks and change in parameters of body composition and insulin resistance, evaluated by linear regression analysis. Corrected models are reported where applicable.

Independent variable[a] Regression model	Dependent variable[a]	R^2	β	95 % Confidence Interval	p-value
Δ IL-1RA (ng/mL)					
Crude model	Δ Fat mass (kg)	0.131	0.017	0.007; 0.027	<0.001
Crude model	Δ HOMA-IR	0.124	0.020	0.008; 0.032	0.001
Crude model	Δ DI	0.054	−0.400	−0.788; −0.012	0.043
Crude model	Δ Matsuda index	0.081	−0.005	−0.008; −0.001	0.013
Crude model	Δ LBM/Fat mass (−)	0.140	0.000	−0.001; 0.000	0.202
Crude model	Δ LBM/Fat mass % (kg/%)	0.129	0.000	−0.001; 0.000	0.238
Crude model	Δ ALM/BMI (m^{-2})	0.201	$-5.3*10^{-5}$	0.000; 0.000	0.068
Crude model	Δ Legpress strength /LLM (−)	0.237	−0.008	−0.016; 0.000	0.059
Crude model	Δ Knee extension power/LLM (Watt/kg)	0.237	0.009	−0.001; 0.018	0.064
Δ Leptin (ng/mL)					
Model with HIIT-load	Δ ALM (kg)	0.057	0.018	−0.007; 0.042	0.156
Model with HIIT-load	Δ LBM (kg)	0.121	0.039	−0.012; 0.090	0.132
Crude model	Δ Fat mass (kg)	0.195	0.133	0.074; 0.192	<0.001
Model with sex:	Δ VAT (cm^2)				
- men		0.296	2.557	1.436; 3.679	<0.001
- women		0.043	0.598	−0.497; 1.693	0.273
Model with RX-load	Δ LBM/Fat mass (−)	0.265	$-5.5*10^{-6}$	0.000; 0.000	0.068
Crude model	Δ ALM/BMI (m^{-2})	0.303	$-9.6*10^{-7}$	0.000; 0.000	0.006
Model with RX-load	Δ HOMA-IR	0.105	0.095	0.013; 0.177	0.024
Model with sex:	Δ DI				
- men		0.107	−4.864	−9.181; −0.547	0.028
- women		0.022	−1.169	−4.220; 1.881	0.438
Model with RX-load	Δ Matsuda index	0.109	−0.026	−0.050; −0.001	0.040

R^2: explained variance, β: regression coefficient, RX-load: relative resistance training load based on leg press performance, HIIT-load: relative high-intensity interval training load based on cycle ergometer performance, Δ: change in, ALM: appendicular lean mass, LBM: lean body mass, VAT: visceral adipose tissue, DI: disposition index, Adipo-IR: adipose tissue insulin resistance index, protein intake: in g/kg FFM per day.

[a] Only those pairs of independent and dependent variables are reported that had significant correlation as evaluated by Spearman's rank correlation coefficient (Supplemental Table 6a and 6b).

4.2. Change in inflammation as a result of the combined lifestyle intervention

Our findings on the total study population partially align with results of other combined lifestyle interventions in individuals with type 2 diabetes. A 12-month intensive lifestyle intervention that included moderate caloric restriction, resistance exercise (2–3 times per week) and aerobic exercise (5–6 times per week) reduced plasma IL-1RA by 30 % compared to standard care (Johansen et al., 2020). Baseline level of IL-1RA was higher compared to our study (294 vs. 130 pg/mL), which may explain the larger reduction. CRP, TNF-α, IL-6 and IL-10 also decreased in that study, but the changes did not differ from standard care. Similarly, in postmenopausal women with type 2 diabetes, plasma CRP and leptin decreased after a 14-week weight loss program including moderate caloric restriction and aerobic exercise (60 min walking 3–4 times per week), while TNF-α, IL-6 and adiponectin did not change (Giannopoulou et al., 2005). Based on CRP level at baseline, at least 35 % of our study population can be classified as having an increased inflammatory profile (Pearson et al., 2003), and levels (with range 1.8 [i.e. LOD/$\sqrt{2}$] to 8.0) were comparable to the study of Johansen et al. (2020) described above. Baseline level of pro-inflammatory cytokine IL-6 (2.5 pg/mL on average, based on back-transformation) was much higher than in the study of Johansen (0.54 pg/mL), and was comparable to the study with postmenopausal women with type 2 diabetes.

The observed reduction in anti-inflammatory cytokine IL-1RA seems disadvantageous. However, chronic low-grade inflammation is characterised by elevated pro-inflammatory and anti-inflammatory cytokine levels (Krabbe et al., 2004; Erusalimsky, 2021), meaning that a reduction in chronic inflammation can be reflected in both anti- and pro-inflammatory cytokines. As such, the decrease in IL-1RA may reflect a reduced inflammatory load from cytokines more upstream in the inflammatory cascade (Johansen et al., 2020). However, we did not find a decrease in pro-inflammatory cytokines IL-6 and TNF-α. Moreover, ratios of pro- and anti-inflammatory markers TNF-α/IL-10 and TNF-α/IL-1RA were increased, indicating an increased pro-inflammatory status as a result of the combined lifestyle intervention. Interestingly, a strong correlation was observed between leptin and IL-1RA in women with rheumatoid arthritis and age matched controls (Ljung et al., 2007), as well as a causal relationship between leptin exposure and production of IL-1RA in vivo (Gabay et al., 2001). This suggests that the change in leptin may explain the observed decrease in IL-1RA. In our study, however, correlation between change in leptin and change in IL-1RA was not significant (rho = 0.143, $p = 0.181$; Spearman's correlation). The decrease in leptin itself supports the notion of a decreased inflammatory profile, as leptin has pro-inflammatory action (Lontchi-Yimagou et al., 2013; López-Jaramillo et al., 2014). Adiponectin, which has anti-inflammatory action (López-Jaramillo et al., 2014; Zamboni et al., 2021), also decreased in our study. The ratio of leptin to adiponectin did not change in response to the combined lifestyle intervention and neither did glycation biomarkers CML and sRAGE. Overall, the combined lifestyle intervention in our study resulted in differential effects on multiple aspects of the inflammatory profile. The reduction in leptin was not accompanied by a decrease in chronic low-grade inflammation as reflected by CRP, nor by a decrease in pro-inflammatory biomarkers. Moreover, the pro- vs. anti-inflammatory cytokine balance seemed to increase towards a more pro-inflammatory state. Differential effects on adipokine and myokine response were previously found in a study of Bruun et al. (2006) in adults with severe obesity, where a 15-week lifestyle intervention with hypocaloric diet and exercise particularly reduced markers of inflammation in adipose tissue, whereas skeletal muscle did not contribute to the attenuation of whole body inflammation. This may also be reflected in our study where we focus on circulatory inflammation biomarkers that is a reflection of whole body inflammatory response, including effects from adipose tissue as well as from muscle tissue.

The reduction in leptin in our study can be explained by the effect of caloric restriction. In an extensive literature review, it was concluded that weight loss induced by caloric restriction is a key factor for reducing the level of pro-inflammatory markers in overweight or obesity, independent of diet composition (Bianchi, 2018). Caloric restriction inhibits the secretion of pro-inflammatory cytokines from the adipose tissue (such as leptin, IL-6, TNF-α, and MCP-1), while the secretion of anti-

inflammatory adiponectin is increased. Adiponectin, in turn, reduces the secretion of CRP by the liver (Bianchi, 2018). Christiansen et al. (2010) concluded that rather large weight losses (> 5–7 %) in individuals with obesity had beneficial effects on circulating inflammatory markers, while 12 weeks of aerobic exercise had not. In our 13-week study, weight loss was approximately 3 % on average (Memelink et al., 2020). The authors also suggested that "more intensive exercise may be necessary to affect systemic inflammation" (Christiansen et al., 2010). Such anti-inflammatory effects of intensive exercise, independent of weight loss, have been shown in individuals with type 2 diabetes. A 12-month combined aerobic and resistance exercise program had the highest reductions in CRP and TNF-α and highest increase in IL-10 compared to aerobic exercise or counselling for low-intensity physical activity (Balducci et al., 2010). The proposed mechanism for the anti-inflammatory effects of exercise is that exercise leads to marked increases in IL-6 and IL-10 and exerts its anti-inflammatory effects by inhibition of TNF-α and by stimulating IL-1RA (Pedersen, 2017).

Effects of exercise on reduction of chronic low-grade inflammation have also been shown for older adults in a recent systematic review (Bautmans et al., 2021). However, reductions of circulating levels of CRP, IL-6, and TNF-α seemed more prominent in healthy older adults compared to older adults with a specific disease or condition. Two studies in older individuals with type 2 diabetes found no changes in inflammatory markers after 12 weeks of resistance exercise (Hsieh et al., 2018) or 12 months of combined training (Zaidi et al., 2019), suggesting that older adults with type 2 diabetes have resistance against the anti-inflammatory effects of exercise. A possible explanation for a more persistent pro-inflammatory status in older adults with type 2 diabetes can be the glucotoxicity and lipotoxicity, that trigger pathological cascades that ultimately result in persistent immune-system stimulation, accumulation of senescent cells, epigenetic re-arrangements, and alterations in microbiota composition (Prattichizzo et al., 2018). Another explanation for inconsistent effects of exercise on inflammation in older patient groups might be participants' adherence to exercise volume and modalities (i.e. frequency, intensity, time and type of exercise) (Bautmans et al., 2021). In the PROBE study, adherence to the exercise program was high (85 %) (Memelink et al., 2020) and neither frequency, nor intensity of exercise was identified as confounder. We did find reductions in inflammatory markers over time (IL-1RA, leptin, adiponectin), which may be caused by the exercise program, caloric restriction, or the combination of both. A previous meta-analysis on the effect of exercise on inflammatory profile in adults with type 2 diabetes showed reductions in CRP, IL-6 and leptin, whereas adiponectin was not altered (Hayashino et al., 2014). Two of the 14 studies included in this meta-analysis involved dietary cointervention. Another, more recent meta-analysis on the effects of exercise modality on inflammation in middle-aged and older adults with type 2 diabetes concluded that long-term aerobic training, resistance training, combined training (aerobic plus resistance), and HIIT all contributed to the reduction of inflammatory status (CRP, TNF-α, IL-6, and IL-10) (Yang et al., 2023). Six of the 18 studies included in this meta-analysis involved dietary cointervention.

4.3. Role for level of chronic low-grade inflammatory profile

CRP decreased in participants with high CLIP, indicating a net positive effect of the combined lifestyle intervention on inflammatory status. Starting from the high cardiovascular risk category, participants' CRP values decreased towards the average risk category (Pearson et al., 2003), indicating a clinically significant reduction of chronic low-grade inflammation in this group. CRP increased in participants in the low to average CLIP subgroup, indicating that the intervention had a net negative effect in this subgroup. A possible reason for such increase could be an increased IL-6 signalling upon training sessions as discussed above. Another reason could be the use of metformin, which may disrupt normal cytokine signalling upon resistance exercise in older adults (Walton et al., 2019). Metformin use seemed unevenly distributed between the two subgroups ($p = 0.050$, 2-sided χ^2), with relatively more users in the low to average CLIP subgroup. Moreover, the subgroups appeared to be different on other aspects as well; biomarkers IL-6, IL-1RA, leptin and sRAGE were higher in the high CLIP subgroup, and insulin sensitivity (Matsuda index) was lower. This points towards a physiological difference between the subgroups, which indicates that the differential results for CRP are not merely a statistical phenomenon of regression to the mean, but are at least in part a physiological phenomenon. In addition, a potential role for the statistical phenomenon of regression to the mean was minimized, by excluding extreme CRP values (> 10 mg/dL) from the analysis and including baseline CRP in the analysis model.

4.4. Association between inflammatory biomarkers and body composition and insulin resistance

Participants who lost more total or visceral fat mass had larger decrease in circulating leptin and HOMA-IR, and had larger increase in DI and Matsuda index. These associations are as expected and the direction of causality has been well described in literature (Johansen et al., 2020; Pi-Sunyer, 2014). Caloric restriction leads to a reduction in fat mass and visceral fat through increased fat oxidation. As a result of that, adipocyte size decreases leading to improved adipocyte function with reduction in the secretion of leptin, IL-6, TNF-α, and MCP-1 among other pro-inflammatory biomarkers (Bianchi, 2018). In addition, exercise reduces visceral fat through IL-6 signalling upon exercise (Wedell-Neergaard et al., 2019). Remarkably, we found an association between leptin and visceral fat for men but not for women. It should however be noted that a relatively high number of women had leptin values above the upper detection limit which may have biased the observed sex-dependence in the association because of affected leptin change values. Participants who lost more total fat mass had larger decrease in anti-inflammatory cytokine IL-1RA. As explained earlier, the decrease in IL-1RA may relate to the decrease in leptin. Our associations found between biomarkers leptin and IL-1RA and indices of insulin resistance were of weak strength and had direction as expected from the mechanism described above. Especially a reduced secretion of TNF-α, through a reduction in fat mass, leads to the improved insulin sensitivity (Petersen and Pedersen, 2005), though we did not find a reduction in circulatory TNF-α.

We did not observe associations between change in inflammatory biomarkers and change in lean mass in our study. Therefore, inflammation does not seem to be part of the pathway leading to the observed preservation of lean mass in our study population (Memelink et al., 2020). The results of our responder analysis confirm this suggestion.

4.5. Strengths and limitations

To our knowledge, this is the first study examining the effect of protein supplementation during a combined lifestyle intervention on inflammatory profile in older individuals with obesity and type 2 diabetes. The broad panel of blood based inflammatory biomarkers enabled a robust evaluation of change in systemic inflammation. Another strength is the elimination of potential influence of acute inflammation by excluding participants with CRP > 10 mg/L from the analysis. The absence of pro-inflammatory cytokine IL-1β in our biomarker panel can be seen as a limitation. Inclusion of this cytokine would have enabled an even better interpretation of pro-inflammatory status by calculating the ratio of IL-1β to its receptor antagonist, IL-1RA. The explorative nature of our analysis could have led to chance findings caused by the multiple testing phenomenon. Therefore our findings must be interpreted with care; hypothesis generation rather than hard evidence. The results on the whole group level cannot be compared with a group that did not receive the hypocaloric diet and the exercise programme. Therefore we cannot conclude on causality between the combined lifestyle

intervention and the change in inflammatory biomarkers observed. Unfortunately, CRP was not analysed as high-sensitivity CRP which forced us to use data modification for values below LOD. This can have affected the observed CRP response, especially in the subgroup with low to average CLIP. The relatively long storage duration of samples potentially has led to cytokine degradation, leading to a general reflection of lower levels compared to the actual values at the time of sampling. However, for the interpretation of our study findings it is crucial to consider the evaluation of pre and post measurements, conducted three months apart, which could be assumed to have undergone similar degradation. Literature suggests that values remain stable when analysed with a three-month interval (Butterfield et al., 2011). Also the use of non-transformed data in our linear regression analysis may be seen as a limitation. However, residuals were normally distributed or did not clearly deviate from normal distribution.

4.6. Clinical implications

We have focused on one of the specific pathways known to be altered in type 2 diabetes, which is inflammation (Forbes and Cooper, 2013). Our intensive combined lifestyle intervention led to changes in inflammatory biomarkers that were associated with reduction in fat mass and insulin resistance. However, inflammation does not seem to play a role in the explanation of the observed improvement in lean mass upon addition of the protein drink in older adults with obesity and type 2 diabetes (Memelink et al., 2020). Furthermore, the observed pro- or anti-inflammatory response depended on the level of chronic low-grade inflammation and showed a clinically significant reduction of chronic low-grade inflammation in the high CLIP subgroup.

4.7. Conclusion

In conclusion, 13 weeks of hypocaloric diet in combination with resistance exercise and high-intensity interval training, either with or without protein drink, led to differential effects on inflammatory profile in older adults with obesity and type 2 diabetes. Circulating leptin, adiponectin and anti-inflammatory cytokine IL-1RA were reduced and inflammatory ratios TNF-α/IL-10 and TNF-α/IL-1RA were increased. Effect on chronic low-grade inflammation was inversely related to baseline inflammatory status.

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CRediT authorship contribution statement

Robert G. Memelink: Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Rose Njemini:** Writing – review & editing, Validation, Project administration, Methodology, Investigation, Data curation. **Minse J.J. de Bos Kuil:** Writing – review & editing, Investigation. **Suzan Wopereis:** Writing – review & editing. **Johan de Vogel-van den Bosch:** Writing – review & editing, Resources, Funding acquisition, Conceptualization. **Josje D. Schoufour:** Writing – review & editing. **Michael Tieland:** Writing – review & editing. **Peter J.M. Weijs:** Writing – review & editing, Resources, Funding acquisition, Conceptualization. **Ivan Bautmans:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Methodology, Funding acquisition, Conceptualization.

Declaration of competing interest

JdV-vdB is an employee of Danone Nutricia Research. SW is an employee of the Netherlands Organisation for Applied Scientific Research (TNO), which is a not-for-profit research organisation collaborating in several public-private partnerships or business-to-business research projects that receive funding from companies. All other authors declare no conflicts of interest related to this research.

Data availability

The dataset for this paper is available from the corresponding author on reasonable request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.exger.2024.112410.

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