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ORIGINAL CONTRIBUTION

Multi-study feasibility analysis on a composite biomarker of inflammatory resilience to quantify the effects of energy restriction on low-grade inflammation in overweight and obese individuals

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Abstract

Purpose Assessing the health impacts of nutritional interventions in metabolically compromised but otherwise healthy individuals is challenging, necessitating sensitive tools. Phenotypic flexibility offers an innovative way to measure homeostatic capacity during challenge tests. A composite biomarker of inflammatory resilience has proven useful in evaluating the health benefits of whole-grain wheat interventions in overweight and obese individuals. Expanding this method to other dietary interventions to combat low-grade inflammation is essential.

Methods This study investigated the feasibility of a composite biomarker of inflammatory resilience through secondary analysis of samples from two independent energy restriction (ER) trials, Bellyfat (NCT02194504) and Nutritech (NCT01684917). In these trials, fasting and postprandial inflammation was analysed using a variety of markers. Four composite biomarker models were developed on the basis of postprandial inflammatory marker responses via the 'health space' model method. These models were statistically evaluated for their sensitivity in detecting the effects of 12 weeks of ER.

Results The minimal composite biomarkers, consisting of IL-6, IL-8, IL-10, and TNF-α, lacked the ability to detect postprandial intervention effects in both ER trials. However, in the Nutritech study, the extended, endothelial, and optimized composite biomarkers of inflammatory resilience displayed significant responses to the ER (all $P < 0.005$). In the latter 3 models, a reduction in the inflammatory score was correlated with a reduction in BMI and body fat percentage.

Conclusion This study underscores the feasibility of employing a composite biomarker of inflammatory resilience to evaluate ER interventions. Further validation in additional nutritional intervention studies is necessary. Once validated, this composite biomarker could offer a novel approach for assessing low-grade inflammation and phenotypic flexibility.

Keywords Inflammation · Resilience · Low-grade inflammation · Obesity · Biomarker

Introduction

Determining the health effects of nutritional intervention strategies in healthy but metabolically compromised persons is challenging, and researchers have recently put effort into the generation of sensitive tools [1]. Traditionally, health effects are measured by using a single biomarker or a few biomarkers in participants after overnight fasting and before and after the intervention [2]. Recently, the definition of 'health' has been redefined as "the ability to adapt or cope with every changing environmental condition" instead of the absence of disease [3]. Biomarkers that capture the capacity to cope with or adapt to nutritional or dietary interventions would therefore be a better strategy to reflect metabolic health [4]. This approach requires alternative strategies to

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measure health, ideally with clusters of composite biomarkers and integrated biological processes without a focus on one biomarker or pathway.

Over the last decade, the concept of resilience has been developed as a novel approach to quantifying the ability to adapt homeostasis to an external stressor such as a standardized meal, temperature change or physical exercise [2, 5–7]. In the metabolism field, phenotypic flexibility refers to the body's ability to adapt its physiological processes in response to metabolic challenges such as food intake [5]. One of these challenges is the PhenFlex Challenge Test (PFT), which was developed as a standardized high-caloric liquid meal test containing lipids, carbohydrates, and proteins, to quantify phenotypic flexibility in health and metabolic diseases [1, 5, 8]. At multiple timepoints after ingestion of the PFT, blood samples are taken to determine various biological parameters. As a reference in this test, similar parameters are measured in response to the PFT in two additional groups: young, lean individuals, representing healthy people, and older, obese individuals, representing those with compromised health [1]. This approach allows for the calculation and visualization of standardized composite biomarkers in a so-called 'health space', reflecting the coping behaviour of specific biological processes in response to a standardized perturbation, such as those relating to liver health, vascular health, metabolism and inflammation within the extremes of the healthy population [1, 9]. In an earlier study, we demonstrated that the health space approach is suitable for evaluating interpretable intervention effects using these composite markers, which were not observed when these markers were analysed after overnight fasting. If the intervention effect causes the composite biomarker to shift in the direction of young, lean individuals, this result indicates a beneficial effect of the intervention, whereas if the intervention effect causes the composite biomarker to shift in the direction of older obese individuals, this finding indicates a detrimental effect of the intervention. This approach supports the idea that health is defined by an individual's ability to respond under metabolic pressure (i.e., phenotypic flexibility) rather than under homeostatic conditions (i.e., overnight fasting) [10].

Low-grade inflammation is recognized as a key pathological feature in most metabolic diseases. Previously, an overview of the utility of inflammatory resilience biomarkers for evaluating the efficacy of nutritional interventions was presented [11]. However, no standardized procedure to quantify an inflammatory resilience biomarker has been proposed. Therefore, we aimed to evaluate the standardized PFT to quantify low-grade inflammation across multiple energy restriction intervention studies, as weight loss is well known to reduce inflammation in obese and overweight individuals [12]. This study aims to develop and compare several configurations of an inflammatory resilience biomarker, which vary in the number and type of inflammatory marker responses to the PFT, in two energy restriction studies.

Materials and methods

Study design

In this multi-study feasibility research, secondary analysis was performed on samples from the Bellyfat and Nutritech studies [13, 14].

The Bellyfat study was a 12-week, randomized, parallel-designed study comparing two energy restriction (ER) interventions and a habitual diet control arm, as described previously [13]. The study was approved by the Medical Ethics Committee of Wageningen University and registered at clinicaltrials.gov as NCT02194504 on the 16th of July, 2014. All the participants provided informed consent prior to their inclusion in the study. It was performed in accordance with the ethical standards defined in the 1964 Declaration of Helsinki and its later amendments. In brief, participants aged 40–70 years with abdominal obesity (BMI > 27 kg/m^2 or a waist circumference > 88 cm for women or > 102 cm for men) were stratified according to BMI, age, and sex. The interventions consisted of a 25% ER diet with a low- or high-nutrient quality diet (further referred to as LQ-ER and HQ-ER, respectively). Compared with the LQ-ER diet, the HQ-ER diet was enriched with MUFAs, n-3 PUFAs, fibre, and plant-based protein and the level of fructose was lower than that of the LQ-ER diet. The participants in the control group were instructed to maintain their habitual diet. Both ER diets resulted in significant average weight loss and BMI reduction: 6.3 kg and 2.1 kg/m^2 in the LQ-ER group and 8.4 kg and 2.8 kg/m^2 in the HQ-ER group. The control group, however, gained 0.8 kg on average, with a BMI increase of 0.3 kg/m^2. The original study was powered to detect changes in intrahepatic lipid accumulation, and the sample sizes were as follows: the control group (n = 30) and the LQ-ER and HQ-ER groups (n = 40 each). Among these 110 individuals, 100 completed the study, with 39 in the LQ-ER diet group, 34 in the HQ-ER diet group, and 27 in the control group [13]. For this multi-study feasibility analysis, the sample sizes were as follows: control group (n = 27), LQ-ER group (n = 39), and HQ-ER group (n = 34).

In the NutriTech study, the subjects were randomized to parallel arms consisting of 12 weeks of ER rather than healthy weight maintenance as a control [14]. The study was approved by the Medical Ethics Committee of West London Ethics Committee and registered at clinicaltrials.gov as NCT01684917 on the 11th of September, 2012. All the participants provided informed consent prior to their inclusion in the study, which was performed in accordance with the ethical standards of the 1964 Declaration of Helsinki and

its later amendments. In brief, participants aged 50–65 years old with a BMI of 25–35 kg/m^2 were stratified by BMI, age and sex. The intervention arm followed a supervised ER diet that reduced caloric intake by 20%, whereas the control group was advised to consume an average healthy European diet. The ER diet resulted in significant average weight loss of 5.6 kg and a BMI reduction of 1.9 kg/m^2, whereas in the control group, participants gained 0.1 kg on average, with a BMI increase of 0.1 kg/m^2. The original study was powered to detect changes in insulin sensitivity. Among the 68 participants who completed the study, 31 were in the control group, whereas 37 were in the ER group [14]. For this multi-study feasibility analysis, the sample sizes were as follows: control group (n = 29) and ER (n = 36). In the Nutritech study, 2 distinct metabotypes were determined based on the hierarchical clustering of the phenotypic data, which revealed two distinct clusters with specific metabolic profiles and biomarker concentrations [15]. Compared with those classified as metabotype A, individuals classified as metabotype B presented slower glucose clearance, greater intra-abdominal fat mass, and elevated liver lipid levels [15].

PhenFlex challenge test and inflammatory marker measurements

Resilience was quantified in both studies by applying the standardized PFT before and after 12 weeks of intervention in overweight and obese participants who fasted for at least 12 h [1, 5, 8, 16]. In brief, PFT is a high-calorie drink that contains 75 g of glucose, 60 g of fat and 18 g of protein concentrate and is ingested within 5 min. No food or beverages were allowed during this period except for water. Plasma samples were taken before and after consumption of the PFT (t = 0, 30, 60, 120, and 240 min). Both inflammatory and anti-inflammatory biomarkers were selected to capture a more complete profile of the inflammatory status. The plasma levels of interleukin (IL)−6, IL-8, IL-10, IL-12p70, IL-13, interferon (IFN)-γ and tumour necrosis factor (TNF)-α were measured using multiplex immunoassays (Multiplex Panel Human; Meso Scale Discovery). For the Nutritech and PhenFlex reference studies, the levels of myeloperoxidase (MPO), leptin, adiponectin, C-reactive protein (CRP), serum amyloid A (SAA), E-selectin, P-selectin, soluble intercellular adhesion molecule (sICAM)−1, soluble vascular adhesion molecule (sVCAM)−1 and plasminogen activator inhibitor (PAI)−1 were also determined. The following multiplexed immunoassays (provider, product number, units) were employed, and they were previously optimized for small plasma volumes using commercially available reference blood donor plasma samples (TCS Bioscience Ltd., Buckingham, UK) [10]: MPO (R&D Systems, DY3174), adiponectin (R&D Systems, DY1065), leptin (R&D Systems, DY398), E-selectin (R&D Systems, DY724), P-selectin (R&D Systems, DY137), sICAM-1 (R&D Systems, DY720), sVCAM-1 (R&D Systems, DY805), SAA (R&D Systems, DY3019), CRP (R&D Systems, DY1707), and total PAI-1 (R&D Systems, DY9387). For each parameter, the linear range and optimal dilution factor were optimized prior to measurement via commercially available reference blood donor plasma samples (TCS Bioscience Ltd., Buckingham, UK).

Data integration into a health space to construct composite biomarkers of inflammatory resilience

We constructed a health space model to develop composite biomarkers of inflammatory resilience on the basis of a previously described methodology [1, 9, 10]. First, we analysed two reference groups: a healthy reference group, which consisted of 20 young individuals aged 20 to 29 years with low to normal body fat percentages (< 20% for men; < 30% for women), representing individuals with optimal health, and a compromised health group, which comprised 20 older individuals aged 60 to 70 years with higher body fat percentages (> 20% for men; > 30% for women), representing people with compromised health.

Next, to construct the health space model, we built a ridge regression model using the *glmnet* package [17] and designed it to classify individuals into two reference groups according to the mean-centred and scaled inflammatory marker responses to the PFT. In brief, this regression was used to analyse the data and create models to identify the most important biomarkers and the time of PFT analysis. This approach gives them 'weights' based on how well they distinguish between the references for 'healthy' and 'compromised'. The 'weight' for each biomarker at each time point represents the importance and direction of influence: positive coefficients highlight risk indicators, and negative coefficients represent beneficial markers. The model calculates a score by multiplying each biomarker value by its corresponding 'weight' in the regression equation and summing up the results for each participant. In the final health score calculation, positive and negative values balance each other, resulting in a net score that reflects overall health status. Scores closer to the health reference group indicate greater resilience (low inflammation score), whereas scores closer to the compromised reference group suggest greater resilience (higher inflammation score).

Four different composite inflammatory resilience biomarkers were developed: a minimal biomarker based on IL-6, IL-8, IL-10 and TNF-α; an extended biomarker based on adiponectin, leptin, CRP, SAA, E-selectin, P-selectin, IFN-γ, IL-6, IL-8, IL-10, IL-12p70, IL-13, TNF-α, MPO, PAI-1, sVCAM-1, and sICAM-1; an endothelial activation composite biomarker based on E-selectin, P-selectin, sICAM-1, sVCAM-1, and PAI-1; and a Nutritech-specified

composite biomarker based on CRP, E-selectin, sICAM-1, PAI-1, and SAA. Health space models contain both pro- and anti-inflammatory biomarkers, with the idea that the balance between pro- and anti-inflammatory components is important for health. By comparing the intervention outcomes between the two reference groups, the model determines either a positive or negative weight in the regression equation. Prior to being input into each model, the data were centred and scaled. Each model generated a regression equation, which we subsequently used to calculate scores for each study subject across the different datasets. Model validation was performed using tenfold cross-validation, during which we optimized the model parameters and assessed their quality using the mean squared error metric.

Statistical analysis

All the statistical analyses and visualizations were performed using R version 4.1.2. All the figures were constructed with the *ggplot2* package. We used the trapezoidal rule to calculate the area under the curve (AUC) for the inflammatory marker data collected during the PFTs. This method involves calculating the areas above and below the baseline fasting value for each feature. These areas are considered separately as positive and negative values. The total AUC is then derived by adding these two areas together. If any data point was missing from a PFT response measurement, we excluded all the data from that response. Statistical analyses with the individual inflammatory markers, the AUCs, and the four composite inflammatory resilience biomarkers as variables were performed using the *lme4* package [18] and *lmerTest* package [19] with the *emmeans* package [20] for post hoc analysis. All the statistical models incorporated the random term 'subject' to account for subject-specific variability as well as fixed effects for 'group' and 'occasion'. Separate models were created for each variable and study. Model residuals were checked for normality, and non-normally distributed data were log-transformed. For all the inflammatory mediators, medians with interquartile ranges (IQRs) are displayed since the data are not normally distributed, and the median is considered a more robust representation of central tendency. The estimated marginal means were back-transformed to their original scale in the case of models with transformed variables. Data points were excluded from the model when the absolute standardized residuals exceeded a threshold of 3.

Results

A minimal composite biomarker of inflammatory resilience is not sensitive to the effects of ER interventions

Figure 1 shows the minimal composite biomarker response of inflammatory resilience at baseline (week 0) and follow-up (week 12) in each intervention group in both studies compared with the metabolically healthy and the metabolically compromised reference groups described earlier [1, 10]. We found no significant difference in the interaction effect in the Bellyfat or the Nutritech studies with the minimal panel (Table 1, $p = 0.356$ and $p = 0.906$, respectively). Moreover, no significant differences between or within intervention groups were observed in either study, indicating insufficient

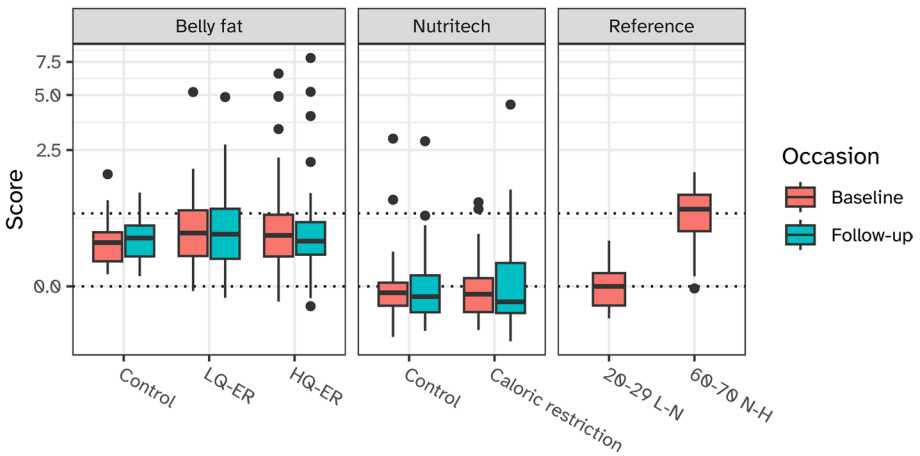

Fig. 1 Minimal composite biomarkers of inflammatory resilience (TNF-α, IL-6, IL-8, and IL-10). Baseline (week 0 in red) and follow-up (week 12 in blue) data of each intervention group in the Bellyfat and Nutritech studies. The groups were compared to the metabolically healthy (aged 20–29 years, lean (L) to normal (N) body fat composition) and the metabolically compromised (aged 60–70 years, normal (N) to high (H) body fat composition) reference groups. *LQ-ER* low-quality energy-restricted diet; *HQ-ER* high-quality energy-restricted diet

Table 1 p values for the interaction effect and post hoc analysis of health space scores in the Nutritech study before (week 0) and after (week 12) the intervention

	Interaction	Between groups ER vs. control		Within Group Week 12 vs. Week 0	
	Treatment x week	Week 0	Week 12	control	ER
Bellyfat study					
Minimal panel	0.356	LQ vs. C: 0.257 HQ vs. C: 0.105 HQ vs. LQ: 0.557	0.317 0.476 0.732	0.331	LQ: 0.744 HQ: 0.292
Nutritech study					
Minimal panel	0.906	0.524	0.567	0.786	0.895
Extended panel	**0.004**	0.872	0.081	0.486	**4.02E− 04**
Metabotype A	0.090	0.102	**0.008**	0.314	0.153
Metabotype B	**0.015**	0.185	0.790	0.921	**3.35E− 04**
Endothelial activation panel	**0.002**	0.726	**0.030**	0.421	**1.73E− 04**
Metabotype A	0.112	0.386	**0.042**	0.658	0.060
Metabotype B	**0.032**	0.731	0.190	0.857	**5.72E− 04**
Nutritech panel	**0.004**	0.983	0.063	0.943	**3.57E− 05**
Metabotype A	**0.022**	0.180	**0.003**	0.261	**0.029**
Metabotype B	**0.028**	0.364	0.416	0.359	**2.79E− 05**

The minimal panel included 4 markers (TNF-α, IL-6, IL-8, and IL-10); the extended panel included 17 markers (adiponectin, leptin, CRP, SAA, E-selectin, P-selectin, IFN-γ, IL-6, IL-8, IL-10, IL-12p70, IL-13, TNF-α, MPO, PAI-1, sVCAM-1 and sICAM-1); the endothelial activation panel included 5 markers (E-selectin, P-selectin, sICAM-1, sVCAM-1 and PAI-1); and the Nutritech panel included 5 markers (E-selectin, sICAM-1, PAI-1, CRP and SAA). Compared with metabotype B (compromised), metabotype A (less compromised) is characterized by faster glucose clearance, lower intra-abdominal fat, and lower liver lipid levels [14]. Bold data show significant interaction effects and post hoc analysis between or within groups

C control diet, *ER* energy-restricted diet, *LQ* low-quality energy-restricted diet, *HQ* high-quality energy-restricted diet

sensitivity to the two ER interventions. These observations were supported by a lack of significant interaction effects in the Bellyfat and Nutritech study overnight fasting levels and the AUCt of TNF-α, IL-6, IL-8, and IL-10 (for Nutritech: Tables 2 and 3; for Bellyfat: Supplemental Table 1).

An extended composite biomarker of inflammatory resilience is sensitive to the effects of ER intervention

Owing to the lack of discriminative effect supporting the effect of weight loss in the health space with the aforementioned minimal composite biomarker, we wanted to determine if another, more extensive panel of inflammatory markers could further improve our health space model sensitivity to quantify effects from ER intervention studies on 'inflammatory resilience'. Therefore, we subsequently measured multiple additional inflammatory markers, namely adiponectin, leptin, CRP, SAA, E-selectin, P-selectin, IFN-γ, IL-6, IL-8, IL-10, IL-12p70, IL-13, TNF-α, MPO, PAI-1, sVCAM-1 and sICAM-1, in the Nutritech and reference groups, which are known to contribute to chronic low-grade inflammation [11, 21]. With this extended panel, a significant interaction effect was observed ($p = 0.004$, Fig. 2 and Table 1), which was explained following post hoc analysis by a significant shift towards the metabolically healthy reference group after 12 weeks within the ER group ($p = 4.02E- 04$), which was not observed in the control group ($p = 0.486$). In the Nutritech study, earlier published multivariate analysis identified 2 distinct metabotypes, which were defined on the basis of plasma markers for fatty acid catabolism separating compromised (type A) and less compromised (type B) metabotypes [15]. Our data revealed a significant interaction effect for metabotype B ($p = 0.032$, Fig. 2 and Table 1) but not for metabotype A. This effect was explained by a significant improvement after 12 weeks within the ER group ($p = 3.35E- 04$).

An endothelial activation composite biomarker of inflammatory resilience is sensitive to the effects of ER intervention

To understand whether the improvement in the extended composite biomarker was explained by vascular inflammation/activation, we developed a separate composite biomarker for endothelial activation based on E-selectin, P-selectin, sICAM-1, sVCAM-1 and PAI-1. A significant interaction effect was observed ($p = 0.002$,

Table 2 Median overnight (O/N) fasting plasma concentrations of inflammatory mediators at baseline (week 0) and after intervention (week 12) in the NutriTech study

	Baseline Week 0		Follow up Week 12		Interaction (O/N) p value
	Control	ER	Control	ER	Treatment x week
TNF-α (pg/ml)	1.07 (0.355)	1.05 (0.55)	0.992 (0.543)	1.01 (0.389)	0.563
IL-6 (pg/ml)	0.825 (0.437)	0.754 (0.381)	0.723 (0.286)	0.702 (0.291)	0.912
IL-8 (pg/ml)	1.84 (0.447)	1.82 (0.67)	**1.98 (0.483)[1]**	1.95 (0.895)	0.399
IL-10 (pg/ml)	0.225 (0.17)	0.268 (0.176)	0.244 (0.133)	0.251 (0.197)	0.796
IL-12p70 (pg/ml)	0.188 (0.105)	0.249 (0.385)	0.171 (0.1)	0.238 (0.311)	0.203
IL-13 (pg/ml)	1.15 (1.44)	1.4 (2.56)	**0.98 (1.52)[1]**	1.3 (1.65)	**0.009**
IFN-γ (pg/ml)	4.27 (2.48)	4.03 (1.52)	4.21 (5.13)	4.42 (2.31)	0.778
Adiponectin (ug/ml)	2.52 (1.84)	2.52 (1.28)	2.23 (1.75)	2.45 (1.4)	0.622
CRP (µg/ml)	1.12 (0.973)	1.04 (0.94)	1.07 (1.06)	**0.602 (0.89)[3]**	**0.004**
E-Selectin (ng/ml)	7.28 (3.31)	7.25 (3.68)	7.76 (3.83)	**5.93 (3.77)[3]**	**7.75E− 09**
sICAM-1 (ng/ml)	134 (51.9)	122 (30.4)	128 (39.8)	**119 (33.9)[2,a]**	0.324
Leptin (ng/ml)	17.7 (16.2)	16.8 (14.2)	17 (13.5)	**10.9 (9.27)[3]**	**1.39E− 11**
MPO (ng/ml)	24.3 (7.47)	25.8 (9.08)	24.8 (8.63)	25.1 (10.1)	0.128
PAI-1 (ng/ml)	12.9 (11)	13.2 (12.3)	13.4 (14.6)	**10.6 (9.49)[3]**	**0.001**
P-Selectin (ng/ml)	22 (10.8)	24.5 (10.1)	18.4 (10.9)	**21 (7.68)[3]**	0.201
SAA (ug/ml)	2.29 (1.5)	2.33 (1.38)	2.14 (1.64)	**1.47 (1.45)[3,a]**	**0.001**
sVCAM-1 (ng/ml)	417 (88.5)	395 (91.3)	415 (96.6)	**414 (131)[1]**	0.247

Data are presented as the median (IQR). Bold data indicate significance

ER energy-restricted diet

Within groups: (1) $P < 0.05$, (2) $P < 0.005$, (3) $P < 0.0005$ vs baseline. Differences between groups at follow-up: (a) $P < 0.05$ vs control

Table 3 p values for total area under the curve (AUCt) values of the interaction effect and post hoc analysis within and between groups of postprandial inflammatory markers in the extended panel of the Nutritech study before (week 0) and after (week 12) intervention

	Interaction (AUCt) p value	Between groups ER vs. control		Within group Week 12 vs. Week 0	
	Treatment x Week	Week 0	Week 12	Control	ER
TNF-α	0.991	0.767	0.776	**1.44E− 04**	**1.69E− 05**
IL-6	0.863	0.807	0.944	0.057	0.052
IL-8	0.834	0.743	0.867	**2.38E− 07**	**1.56E− 08**
IL-10	0.695	0.255	0.366	**7.15E− 04**	**9.37E− 04**
IL-12p70	0.686	0.158	0.222	**0.011**	**0.018**
IL-13	0.168	0.795	0.428	0.572	**0.007**
IFN-γ	0.771	0.418	0.625	**0.016**	**0.001**
Adiponectin	0.642	0.997	0.899	0.338	0.668
CRP	**0.042**	0.899	0.113	0.875	**0.001**
E-selectin	**4.01E− 05**	0.649	0.154	0.424	**1.59E− 07**
sICAM-1	0.071	0.147	**0.013**	0.560	**0.033**
Leptin	**1.01E− 06**	0.539	**0.036**	0.742	**4.74E− 11**
MPO	0.85	0.613	0.743	0.750	0.492
PAI-1	0.085	0.843	0.248	0.583	**0.001**
P-Selectin	0.944	0.430	0.506	0.284	0.183
SAA	**0.004**	0.739	**0.014**	0.728	**6.68E− 06**
sVCAM-1	0.669	0.875	0.948	0.057	**0.004**

Bold data show significance for the interaction effect and post hoc analysis

ER energy−restricted diet

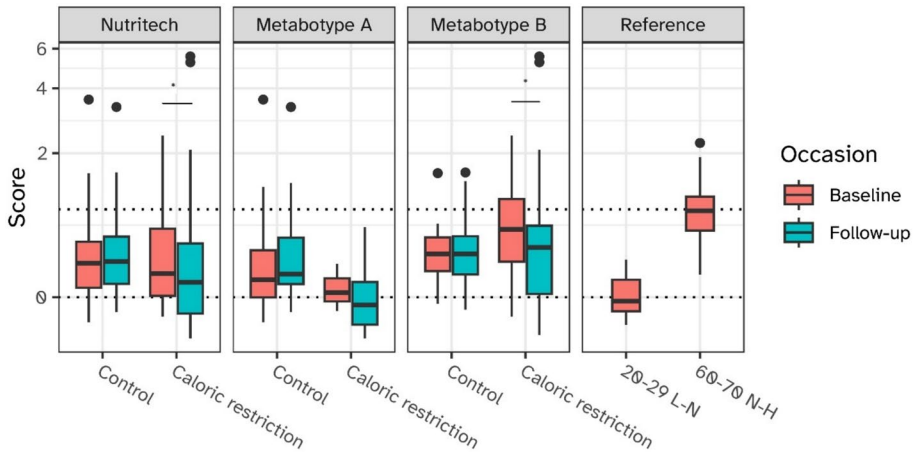

Fig. 2 Extended composite biomarkers of inflammatory resilience (adiponectin, leptin, CRP, SAA, E-selectin, P-selectin, IFN-γ, IL-6, IL-8, IL-10, IL-12p70, IL-13, TNF-α, MPO, PAI-1, sVCAM-1 and sICAM-1). Baseline (week 0 in red) and follow-up (week 12 in blue) data of each intervention group in the Nutritech study, both for the total population and for metabotype A and metabotype B. Compared with metabotype B, metabotype A (less compromised) is characterized by faster glucose clearance, lower intra-abdominal fat, and lower liver lipid levels (compromised) [15]. The groups are compared to the metabolically healthy (aged 20–29 years, lean (L) to normal (N) body fat composition) and the metabolically compromised (aged 60–70 years, normal (N) to high (H) body fat composition) reference groups. *$p < 0.0005$ between occasions

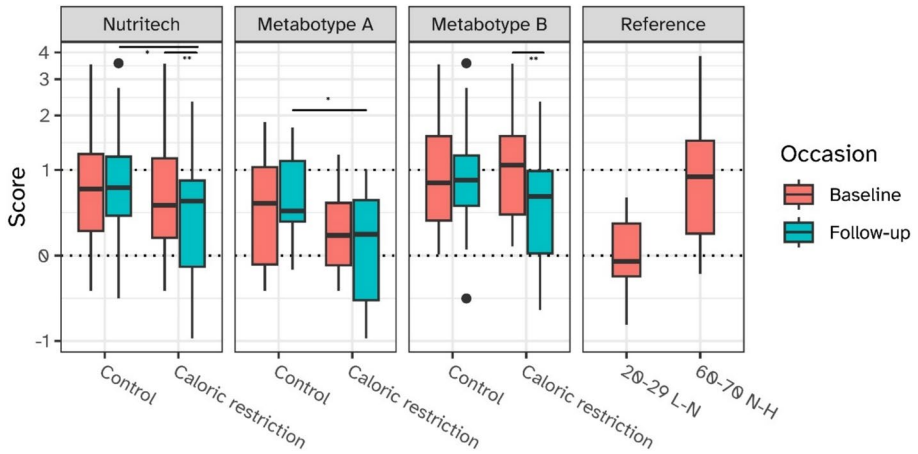

Fig. 3 Endothelial activation composite biomarkers of inflammatory resilience (E-selectin, P-selectin, sICAM-1, sVCAM-1 and PAI-1). Baseline (week 0 in red) and follow-up (week 12 in blue) data of each intervention group in the Nutritech study, both for the total population and for metabotype A and metabotype B. Compared with metabotype B, metabotype A (less compromised) is characterized by faster glucose clearance, lower intra-abdominal fat, and lower liver lipid levels (compromised) [15]. The groups are compared to the metabolically healthy (aged 20–29 years, lean (L) to normal (N) body fat composition) and the metabolically compromised (aged 60–70 years, normal (N) to high (H) body fat composition) reference groups. *$p < 0.05$ between interventions. **$p < 0.0010$ between occasions

Fig. 3 and Table 1), which was explained by a significant shift towards the metabolically healthy reference after 12 weeks of ER ($p = 1.73E-04$) and a significant difference between the ER and control groups at week 12 ($p = 0.030$). Metabotype B had a significant interaction effect B ($p = 0.032$, Fig. 3, and Table 1), which was explained by a significant decrease after 12 weeks in the ER group ($p = 5.72E-04$).

A specified composite biomarker of inflammatory resilience is most sensitive to the effects of ER intervention

A specified composite biomarker of inflammatory resilience was developed based on a selected panel of markers with (near) significant interaction effects on the fasting and/or postprandial response in the Nutritech study ($p < 0.1$, Tables 2 and 3), with a focus on systemic and

vascular inflammation (E-selectin, sICAM-1, PAI-1, CRP and SAA, further referred to as the Nutritech panel). A significant interaction effect was observed ($p < 0.004$, Fig. 4 and Table 1), which was explained by a significant decrease after 12 weeks of ER ($p = 3.57E-05$). In addition, a significant interaction effect was observed for both metabotype A ($p = 0.023$, Fig. 4 and Table 1) and metabotype B ($p = 0.028$, Fig. 4 and Table 1). This finding was explained by post hoc analysis as a decrease in the composite biomarker with 12 weeks of ER intervention for metabotype A ($p = 0.029$) and metabotype B ER ($p = 2.79E-05$) individuals. Additionally, a significantly lower composite biomarker level in the ER intervention group than in the control group was observed for metabotype A at week 12 ($p = 0.003$).

Inflammatory resilience score is correlated with weight loss

In our final analysis, we assessed whether the reduction in the inflammation score was correlated with changes in body fat percentage as determined by bioelectric impedance or weight loss, which was calculated as the BMI [14]. We found a significant correlation between a reduction in BMI or body fat percentage and a decrease in the inflammation score in the extended, endothelial activation and Nutritech panels (Table 4).

Table 4 p values and correlation coefficients between differences in inflammation scores and either BMI or body fat percentage before (week 0) and after (week 12) the intervention

	Correlation Coefficient	p value
Minimal panel		
BMI	− 0.087	0.515
Body fat %	− 0.073	0.586
Extended panel		
BMI	0.47	**1.61E− 04**
Body fat %	0.28	**0.025**
Endothelial activation panel		
BMI	0.58	**1.30E− 06**
Body fat %	0.43	**6.13E− 04**
Nutritech panel		
BMI	0.53	**1.41E− 05**
Body fat %	0.42	**8.00E− 04**

The minimal panel contained 4 markers (TNF-α, IL-6, IL-8, and IL-10); the extended panel contained 17 markers (adiponectin, leptin, CRP, SAA, E-selectin, P-selectin, IFN-γ, IL-6, IL-8, IL-10, IL-12p70, IL-13, TNF-α, MPO, PAI-1, sVCAM-1 and sICAM-1); the endothelial activation panel contained 5 markers (E-selectin, P-selectin, sICAM-1, sVCAM-1 and PAI-1); and the Nutritech panel contained 5 markers (E-selectin, sICAM-1, PAI-1, CRP and SAA). Bold data indicate significance

BMI body mass index

Discussion

In this multi-study analysis, we aimed to evaluate composite biomarkers of inflammatory resilience in two independent

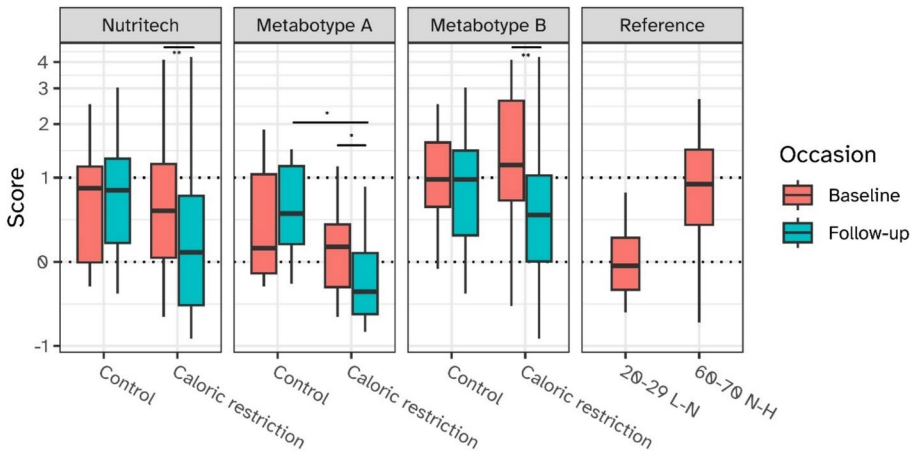

Fig. 4 Specific Nutritech composite biomarkers of inflammatory resilience (E-selectin, sICAM-1, PAI-1, CRP and SAA). Baseline (week 0 in red) and follow-up (week 12 in blue) data of each intervention group in the Nutritech study, both for the total population and for metabotype A and metabotype B. Compared with metabotype B, metabotype A (less compromised) is characterized by faster glucose clearance, lower intra-abdominal fat, and lower liver lipid levels (compromised) (15). The groups are compared to the metabolically healthy (aged 20–29 years, lean (L) to normal (N) body fat composition) and the metabolically compromised (aged 60–70 years, normal (N) to high (H) body fat composition) reference groups. *$p < 0.05$ between or within groups. **$p < 0.0005$ between occasions

randomized controlled ER intervention studies. We found that the original minimal composite biomarker, which is based on four cytokines [10], was not sensitive enough to detect an intervention effect of energy-restricted diets in the Bellyfat and Nutritech studies. In contrast, extended, endothelial activation and specific composite biomarkers were able to show a significant improvement of 'inflammatory resilience' following 12 weeks of ER in the Nutritech study.

Nutritional and dietary intervention strategies have shown promise in mitigating low-grade inflammation associated with obesity, suggesting that specific changes in diet and nutrition can alleviate inflammatory responses in individuals with excess weight [22–24]. According to a recent systematic review meta-analysis, ER diets are known to reduce the levels of inflammatory markers such as the acute phase protein CRP and, to some extent, the cytokine IL-6 [25]. Notably, a reduction in CRP levels was especially observed when energy restriction was maintained for more than 12 weeks [25]. However, the overall results of an ER diet on circulating levels of TNFα, IL-8, and IL-12 in fasted subjects are inconsistent, possibly due to heterogeneity between studies and the limited number of studies [11, 25]. Similarly, in our multi-study evaluation, we found no cytokine markers that, individually, displayed significant and mutual interaction effects in both studies.

Obesity is associated with the shedding of VCAM-1, ICAM-1, and E-selectin from the endothelium, leading to increased levels of these mediators [26–29]. The shedding of these markers is known to be associated with various inducers, such as adipose tissue-related inflammation, metalloproteinases, circulating cytokines and reactive oxygen species [11]. Similar to the observations of the Nutritech study, earlier studies have shown that adherence to an ER diet by (diabetic) obese patients led to reduced levels of the acute phase proteins CRP and SAA, the endothelium activation marker E-selectin and the procoagulant factor PAI-1 [11]. These findings suggest that an ER diet, as in the case of the Nutritech study, improves vascular health by inhibiting endothelial and coagulation activity, which is associated with systemic and local inflammation.

The measurement of inflammatory resilience following a mixed-meal or high-fat challenge has been a topic of debate due to inconsistent results [11, 30]. Mounting evidence shows that there are no or only subtle postprandial responses for the majority of circulating cytokines [10, 30–34]. IL-6 is a single inflammatory marker that is consistently elevated following a mixed- or high-fat meal [30]. However, this result is likely attributed to the cannulation procedure rather than to the body's metabolic response to food [35, 36]. In both the Bellyfat and the Nutritech studies, the subjects received a catheter for blood sample collection during the mixed-meal challenge [13, 15]. Current and previous analyses have demonstrated that measuring circulatory cytokines postprandially alone is likely insufficient to reflect inflammatory resilience in overweight or obese individuals. In contrast, the extended, endothelial activation, and Nutritech specified composite biomarkers showed responsiveness of inflammatory resilience to ER intervention. The extended composite biomarker, which is based on 17 inflammatory markers linked to adipose tissue inflammation, endothelial dysfunction, and the activation of inflammatory pathways in obesity, can be used for future studies to explore inflammatory resilience as a basis to define a more pragmatic, simpler version. The findings of the present study led to the development of a specific composite biomarker of inflammatory resilience to evaluate ER intervention in the Nutritech study. The specified composite biomarker levels in the Nutritech study fall within the range of our metabolically healthy and metabolically compromised reference groups (as described in the methods section), indicating content validity. Using this composite biomarker of inflammatory resilience in relation to the reference groups helps us to understand the beneficial health impact of the intervention.

For example, the Graandioos study did not find an effect of whole-grain wheat versus refined wheat on the postprandial responses of E-selectin, P-selectin, sICAM-1, and sVCAM-1, which are markers of endothelial activation [10]. However, a significant effect was observed on a composite biomarker based on postprandial responses of TNF-α, IL-6, IL-8, and IL-10. These findings suggest that the mechanism by which whole-grain wheat improves inflammatory resilience differs from that of the ER. To capitalize on the use of inflammatory resilience biomarkers to support next-generation health claims [37], nutritional studies need to show the statistical significance, clinical relevance, and biological plausibility of interventions in improving inflammatory resilience. Inflammatory resilience is driven by multiple biological systems and processes, and the abovementioned examples show that different types of dietary intervention may require different composite biomarkers of inflammatory resilience. This work exemplifies this idea by proposing a composite biomarker based on acute phase proteins and endothelial activation markers for the evaluation of the ER, as opposed to the earlier proposed biomarker with circulatory cytokines for the evaluation of whole grain wheat.

The strengths of this study include the investigation of multiple inflammatory markers from 2 different studies, both in terms of overnight fasting levels and postprandial response. In addition, we investigated multiple composite biomarker panels to explore and optimize the health space model for 'inflammatory resilience' following ER. This approach makes it possible to evaluate the intervention effect on phenotypic flexibility objectively. A limitation of this study was the absence of comprehensive measurements of the extended inflammatory panel in the Bellyfat

study, which precluded validation of the findings from the Nutritech study.

In conclusion, this study demonstrated the feasibility of a composite biomarker of inflammatory resilience for the evaluation of ER interventions. Pending further validation in additional ER restriction studies, it is envisioned that this composite biomarker constitutes a next-generation biomarker for the evaluation of subtle ER interventions for low-grade inflammation.

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Data availability We can (temporarily) grant access to someone via a Data Transfer Agreement (DTA) or a similar agreement. All information is available upon request in our Phenotype database.

Declarations

Conflict of interest Gary Frost has received research funding from Nestle, Quorn and Sosei Heptares but not in relation to this work; he is also a director of the metabolomic profiling company Melico Sciences. Lydia Afman, Milena Rundle: no disclosures. TNO auteurs: This research was funded by a public−private partnership entitled "PhenFlex-based resilience as a measure for health effects of diet" financed by Topsector Agri & Food (TKI-AF-16035) and cofunded by the PPP allowance made available by Health ~ Holland, Top Sector Life Sciences & Health, to stimulate public–private partnerships. This project was sponsored by TNO roadmap Biomedical Health and cofunded by Pfizer, Inc., BASF SE, By-Health, Roquette, Biofortis Merieux NutriSciences and CIRO. Neither the sponsors nor the co-funders had a role in the analysis, interpretation of the data or writing of the publication.

Ethical approval All the participants provided informed consent prior to their inclusion in the Bellyfat and Nutritech studies [13, 14]. Studies were approved by the appropriate ethics committee and were performed in accordance with the ethical standards of the 1964 Declaration of Helsinki and its later amendments.

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