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Cardiometabolic health improvements upon dietary intervention are driven by tissue-specific insulin resistance phenotype: A precision nutrition trial

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Clinical and Translational Report

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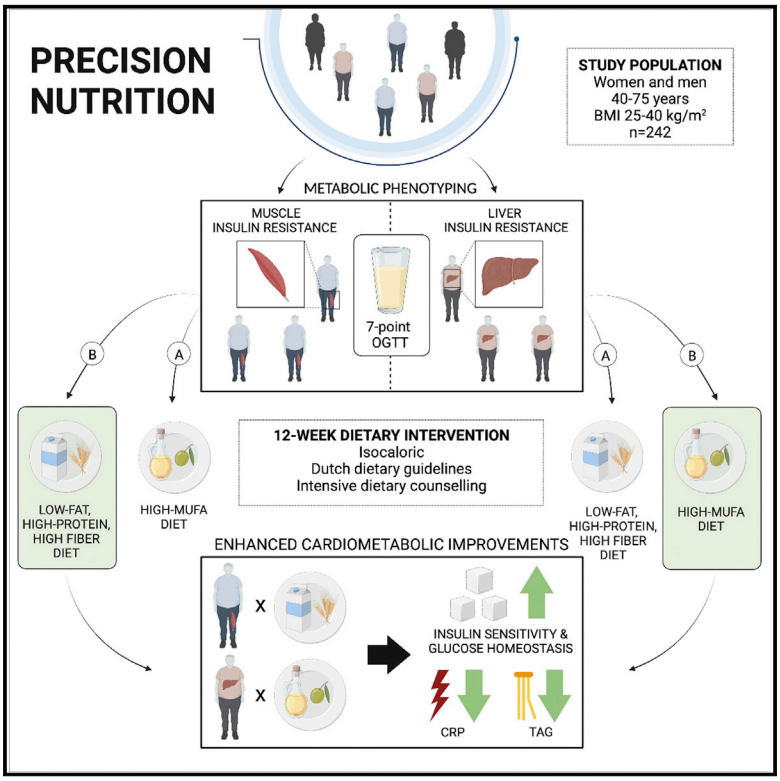
Graphical abstract



Highlights

- 242 adults with tissue-specific IR participated in a 12-week precision nutrition trial
- Health improvements upon the dietary intervention were driven by IR phenotype
- These cardiometabolic health improvements were independent of weight loss
- Precision nutrition based on IR phenotype enhances diet-induced health improvements

Authors

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In brief

Trouwborst, Gijbels, and Jardon et al. included 242 adults with tissue-specific insulin resistance in a 12-week precision nutrition trial. Here, they demonstrate that modulation of macronutrient composition within the dietary guidelines based on tissue-specific insulin resistance phenotype enhances cardiometabolic health improvements.

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Clinical and Translational Report

Cardiometabolic health improvements upon dietary intervention are driven by tissue-specific insulin resistance phenotype: A precision nutrition trial

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SUMMARY

Precision nutrition based on metabolic phenotype may increase the effectiveness of interventions. In this proof-of-concept study, we investigated the effect of modulating dietary macronutrient composition according to muscle insulin-resistant (MIR) or liver insulin-resistant (LIR) phenotypes on cardiometabolic health. Women and men with MIR or LIR (n = 242, body mass index [BMI] 25–40 kg/m^2, 40–75 years) were randomized to phenotype diet (PhenoDiet) group A or B and followed a 12-week high-monounsaturated fatty acid (HMUFA) diet or low-fat, high-protein, and high-fiber diet (LFHP) (PhenoDiet group A, MIR/HMUFA and LIR/LFHP; PhenoDiet group B, MIR/LFHP and LIR/HMUFA). PhenoDiet group B showed no significant improvements in the primary outcome disposition index, but greater improvements in insulin sensitivity, glucose homeostasis, serum triacylglycerol, and C-reactive protein compared with PhenoDiet group A were observed. We demonstrate that modulating macronutrient composition within the dietary guidelines based on tissue-specific insulin resistance (IR) phenotype enhances cardiometabolic health improvements. Clinicaltrials.gov registration: NCT03708419, CCMO registration NL63768.068.17.

INTRODUCTION

The unprecedented prevalence of obesity and related cardiometabolic disturbances calls for effective prevention strategies. A well-known strategy to improve cardiometabolic health is healthy nutrition, even in the absence of weight loss.[1,2] Nevertheless, a considerable proportion of individuals does not show clinically relevant improvements upon a dietary intervention.[3–5] These differential responses to diet may be explained by inter-individual heterogeneity in both exogenous and endogenous factors such as sex, dietary habits, gut microbiota composition, and metabolic phenotype.[6,7] Precision nutrition based on individual traits may increase the effectiveness of dietary interventions to improve metabolic health.[8]

There are indications that parameters related to glucose metabolism and insulin action or resistance, such as plasma glucose and insulin concentrations and indices based on these concentrations, may predict the response to dietary modification.[5,9,10] Importantly, insulin resistance (IR) can develop separately in insulin-sensitive tissues such as skeletal muscle and the liver, representing different etiologies toward cardiometabolic diseases. We have recently shown that individuals with more pronounced liver IR (LIR) have a distinct metabolome,[11] lipidome,[12] adipose tissue transcriptome,[13] and systemic inflammatory

profile[13] compared with individuals with more pronounced muscle IR (MIR). Therefore, individuals with these distinct tissue-specific IR phenotypes may respond differentially to dietary intervention.

Indeed, in a post hoc analysis of the CORDIOPREV-DIAB study, individuals with predominant MIR responded more favorably to a diet high in monounsaturated fatty acids (MUFAs), while individuals with predominant LIR responded more favorably to a low-fat, high-complex carbohydrate diet with regard to the disposition index, a composite marker of whole-body insulin sensitivity and insulin secretion.[14] In addition, both high-protein[15–17] and high-fiber diets,[18] as well as the Mediterranean diet,[19,20] have been shown to reduce liver fat content, which in turn may improve hepatic insulin sensitivity.[21,22] Furthermore, dietary fat quality may specifically impact skeletal muscle lipid metabolism and peripheral insulin sensitivity.[23] Importantly, however, well-designed, prospective, randomized, isocaloric dietary intervention trials to test the effectiveness of precision nutrition based on tissue-specific IR phenotype are currently lacking.

In this personalized glucose optimization through nutritional intervention (PERSON) study,[24] we investigated the efficacy of modulation of dietary macronutrient composition according to MIR and LIR phenotypes on parameters of glucose homeostasis, cardiometabolic health, health-related quality of life, and perceived well-being. We hypothesized that individuals with the MIR phenotype would benefit most from a diet rich in MUFA, and individuals with the LIR phenotype from a diet low in fat and rich in protein and fiber. Interestingly, these findings demonstrate that individuals with the MIR phenotype showed a more pronounced cardiometabolic health improvement upon a low-fat, high-protein, and high-fiber (LFHP) diet, while individuals with the LIR phenotype had the greatest cardiometabolic health benefit from a high-MUFA (HMUFA) diet. Although not in concert with the initial hypothesis, these findings for the first time provide the proof-of-concept that modulating dietary macronutrient composition based on tissue-specific IR phenotype with healthy, isocaloric diets can induce more pronounced, clinically relevant improvements in cardiometabolic health, independent of changes in body weight.

RESULTS

Study design and participant characteristics

Between May 2018 and November 2021, 990 men and women aged 40–75 years and with a body mass index (BMI) 25–40 kg/m^2 were enrolled, of whom 877 were fully screened for eligibility (Figure S1, CONSORT diagram). At screening, tissue-specific IR was assessed using the muscle insulin sensitivity index (MISI) and hepatic IR index (HIRI), which were calculated from the plasma glucose and insulin responses during a 7-point oral glucose tolerance test (OGTT).[25,26] Tertile cutoffs for MISI and HIRI from a previous study (the Maastricht study[11,27]) were used to identify individuals with predominant MIR or LIR.

In total, 242 participants (123 at Maastricht University Medical Center+ [MUMC+] and 119 at Wageningen University [WUR]) were included and randomized to phenotype diet (PhenoDiet) group A or B (n = 121 in both groups). PhenoDiet group A included individuals with MIR following an HMUFA diet and individuals with LIR following an LFHP diet. PhenoDiet group B included individuals with MIR following an LFHP diet and individuals with LIR following an HMUFA diet. The targeted macronutrient composition of both diets is described in Table S1. The dietary intervention strategy was based on weekly dietary counseling and provision of key products. Both diets were in line with the Dutch dietary guidelines, and we aimed for both diets to be eucaloric to keep participants on a stable body weight throughout the study. At baseline (week 0) and after 12 weeks of dietary intervention, participants underwent extensive metabolic phenotyping in a characterization week (Figure 1).

Overall, 58% of the randomized participants were women, mean age was 60 years, and mean BMI 29.9 kg/m^2. Baseline characteristics were well balanced in the two groups (Table 1). The majority of the participants (76%) was considered normal glucose tolerant at baseline according to fasting and 2-h glucose levels in response to an OGTT. Baseline characteristics with stratification for IR phenotype and diet intervention are described in Table S2. BMI was slightly higher in individuals with the LIR compared with MIR phenotype (p MIR versus LIR = 0.037) and the use of anti-inflammatory medication was higher in MIR compared with LIR (p MIR versus LIR = 0.041).

In PhenoDiet group A, 94% (n = 114 of 121) and in PhenoDiet group B 88% (n = 107 of 121) completed the study (Figure S1). Twenty-two participants (13 in PhenoDiet group A, 9 in PhenoDiet group B) completed the study according to an adjusted protocol employed during the COVID-19 lockdown (only limited post-intervention measurements; STAR Methods). No major difference between the characteristics of completers and dropouts was observed at baseline (Table S3).

Habitual dietary intake at baseline was comparable between PhenoDiet group A and B

Self-reported habitual dietary intake before start of the intervention was assessed with a food frequency questionnaire (FFQ). After exclusion of data due to energy under- (n = 27) and overreporting (n = 1), FFQ data from 213 participants were included in these analyses. Habitual dietary intake was comparable between the groups, except for energy intake, which was higher in PhenoDiet group A (median [IQR]; 9.6 [7.8, 10.9] MJ) compared with PhenoDiet group B (8.6 [7.4, 10.6] MJ) (Table S4). Average intakes of calories from fat, protein, and carbohydrates were 37.7%, 15.6%, and 41.5%, respectively.

Adherence to the MHUFA and LFHP diets was high, with no differences between PhenoDiet group A and B

Compliance to the dietary interventions was evaluated with three 1-day food records that were randomly requested for throughout weeks 2–11 of the intervention via a mobile app,[28] as well as with pre- and post-measurement of plasma fatty acid profile. After exclusion of data from 20 participants (MIR – HMUFA, n = 10; LIR – LFHP, n = 2; LIR – HMUFA, n = 4; MIR – LHFP, n = 4) due to energy underreporting, food record data from 206 participants were included in these analyses. Advised macronutrient composition of the two intervention diets and reported intake can be found in Table S5. Macronutrient composition of the two different intervention diets was comparable in PhenoDiet group A and B. Individuals randomized to the HMUFA diet reported higher intake of calories from total fat and MUFA, lower intake of protein and fiber, and similar intake of saturated fatty

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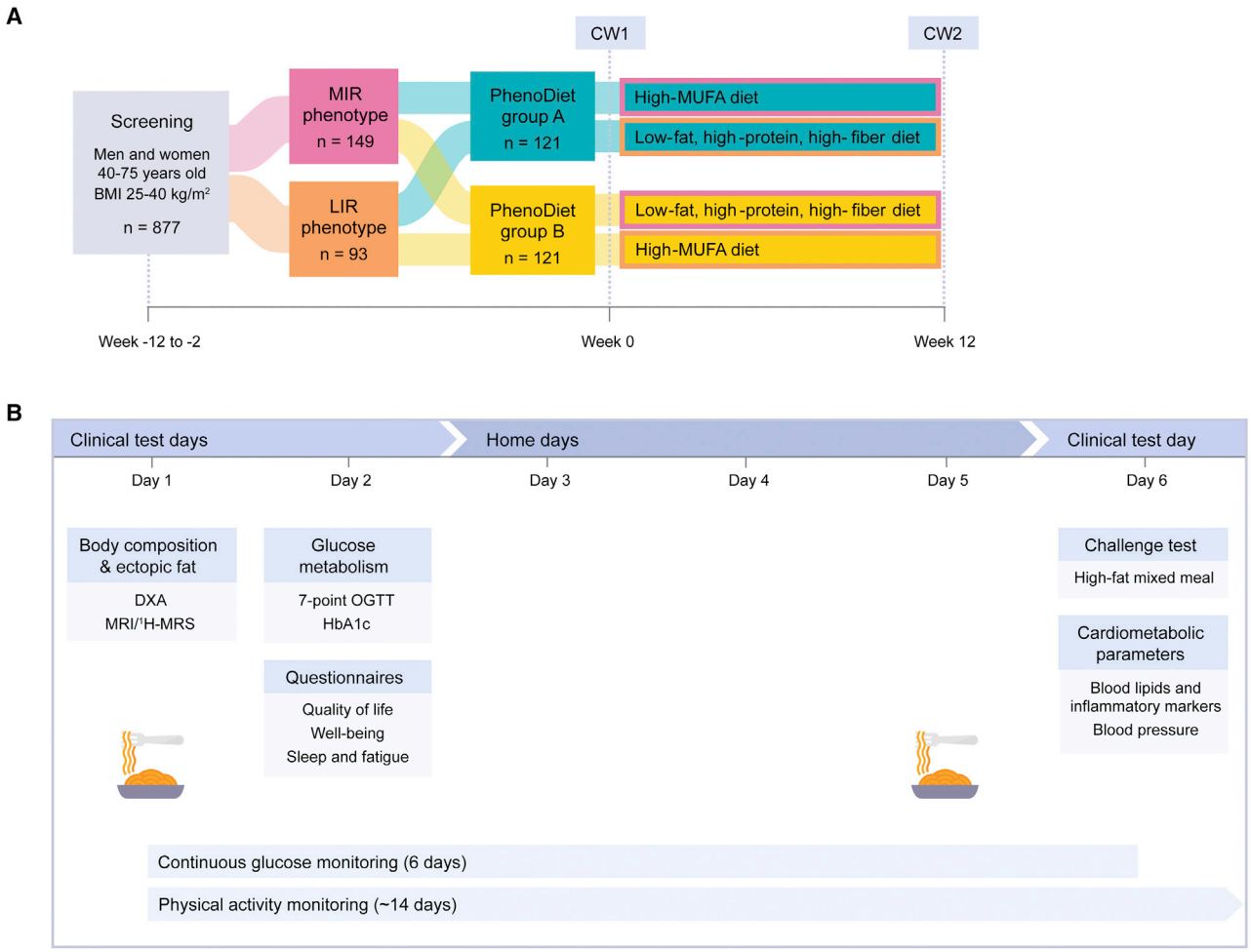

Figure 1. Study design of the PERSON study

(A) Tissue-specific insulin resistance was assessed at screening using a 7-point oral glucose tolerance test. Individuals with predominant muscle insulin resistance (MIR) or liver insulin resistance (LIR) were randomized to phenotype diet (PhenoDiet) group A or B. PhenoDiet group A consisted of individuals with MIR following a high-monounsaturated fatty acid (HMUFA) diet and individuals with LIR following a low-fat, high-protein, and high-fiber (LFHP) diet. PhenoDiet group B consisted of individuals with LIR following an HMUFA diet and MIR following an LFHP diet.

(B) In clinical investigation week (CIW) 1 and 2, in weeks 0 and 12, respectively, participants underwent several clinical and at-home measurements.

acid (SFA) and carbohydrates compared with those on the LFHP diet. The contribution of MUFA to total plasma fatty acid concentrations increased in individuals on the HMUFA diet, while it decreased in those on the LFHP diet (Table S6). Plasma SFA concentrations were reduced after both diets.

The change in the primary outcome disposition index was not significantly different between intervention groups

Glucose homeostasis and insulin sensitivity were assessed with a 7-point venous OGTT (75 g of glucose) before and at the end of the intervention. The primary outcome was the disposition index, which is a composite measure of insulin sensitivity and insulin secretion. The disposition index was 412 (369–460) (estimated marginal mean with adjustment for age, sex, and center [95% CI]) before intervention and 406 (365–451) after intervention in PhenoDiet group A, and 357 (321–398) before intervention and 380 (343–423) after intervention in PhenoDiet group B. Differences between groups did not reach statistical significance ($p = 0.109$, group × time) (Table 2; Figure 2A). Also, there was no change over time in either of the intervention groups ($p = 0.640$, time).

Greater improvements in insulin sensitivity and glucose homeostasis in PhenoDiet group B

Fasting insulin, 2-h glucose, 2-h insulin, and HOMA-IR decreased, and MISI increased significantly in PhenoDiet group B, but not in PhenoDiet group A (all $p < 0.05$, group × time) (Figures 2B–2E). The Matsuda index, which reflects whole-body insulin sensitivity, also increased significantly in PhenoDiet group B (from 4.2 [3.9–4.6] to 5.1 [4.6–5.5]) compared with PhenoDiet group A (from 4.8 [4.4–5.3] to 5.1 [4.6–5.6]) ($p = 0.004$, group × time) (Figure 2F). HIRI decreased significantly in both groups ($p = 0.021$, time), with no difference between the groups ($p = 0.25$, group × time). HbA1c tended to decrease slightly in PhenoDiet group B compared with PhenoDiet group A ($p = 0.091$, group × time)

Table 1. Baseline characteristics of participants allocated to PhenoDiet group A or B

	PhenoDiet group A (n = 121)	PhenoDiet group B (n = 121)
MIR/LIR phenotype, n	76/45	73/48
Age, years	60 ± 8	60 ± 8
Women, n (%)	66 (54.5%)	75 (62.0%)
BMI, kg/m^2	30.1 ± 3.5	29.8 ± 3.5
Medication use, n (%)		
Antidepressants	5 (4.1%)	12 (9.9%)
Antihypertensives	27 (22.3%)	16 (13.2%)
Anti-inflammatory medication	14 (11.6%)	9 (7.4%)
Statins	9 (7.4%)	7 (5.8%)
Other	42 (34.7%)	37 (30.6%)
Family history of diabetes, n (%)	22 (18.2%)	32 (26.4%)
Glucose status (%) (n = 240)		
NGT	94 (79.0%)	88 (72.7%)
IFG	5 (4.2%)	4 (3.3%)
IGT	12 (10.1%)	16 (13.2%)
Combined IFG/IGT	3 (2.5%)	4 (3.3%)
T2DM	5 (4.1%)	9 (7.4%)
Habitual physical activity, Baecke score	8.4 ± 1.2	8.3 ± 1.2
Employment status (%) (n = 236)		
Paid job	69 (59.5%)	55 (45.8%)
Retired	34 (29.3%)	43 (35.8%)
Other	13 (11.2%)	22 (18.3%)
Education level (%) (n = 235)		
Low	17 (14.7%)	18 (15.1%)
Intermediate	44 (37.9%)	48 (40.3%)
High	55 (47.4%)	53 (44.5%)

Values are n (%) or mean ± SD. MIR, muscle insulin resistance; LIR, liver insulin resistance; BMI, body mass index; NGT, normal glucose tolerance; IFG, impaired fasting glucose; IGT, impaired glucose tolerance; T2DM, type 2 diabetes mellitus.

(Table 2). Additional statistical adjustment for weight change did not affect these results (data not shown).

We additionally compared changes in glucose and insulin areas under the curve (AUCs) in response to the OGTT between the two groups. The AUCs of postprandial glucose showed a larger reduction (p = 0.004, group × time) and a trend for larger reduction in postprandial insulin (p = 0.076, group × time) in PhenoDiet group B compared with PhenoDiet group A (Figure S2).

The greater improvements in insulin sensitivity and glucose homeostasis in PhenoDiet group B were observed both in individuals with the MIR and LIR phenotype

We performed post hoc analyses with stratification for IR phenotype for the outcomes with significant group × time interaction (Figure 3; Table S7). Fasting insulin, HOMA-IR, Matsuda index, and MISI improved in both individuals with the MIR and individuals with the LIR phenotype in PhenoDiet group B, whereas these parameters did not improve in individuals with either IR phenotype within PhenoDiet group A. Within PhenoDiet group B, 2-h glucose and insulin decreased significantly in the MIR group following the LFHP diet, but the decreases did not reach significance in the LIR group on the HMUFA diet.

Glycemic variability was not affected in either of the groups

In addition to measuring glucose parameters in response to a laboratory challenge test, we assessed glycemic variability in daily-life settings for 6 days using continuous glucose monitoring (CGM). Mean glucose, glucose standard deviation (SD), glucose coefficient of variation (CV) %, % glucose time in range 3.9–7.8 mmol/L, and mean amplitude of glucose excursions (MAGE) were not affected in either of the groups (Table 3).

Minor weight loss and reduction in body fat and ectopic fat in both groups

Body weight decreased to a similar extent in both groups, with ~2.0% and ~2.7% in PhenoDiet group A and B, respectively (p < 0.001, time; p = 0.22, group × time) (Table 2). We performed a dual X-ray absorptiometry (DXA) to assess body composition. The weight loss was caused by a reduction in body fat mass, which tended to be greater in PhenoDiet group B compared with PhenoDiet group A (p = 0.058, group × time). Both android and gynoid fat mass decreased in both groups (p < 0.001, time), but the reduction in gynoid fat mass was slightly larger in PhenoDiet group B, compared with PhenoDiet group A (p = 0.035, group × time). Additionally, at MUMC+, visceral adipose tissue (VAT), liver fat, and muscle fat were assessed using a whole-body magnetic resonance imaging (MRI) scan ,and at WUR, VAT was assessed using single-slice MRI and liver fat was measured using proton magnetic resonance spectroscopy (^{1}H-MRS). VAT decreased in both groups in both centers, without significant differences between groups (p = 0.49 [whole-body MRI] and 0.97 [single-slice MRI], group × time) (Table 2). Liver fat and muscle fat decreased to a similar extent in both groups, with no significant differences between groups (p = 0.58 [liver fat measured by MRI], 0.15 [liver fat measured by MRS], and 0.73 [muscle fat], respectively, group × time) (Table 3).

Larger reduction in serum TAG in PhenoDiet group B and similar reductions in cholesterol, FFA, and blood pressure in both groups

Both groups showed a decrease in fasting serum total cholesterol and high-density lipoprotein (HDL) cholesterol levels, with a tendency for a greater decrease in total cholesterol in PhenoDiet group B (p < 0.001, time; p = 0.078, group × time) (Table 2). Fasting serum triacylglycerol (TAG) decreased in PhenoDiet group B, whereas it did not change in PhenoDiet group A (p = 0.028, group × time) (Figure 2H). The lack of improvement in serum TAG in PhenoDiet group A was mainly driven by a lack of improvement of individuals with the MIR phenotype on the HMUFA diet (Figure 3G). Fasting free fatty acids (FFAs) decreased in both groups to a similar extent (p = 0.013, time; p = 0.68, group × time) (Table 2). Both interventions significantly reduced

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Table 2. Primary and secondary outcomes at baseline and after 12 weeks in PhenoDiet groups A and B

			PhenoDiet group A (n = 121)		PhenoDiet group B (n = 121)		p value		
		n	Week 0	Week 12	Week 0	Week 12	Group	Time	Group × time
Primary outcome	disposition index (AU)	199	412 (369–460)	406 (365–451)	357 (321–398)	380 (343–423)	0.068	0.640	0.109
Secondary outcomes	Glucose metabolism								
	fasting glucose (mmol/L)	199	5.3 (5.2–5.5)	5.3 (5.2–5.4)	5.5 (5.3–5.6)	5.3 (5.2–5.4)	0.179	0.146	0.238
	fasting insulin (pmol/L)	199	47.5 (44.0–51.4)	46.0 (42.4–49.9)	52.7 (48.9–56.9)	46.0 (42.4–49.9)	0.063	0.285	0.019[c]
	2-h glucose (mmol/L)	199	6.1 (5.8–6.5)	6.2 (5.8–6.5)	6.5 (6.1–6.9)	6.1 (5.8–6.5)	0.123	0.561	0.020[c]
	2-h insulin (pmol/L)	199	349.7 (308.3–396.3)	337.0 (297.9–381.1)	397.0 (350.8–449.8)	322.9 (285.1–365.6)	0.154	0.569	0.023[c]
	HOMA-IR (AU)	199	1.6 (1.5–1.8)	1.6 (1.4–1.7)	1.8 (1.7–2)	1.6 (1.4–1.7)	0.052	0.203	0.017[c]
	HOMA-β (AU)	199	76.5 (71.3–81.8)	76.2 (70.8–82)	79.8 (74.5–85.5)	73.9 (68.5–79.6)	0.301	0.931	0.079
	Matsuda index (AU)	199	4.8 (4.4–5.3)	5.1 (4.6–5.6)	4.2 (3.9–4.6)	5.1 (4.6–5.5)	0.032[c]	0.150	0.004[c]
	insulinogenic index (AU)	199	32.2 (29.6–35)	30.4 (27.8–33.2)	32.3 (29.8–35.1)	28.8 (26.4–31.5)	0.957	0.072	0.234
	MISI (AU)	191	0.123 (0.11–0.138)	0.130 (0.114–0.147)	0.116 (0.104–0.13)	0.151 (0.133–0.171)	0.424	0.583	0.038[c]
	HIRI (AU)	198	383 (348–421)	346 (311–385)	404 (367–444)	340 (305–378)	0.505	0.021[c]	0.253
	HbA1c (mmol/mol)	199	36.0 (35.2–36.7)	36.0 (35.4–36.7)	36.5 (35.7–37.2)	35.9 (35.2–36.6)	0.635	0.976	0.091
	Anthropometrics								
	weight (kg)	221	86.9 (84.9–88.9)	85.2 (83.4–87.1)	87.9 (85.9–89.7)	85.5 (83.6–87.5)	0.408	<0.001[c]	0.224
	waist circumference (cm)	221	101.2 (99.5–102.8)	99.1 (97.7–100.7)	102.4 (100.9–104)	100.5 (99.1–102.1)	0.187	<0.001[c]	0.789
	waist-to-hip ratio	221	0.93 (0.92–0.94)	0.92 (0.91–0.94)	0.94 (0.93–0.95)	0.94 (0.92–0.95)	0.156	0.149	0.947
	Body composition								
	body fat mass (%)	195	36.1 (35.2–37.1)	35.0 (34.0–36.1)	37.0 (36.1–37.9)	35.4 (34.4–36.4)	0.186	<0.001[c]	0.078
	body fat mass (kg)	195	31.4 (30.1–32.8)	29.8 (28.4–31.3)	32.7 (31.3–34)	30.5 (29.1–31.9)	0.193	<0.001[c]	0.058
	lean body mass (kg)	195	51.6 (50.6–52.7)	51.4 (50.4–52.5)	52.0 (51.1–53.1)	52.0 (50.9–53.1)	0.604	0.130	0.466
	android fat mass (kg)	195	3.2 (3–3.3)	3.0 (2.8–3.1)	3.3 (3.1–3.4)	3.0 (2.9–3.2)	0.399	<0.001[c]	0.535
	gynoid fat mass (kg)	195	4.9 (4.6–5.1)	4.7 (4.4–4.9)	5.1 (4.9–5.4)	4.8 (4.6–5.0)	0.114	<0.001[c]	0.035[c]
	android/gynoid ratio	195	1.21 (1.18–1.23)	1.19 (1.16–1.22)	1.19 (1.16–1.21)	1.18 (1.15–1.21)	0.261	0.031[c]	0.498
	VAT (L)[a]	70	5.4 (4.9–6.0)	5.0 (4.5–5.5)	5.3 (4.8–5.8)	5.0 (4.6–5.5)	0.808	<0.001[c]	0.489
	VAT (cm^2)[b]	88	158 (146–170)	145 (134–158)	176 (163–191)	162 (149–176)	0.047[c]	<0.001[c]	0.972
	Cardiometabolic parameters								
	total cholesterol (mmol/L)	198	5.3 (5.1–5.5)	4.8 (4.7–5)	5.4 (5.2–5.6)	4.8 (4.6–5)	0.432	<0.001[c]	0.078
	HDL cholesterol (mmol/L)	198	1.3 (1.2–1.3)	1.2 (1.2–1.3)	1.3 (1.2–1.3)	1.2 (1.1–1.2)	0.266	<0.001[c]	0.101
	total cholesterol:HDL ratio	198	4.2 (4.0–4.4)	4.0 (3.8–4.2)	4.4 (4.2–4.6)	4.2 (4.0–4.4)	0.146	<0.001[c]	0.980
	TAG (mmol/L)	196	1.3 (1.2–1.4)	1.2 (1.2–1.3)	1.5 (1.4–1.6)	1.3 (1.2–1.4)	0.033[c]	0.103	0.028[c]
	FFA (mmol/L)	196	0.5 (0.4–0.5)	0.4 (0.4–0.5)	0.5 (0.4–0.5)	0.4 (0.4–0.5)	0.884	0.013[c]	0.684
	SBP (mmHg)	198	123.7 (121.1–126.2)	121.5 (119.1–123.9)	126.5 (123.9–129.1)	121.6 (119.1–124.2)	0.137	0.033[c]	0.077
	DBP (mmHg)	198	77.9 (76.2–79.7)	76.3 (74.6–78.0)	79.4 (77.7–81.1)	77.1 (75.4–78.7)	0.257	0.013[c]	0.495
	Inflammatory profile								
	CRP (mg/L)	197	0.98 (0.81–1.17)	0.97 (0.78–1.19)	1.12 (0.94–1.34)	0.88 (0.71–1.08)	0.298	0.892	0.034[c]

Values are estimated marginal means with 95% confidence intervals, adjusted for age, sex, and center. CRP, C-reactive protein; DBP, diastolic blood pressure; FFA, free fatty acid; HDL, high-density lipoprotein; HIRI, hepatic insulin resistance index; MISI, muscle insulin sensitivity index; SBP, systolic blood pressure; TAG, triacylglyceride; VAT, visceral adipose tissue. n represents number of individuals for whom data were available from both week 0 and week 12.

[a]At MUMC+, VAT was assessed using a whole-body MRI scan

[b]At WUR, VAT was assessed using single-slice MRI

[c]$p < 0.05$

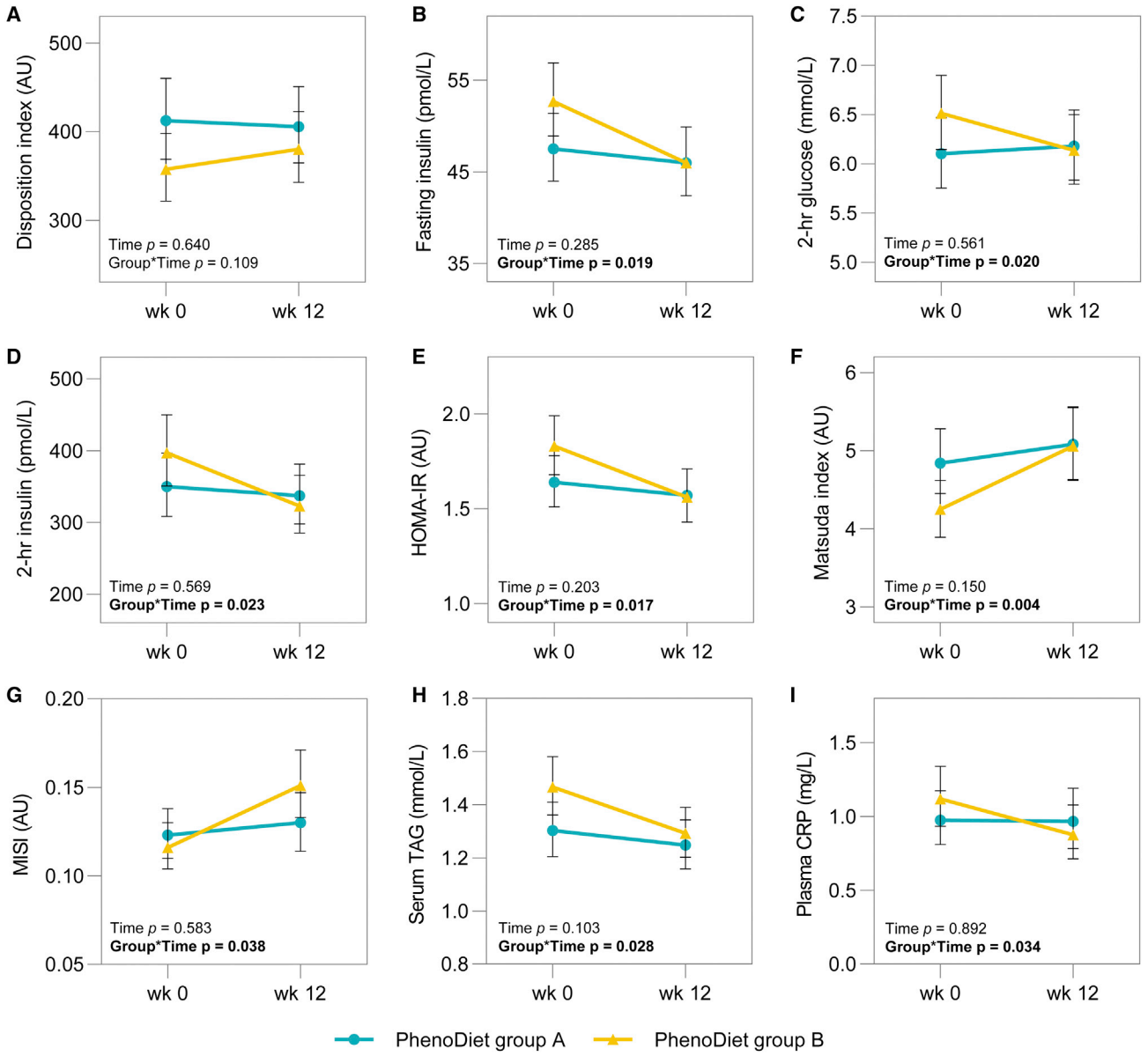

Figure 2. Greater improvements in insulin sensitivity, glucose tolerance, fasting TAG, and CRP in PhenoDiet group B compared with PhenoDiet group A

Individuals in PhenoDiet group B (n = 121) had more pronounced improvements in fasting insulin (B), 2-h glucose (C), 2-h insulin (D), HOMA-IR (E),Matsuda index (F), muscle sensitivity index (MISI) (G), serum triacylglycerol (TAG) (H), and plasma C-reactive protein (CRP) (I), but not disposition index (A), after 12 weeks of dietary intervention compared with PhenoDiet group A (n = 121). Data are presented as estimated marginal means with 95% confidence intervals, adjusted for age, sex, and center. p < 0.05 highlighted in bold. Intervention effects were tested using a repeated measures linear mixed model.

systolic blood pressure (SBP) and diastolic blood pressure (DBP) (Table 2). The reduction in SBP tended to be larger in PhenoDiet group B (p = 0.077, group × time).

Systemic inflammation marker CRP decreased only in PhenoDiet group B

Plasma C-reactive protein (CRP) decreased significantly from 1.12 (0.94–1.34) to 0.88 (0.71–1.08) mg/L in PhenoDiet group B, whereas it did not change in PhenoDiet group A (from 0.98 [0.81–11.7] to 0.97 [0.78–119]) (p = 0.034, group × time) (Table 2; Figure 2I). Post hoc analysis revealed that plasma CRP only improved in individuals with the MIR phenotype in the LFHP diet but did not significantly improve in other combinations of diet and phenotype (Figure 3H).

Similar reductions in postprandial glucose, insulin, TAG, and FFA upon a high-fat mixed meal in both groups

In addition to an OGTT, we also performed a liquid high-fat mixed meal (HFMM) test to assess postprandial responses to a meal containing fat, carbohydrates, and protein. The AUCs for

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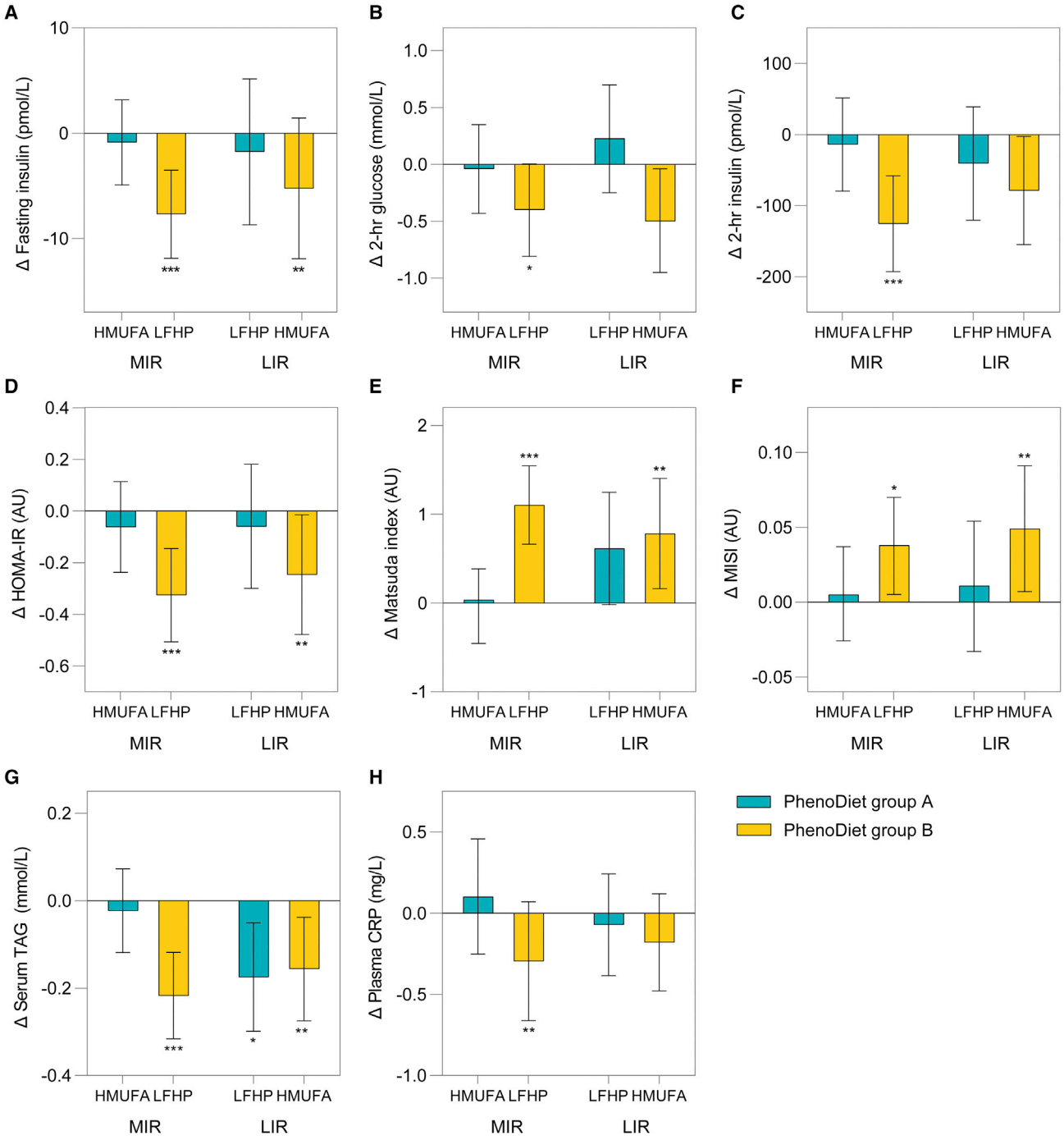

Figure 3. The greater improvements in PhenoDiet group B were observed both in the MIR and LIR phenotype

(A–F) Greater improvements in fasting insulin (A), 2-h glucose (B), 2-h insulin (C), HOMA-IR (D), Matsuda index (E), and MISI (F) were observed in PhenoDiet B (n = 121) in both individuals with MIR and LIR, whereas PhenoDiet A (n = 121) did not affect outcomes in either IR phenotype.

(G) Serum TAG was reduced after 12 weeks in PhenoDiet group B in both individuals with MIR and LIR, and in PhenoDiet group A in LIR individuals only.

(H) Plasma CRP was reduced in PhenoDiet group B in individuals with MIR and was not affected in the other groups.

Data are presented as estimated marginal means with 95% confidence intervals, adjusted for age, sex, and center. *p < 0.05, **p < 0.01, ***p < 0.001 for time effect, as tested with a repeated measures linear mixed model, stratified for IR phenotype (post-hoc analysis).

Table 3. Secondary outcomes at baseline and after 12 weeks in PhenoDiet groups A and B

		PhenoDiet group A (n = 121)		PhenoDiet group B (n = 121)		p value		
	n	Week 0	Week 12	Week 0	Week 12	Group	Time	Group × time
Glycemic variability								
Mean glucose (mmol/L)	211	6.0 (5.9–6.1)	6.0 (5.9–6.1)	6.2 (6.1–6.3)	6.1 (6–6.2)	0.031[c]	0.545	0.178
SD glucose (mmol/L)	211	0.85 (0.80–0.91)	0.89 (0.83–0.94)	0.93 (0.88–0.99)	0.91 (0.86–0.97)	0.046[c]	0.185	0.148
CV glucose (%)	211	14.2 (13.4–15.0)	14.8 (14.0–15.6)	15.1 (14.3–15.9)	15.0 (14.2–15.8)	0.115	0.134	0.227
Time in range 3.9–7.8 mmol/L (%)	211	93.6 (86.7–101.2)	93.3 (90.6–95.9)	84.3 (78–91)	89.6 (87.1–92.3)	0.055	0.887	0.102
MAGE (mmol/L)	211	2.1 (2–2.3)	2.2 (2.1–2.4)	2.4 (2.2–2.5)	2.3 (2.1–2.4)	0.045[c]	0.438	0.159
Ectopic fat								
Liver fat (%) (MRI)[a]	69	5.2 (3.9–6.8)	3.4 (2.5–4.5)	6.1 (4.7–7.9)	4.2 (3.2–5.5)	0.367	<0.001[c]	0.580
Liver fat (%) (^{1}MRS)[b]	84	2.6 (2.0–3.5)	1.3 (1.0–1.7)	3.2 (2.4–4.4)	1.3 (0.9–1.7)	0.347	<0.001[c]	0.154
Muscle fat (%)	70	7.7 (7.2–8.2)	7.6 (7.1–8.1)	7.4 (7.0–7.9)	7.3 (6.9–7.8)	0.427	0.036[c]	0.728
Physical activity								
LPA (h/day)	187	5.1 (4.8–5.3)	4.8 (4.6–5.1)	5.1 (4.9–5.4)	5.0 (4.7–5.3)	0.942	0.030[c]	0.233
MVPA (h/day)	187	1.2 (1.1–1.3)	1.2 (1.1–1.3)	1.2 (1.1–1.3)	1.2 (1.1–1.3)	0.562	0.241	0.297
Quality of life								
RAND-36 PCS	220	65.7 (64.2–67.3)	65.8 (64.3–67.4)	65.3 (63.8–66.9)	66.3 (64.6–67.9)	0.721	0.451	0.543
RAND-36 MCS	220	60.4 (58.9–61.9)	59.5 (58.2–60.9)	59.4 (58–60.9)	60.2 (58.8–61.6)	0.353	0.140	0.946
Sleep and fatigue								
Global PSQI score	220	5.0 (4.3–5.2)	5.1 (4.6–5.6)	4.7 (4.3–5.2)	5.2 (4.7–5.7)	0.700	0.189	0.534
Epworth sleepiness scale score	220	7.1 (6.4–7.7)	6.5 (5.8–7.1)	7.2 (6.6–7.9)	7.2 (6.4–7.9)	0.325	0.044[c]	0.115
Chalder fatigue score	220	11.7 (11.2–12.3)	11.4 (10.7–12.1)	11.7 (11.1–12.3)	11.1 (10.4–11.8)	0.892	0.576	0.090
Perceived stress								
Perceived stress score	220	8.8 (7.9–9.6)	8.2 (7.4–9)	8.6 (7.8–9.4)	9.4 (8.5–10.4)	0.333	0.592	0.003[c]

Values are estimated marginal means with 95% confidence intervals, adjusted for age, sex, and center. CV, coefficient of variation; LPA, light physical activity; MAGE, mean amplitude glucose excursion; MCS, mental component summary; MRI, magnetic resonance imaging; MRS, magnetic resonance spectroscopy; MVPA, moderate-to-vigorous physical activity; PCS, physical component summary; PSQI, Pittsburgh sleep quality index; SD, standard deviation. n represents number of individuals of which data were available from both week 0 and week 12.

[a]At MUMC+, liver fat was assessed using a whole-body MRI scan

[b]At WUR, liver fat was measured using ^{1}H-MRS

[c]$p < 0.05$

postprandial glucose, insulin, and FFA response decreased for both interventions (all $p < 0.05$, time) without differences between PhenoDiet groups A and B (Figures S3 and S4). The postprandial increase in serum TAG decreased slightly in PhenoDiet group B compared with PhenoDiet group A, but this did not reach statistical significance ($p = 0.11$, group × time) (Figure S4).

The interventions had mixed effects on perceived well-being

Next to physiological measures, we included questionnaires to assess perceived well-being. Health-related quality of life was not affected in either of the groups (Table 3). Of the questionnaires related to sleep and fatigue, only the Epworth sleepiness scale score significantly decreased in both groups, indicating a reduction in daytime sleepiness, but with no difference between the groups ($p = 0.044$, time; $p = 0.12$, group × time). The Chalder fatigue score tended to decrease in PhenoDiet group B only, indicating a reduction in self-reported fatigue ($p = 0.58$, time; $p = 0.090$, group × time). The perceived stress score increased in PhenoDiet group B, indicating an increase in perceived stress compared with PhenoDiet group A ($p = 0.003$, group × time).

Light-intensity physical activity decreased slightly in both groups

Physical activity was objectively measured throughout ~7 days in free-living conditions at the start and end of the intervention period using a thigh-worn accelerometer. In both groups, light-intensity physical activity decreased from baseline to week 12, with no difference between the groups ($p = 0.030$, time; $p = 0.23$, group × time) (Table 3). Moderate-to-vigorous physical activity (MVPA) did not change in either of the groups.

DISCUSSION

In this study, we show for the first time that improvements in cardiometabolic health after modulation of dietary macronutrient composition are dependent on tissue-specific IR phenotype.

We defined two PhenoDiet groups, with PhenoDiet group A including individuals with MIR following HMUFA diet and individuals with LIR following a LFHP diet, and PhenoDiet group B including individuals with LIR following a HMUFA diet and MIR following a LFHP diet. The data demonstrate pronounced and clinically relevant improvements in insulin sensitivity, fasting plasma insulin and TAG concentrations, glucose tolerance, and CRP in PhenoDiet group B compared with PhenoDiet group A. These findings provide evidence for a greater effectiveness of a precision nutrition strategy based on tissue-specific IR phenotypes over a ''one-size-fits-all'' dietary approach within the general dietary guidelines in improving cardiometabolic health.

Here, we demonstrate for the first time in a prospective study that individuals with distinct tissue-specific IR phenotypes respond differentially to dietary macronutrient modification. Interestingly, peripheral, rather than hepatic, insulin sensitivity showed a distinct differential response between PhenoDiet groups A and B. The Matsuda index significantly improved by ~20% in PhenoDiet group B compared to ~5% in PhenoDiet group A. Besides MISI, 2-h glucose and 2-h insulin concentrations improved more in PhenoDiet group B, independent of IR phenotype, while no distinct responses between PhenoDiet groups A and B were observed for HIRI and fasting plasma glucose. The Matsuda index[29] and MISI[26] have previously been validated against the glucose disposal rate, as determined by the gold-standard two-step hyperinsulinemic-euglycemic clamp. Both indices represent primarily peripheral, or skeletal muscle, insulin sensitivity.

The underlying mechanisms for the more pronounced improvements in particularly peripheral insulin sensitivity and overall cardiometabolic health in individuals with the MIR phenotype on the LFHP diet and individuals with the LIR phenotype on the HMUFA diet remain to be elucidated. Interestingly, modification of microbial composition by either fecal transplantation from lean donors to men with the metabolic syndrome, or dietary fiber intervention improved peripheral but not hepatic insulin sensitivity.[30,31] These data suggest that modulation of gut microbial composition may primarily affect peripheral insulin sensitivity, and this may thus be a putative underlying mechanism for the more pronounced effects on peripheral insulin sensitivity in individuals with the MIR phenotype on the LFHP diet and individuals with the LIR phenotype on the HMUFA diet. The high content of slowly fermentable fibers in the LHFP diet (such as β-glucan, the fiber that was provided in the present LFHP diet) may ferment more distally in the colon, whereby the produced short-chain fatty acids (SCFAs) may bypass the liver and elicit metabolic effects more peripherally.[32] Additionally, high-fermented foods, including yogurt and quark (largely provided within the LFHP diet), can increase microbial diversity and decrease inflammatory markers.[33] Together, several components within the LFHP diet may have elicited improvements in peripheral insulin sensitivity and inflammation, possibly via modulation of the gut microbiota.

Despite these indications that microbial modulation may target peripheral insulin sensitivity specifically, a role of microbiota composition in hepatic metabolism, possibly depending on initial microbial composition as well as site of colonic fermentation, cannot be excluded.[34] A diet rich in MUFA and thereby rich in polyphenols may also affect microbial composition and liver lipid metabolism.[34–36] Besides that, we have previously shown that a meal high in PUFA or MUFA acutely decreased circulating VLDL-TAG levels (liver-derived TAG), increased the fractional synthetic rate of TAG in the skeletal muscle, and increased postprandial insulin sensitivity, compared with SFA.[23] In line, in this study the HMUFA diet reduced fasting TAG levels and tended to reduce postprandial TAG levels in individuals with LIR compared with MIR. These data suggest that the HMUFA diet may affect hepatic lipid metabolism, thereby possibly contributing to improved peripheral insulin sensitivity through inter-organ crosstalk.

The findings are in line with a recent post hoc analysis of the CORDIOPREV-DIAB study, which showed that individuals with distinct tissue-specific IR phenotypes benefit most from diets that differ in macronutrient composition.[14] Based on the CORDIOPREV-DIAB study, we hypothesized that individuals with the MIR phenotype would benefit more from an HMUFA diet and individuals with the LIR phenotype more from an LFHP diet. We, however, observed a nonsignificant tendency for an improved disposition index and a more pronounced improvement in cardiometabolic health in individuals with the MIR phenotype on an LFHP diet and individuals with the LIR phenotype on an HMUFA diet (PhenoDiet group B as compared with PhenoDiet group A). These conflicting findings may relate to several factors, including differences in study populations (overall more healthy population in the present PERSON study), in assessment of LIR, and in composition of diet interventions. These contrasting results illustrate the complexity of precision nutrition. Further advancement of the field of precision nutrition requires more well designed, clinical trials with deep phenotyping to better understand the mechanisms that underlie inter-individual variation in response to diet. Such studies are needed to identify the most important factors that explain individual response to diet, as well as to validate precision nutrition-based strategies.

Interestingly, 76% of both individuals with MIR and LIR were considered normal glucose tolerant at baseline. Nevertheless, based on elevated waist circumference, body fat percentage, and total cholesterol levels observed in this study population, individuals with MIR or LIR may already be at increased risk for metabolic perturbations before the onset of disturbed glucose homeostasis as defined by established clinical cutoff values for impaired fasting glucose (IFG) and impaired glucose tolerance (IGT). Previous findings also show that tissue-specific IR phenotypes are related to disturbances in metabolome, lipidome, and inflammatory profiles.[11–13] An important finding in this study is that individuals in both study arms showed improvements in body composition, body fat distribution, ectopic fat, and several cardiometabolic parameters, regardless of intervention group (PhenoDiet group A or B) and without substantial weight loss (average weight loss: 2.3% or ~2 kg). To illustrate, liver fat decreased by more than 40% on average in the total population and total cholesterol levels decreased on average to values within the healthy range (<5.0 mmol/L). These results highlight the effectiveness and clinical relevance of a healthy diet in individuals with tissue-specific IR. Importantly, however, we demonstrate that health improvements can be remarkably enhanced when modulating dietary macronutrient composition based on tissue-specific IR phenotype.

We included questionnaires related to perceived well-being to explore the relationship between objective (clinical parameters) health and subjective health and well-being. Although slight changes in fatigue and perceived stress were observed, the effects on subjective health and well-being were not consistent. These findings suggest that improvements in cardiometabolic health were not reflected in detectable improvements in perceived well-being.

A major strength of this study is that it is the first to investigate the effects of modulating dietary macronutrient composition according to tissue-specific IR with a prospective, double-blind, randomized design in a large number of individuals. Another strength of this study is the classification of individuals by using only one measurement (7-point OGTT), paving the way for implementation of precision nutrition into clinical practice, although even more easily measurable biomarkers may be identified in future research. Finally, the dietary interventions were implemented by intensive dietary counseling and provision of key products. Dietary compliance was high, with substantial differences in reported MUFA, protein, and fiber intake between the HMUFA and LFHP diets, while keeping carbohydrate and SFA intake similar between the diets. The macronutrient composition that we aimed for was largely achieved in both diets, although reported MUFA and fiber intakes were slightly lower than advised in the HMUFA and LFHP diets, respectively. This may be due to either lower actual intake or misreporting.[37] Nevertheless, the two intervention diets clearly differed in key macronutrients, and both diets were a considerable modification to the participants' habitual diet.

In conclusion, we here demonstrate for the first time that clinically relevant improvements in cardiometabolic health after dietary macronutrient intervention are driven by IR phenotype, with the optimal macronutrient composition for each phenotype leading to a more pronounced improvement in cardiometabolic health, independent of weight loss. Our findings indicate that precision nutrition based on metabolic phenotype may be superior to a one-size-fits-all diet based on general guidelines with respect to improving cardiometabolic health.

Limitations of study

We acknowledge several limitations of this study. First, more individuals with the MIR phenotype were included in the study compared with LIR (149 versus 93). Due to equal distribution of phenotypes between PhenoDiet groups A and B, by design, more individuals followed the HMUFA diet in PhenoDiet group A and more LFHP in PhenoDiet group B. Still, post hoc analyses revealed that the more pronounced improvements in PhenoDiet group B as compared with PhenoDiet group A were driven by improvements in both individuals with the MIR on the LFHP diet and individuals with the LIR phenotype on the HMUFA diet. Furthermore, it appeared that the individuals in PhenoDiet group A were by chance somewhat more insulin sensitive at baseline compared with PhenoDiet group B. Nevertheless, statistical adjustments for baseline differences were made, indicating that the conclusions of larger improvements observed in PhenoDiet group B cannot be explained by a more unfavorable metabolic profile at baseline. Tissue-specific IR was assessed with a 7-point OGTT. This method has been validated again the gold-standard hyperinsulinemic-euglycemic clamp technique.[25,26] Nevertheless, contrary to the highly standardized hyperinsulinemic-euglycemic clamp technique, OGTT-derived measurement of tissue-specific IR may partially be affected by biological processes associated with the oral ingestion of glucose, including differences in gastrointestinal factors, such as the rate of glucose absorption by the gut and the related incretin response.[38] Furthermore, glucose and insulin responses to an OGTT may be affected by an individual's body size, as the dose of ingested glucose is the same for all. In addition, all blood samples were taken from a venous forearm catheter. Therefore, it should be noted that the degree of forearm glucose uptake may have contributed to inter-individual variation in venous plasma glucose concentrations.[39] Importantly, however, we have shown that based on just one OGTT, regardless of whether we were truly able to distinguish LIR and MIR, we identified distinct metabolic phenotypes, which could be replicated in independent cohorts[11–13] and which in this prospective study responded differentially to dietary intervention. We hereby provide support for the efficacy of the clinical use of (7-point) OGTT-derived measures of metabolic heterogeneity. Finally, this study is a proof-of-concept study, focused on specific IR phenotypes that are prevalent in ~30% of the overweight population. Future research has to demonstrate whether more metabolic and IR phenotypes that respond differentially to dietary macronutrient modulation can be defined.

STAR★METHODS

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SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.cmet.2022.12.002.

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AUTHOR CONTRIBUTIONS

I.T., A.G., and K.M.J. drafted the manuscript and were responsible for data analysis and execution of the study. G.B.H. was responsible for data management of the study. B.E. supported in the data processing and analysis. L.W. contributed to data collection and contributed to data analysis related to physical activity. E.S. was responsible for setting up the dietary intervention protocol and dietetics support during the study. E.E.B. was project leader and principal investigator and obtained funding for the project. E.E.B., L.A.A., G.H.G., E.J.M.F., D.H.J.T., and I.C.W.A. co-designed the study. All authors actively participated in project development, discussion of results, and revision of the article, and approved the final version of the manuscript.

DECLARATION OF INTERESTS

S.P. is an employee at DSM Nutritional Products, C.M.S.-P. is an employee at FrieslandCampina, and J.d.V.-v.d.B. is an employee at Danone Nutricia Research.

INCLUSION AND DIVERSITY

We support inclusive, diverse, and equitable conduct of research.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Critical commercial assays		
Human Insulin kit	Meso Scale Discovery	Cat# K151BZC; RRID:AB_2819057
Glucose HK CP ABX Pentra	Horiba ABX	Cat# A11A01667
Triglycerides CP ABX Pentra	Horiba ABX	Cat# A11A01640
NEFA-HR(2) Assay	FUJIFILM Wako Chemicals Europe GmbH	Cat# 0419D4WP
Cholesterol CP ABX Pentra	Horiba ABX	Cat# A11A01634
HDL Direct CP ABX Pentra	Horiba ABX	Cat# A11A01636
CRP Luminex Assay	Bio-Techne	Cat# LXSAHM, RRID:AB_2924693
Software and algorithms		
MISI calculator	O'Donovan et al.[25]	https://www.maastrichtuniversity.nl/macsbio-misi-calculator
CareLink	Medtronic	https://carelink.medtronic.com/
iglu v3.3.0 in R v4.0.2	Broll et al.[40]	https://cran.r-project.org/web/packages/iglu/index.html
Script for activPAL data processing	Winkler et al.[41]	N/A
SliceOmatic v5.0	TomoVision	https://www.tomovision.com/products/sliceomatic.html
jMRUI v5.2	Stefan et al.[42]	http://www.jmrui.eu/license-and-download/download/
Computational modeling method for quantification of body composition from MRI	AMRA medical	https://amramedical.com/science/technology/
SPSS v28.0	IBM	https://www.ibm.com/products/spss-statistics
Other		
CGM (iPro2 and Enlite Glucose Sensor)	Medtronic	https://www.medtronicdiabetes.com
ActivPAL3 micro	PAL Technologies Ltd.	https://www.palt.com/pals/

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Ellen Blaak (e.blaak@maastrichtuniversity.nl).

Materials availability

This study did not generate new unique reagents.

Data and code availability

The published article and supplemental information include the data used to generate the figures in the paper (Data S1). This paper does not report original code. Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

The PERSON study (PERSonalized glucose Optimization through Nutritional intervention) was a two-center, randomized, double-blinded, 12-week dietary intervention study with a parallel design (Figure 1). The rationale and methodology of the PERSON study have been described in detail previously.[24] The study was conducted from May 2018 until November 2021 at Maastricht University Medical Center+ (MUMC+) and Wageningen University (WUR) in the Netherlands, in line with the principles of the Declaration of

Helsinki. The protocol was approved by the Medical Ethical Committee of the MUMC+(NL63768.068.17) and registered at ClinicalTrials.gov (NCT03708419). All participants gave written informed consent.

Study participants

Participants were recruited via a volunteer database, flyers, and advertisements in local and online media. Inclusion criteria were: age 40–75 years, BMI 25–40 kg/m^2, body weight stability for at least 3 months (no weight gain or loss >3 kg), and tissue-specific IR, characterized as predominant LIR or MIR, as assessed by a 7-point oral glucose tolerance test (OGTT) based on venous plasma glucose and insulin concentrations. Exclusion criteria included among others pre-diagnosis of type 2 diabetes mellitus (T2DM), diseases or use of medication that affect glucose and/or lipid metabolism, major gastrointestinal diseases, history of major abdominal surgery, uncontrolled hypertension, smoking, alcohol consumption >14 units/week, and >4 h/week moderate-to-vigorous physical activity.[24] See CONSORT diagram (Figure S1).

Assessment of eligibility

Compliance with in- and exclusion criteria was assessed according to standard protocols during a screening visit as described previously.[24] Data on demographics, medical history, family history of DM ($\geq$1 first-degree relative with DM), and medication use were collected by a screening questionnaire. Education level was categorized into low (no education, primary education, lower or preparatory vocational education, lower general secondary education), medium (intermediate vocational education, higher general senior secondary education or pre-university secondary education) and high (higher vocational education, university).

Tissue-specific IR was assessed based on the plasma glucose and insulin concentrations during a 7-point OGTT. Participants ingested 200 ml of a ready-to-use 75 g glucose solution (Novolab) within 5 min, and blood samples were collected from the antecubital vein via an intravenous cannula under fasting conditions (t = 0 min) and after ingestion of the glucose drink (t = 15, 30, 45, 60, 90, and 120 min) for determination of plasma glucose and insulin concentrations. LIR and MIR were estimated using calculations for the hepatic insulin resistance index (HIRI) and muscle insulin sensitivity index (MISI) respectively, by Abdul-Ghani and colleagues.[26] The MISI calculation has been optimized using the cubic spline method.[25] HIRI and MISI were calculated as follows:

$$\text{HIRI} = \text{glucose } 0 - 30[\text{AUC in mmol/L x h}] \text{ x insulin } 0 - 30[\text{AUC in pmol/L x h}]$$

$$\text{MISI} = (\text{dGlucose/dt})/\text{insulin}[\text{mean during OGTT in pmol/L}]$$

In the calculation for MISI, dGlucose/dt is the rate of decay of plasma glucose concentration (mmol/L) during the OGTT, calculated as the slope of the least square fit to the decline in plasma glucose concentration from peak to nadir. Deviating glucose curves that were flagged by the calculator were visually inspected for MIR and LIR classification. Individuals were classified as "No MIR/LIR," "MIR," "LIR," or "combined MIR/LIR," using tertile cutoffs for MISI and HIRI. The lowest tertile of MISI represented individuals with MIR, while the highest tertile of HIRI represented individuals with LIR. The cutoffs for these tertiles were based on values of a selected study population of The Maastricht Study,[27] which resembles the target population of the PERSON study. After inclusion of 163 participants, the median HIRI of the current study screening population was used for classification due to an apparent discrepancy in LIR prevalence between the two populations. Additional OGTT-derived indices and other outcomes were determined as described below and as previously reported.[24] Eligible participants started the study within 3 months after screening.

Randomization

Eligible participants were randomly assigned to either PhenoDiet group A or PhenoDiet group B, which consisted of unique combinations of the MIR and LIR metabolic phenotypes and two distinct diets meeting the Dutch dietary guidelines.[43] PhenoDiet group A included individuals with MIR following a high-monounsaturated fatty acids (HMUFA), and individuals with LIR following a low-fat, high-protein, and high-fiber (LFHP) diet. PhenoDiet group B included individuals with LIR and MIR on HMUFA and LFHP diets, respectively.

Random allocation to either PhenoDiet group A or B in 1:1 ratio was conducted by an independent researcher using center-specific minimization,[44,45] with randomization factors of 1.0 for the LIR/MIR phenotype, and 0.8 for age and sex, and a base probability of 0.7 by means of biased-coin.[46] Both researchers and participants were blinded to the participants' metabolic phenotype (LIR or MIR), and thus blinded to whether participants were allocated to PhenoDiet A or B.

METHOD DETAILS

Dietary intervention

The HMUFA diet had a targeted macronutrient composition of 38% of energy from fat (20% MUFA, 8% PUFA, 8% SFA), 48% of energy from carbohydrates (CHO) (30% polysaccharides; 3 g/MJ fiber), and 14% of energy from protein. The macronutrient composition of the LFHP diet was targeted at 28% of energy from fat (10% MUFA, 8% PUFA, 8% SFA), 48% of energy from CHO (30% polysaccharides; >4 g/MJ fiber), and 24% of energy from protein (Table S1). Energy from CHO was similar between diets. Key products that largely distinguished the two diets with regards to macronutrient composition were provided in pre-measured

amounts. For the HMUFA diet, key products included olive oil, olives, olive tapenade, and low-fat margarine with olive oil. Key products for the LFHP diet included low-fat yogurt and quark, reduced-fat cheese, very low-fat spread, pumpkin seeds, baking margarine with olive oil, and a dietary fiber supplement (2 g β-glucan per 6 g, DSM Nutritional Products, Basel, Switzerland) providing 6–12 g of additional fiber per day. Participants were instructed to consume a certain amount of every provided product each day. Apart from the fiber supplement, all products were commercially available. Alcohol consumption was restricted to ≤ 1 glass/day, in agreement with the current Dutch dietary guidelines.[43]

Participants were assigned to one of eight energy groups ranging from 6 to 13 MJ/d according to their estimated individual energy requirement, which was calculated by averaging self-reported energy intake from a food frequency questionnaire (FFQ)[47] with the product of the predicted BMR, as calculated with Schofield equations,[48] and self-reported physical activity level.

Individual counseling sessions with a dietician or research nutritionist were scheduled weekly at the research facilities to monitor adherence to the diet, adverse events and body weight to assess weight stability. Additional support was provided via e-mail or phone if needed. In case of weight instability, the participant's energy group was adjusted to avoid further weight change. During the period of COVID-19 restrictions, all counseling sessions took place via phone or video call. The dietary intervention strategy has been described in more detail before.[24]

Dietary compliance

During the 12-week intervention, dietary compliance was assessed by three unannounced 1-day food records on two non-consecutive weekdays and one weekend day using the mobile app "Traqq"[28]. In addition, plasma fatty acid profile was measured by nuclear magnetic resonance spectroscopy as a biomarker for MUFA, PUFA and SFA consumption.[49]

Habitual dietary intake

A validated 163-item semi-quantitative FFQ[47] was used to assess habitual dietary intake before the start of the dietary intervention period. Dietary misreporting was evaluated by Goldberg's method,[50,51] using the ratio of daily energy intake (EI) to estimated basal metabolic rate (BMR). Energy under- (EI/BMR < 0.87) and over reporters (EI/BMR > 2.75) were excluded from data analyses.

Measurements

In the week before start of the dietary intervention (baseline) and in the last week of the 12-week intervention (week 12), participants were extensively phenotyped during a characterization week. This week included three or four (depending on study center and participation in additional subgroup measurements) clinical test days including a broad spectrum of laboratory analyses and three at-home days for additional data collection in daily-life settings.[24] On the clinical test days, participants were instructed to travel to the facility by car or public transport. The day prior to and during the characterization weeks, participants were requested to refrain from alcohol and vigorous physical activity.

7-point oral glucose tolerance test

A 7-point OGTT was performed according to the same procedures as during the screening visit. Participants consumed a standardized low-fat macaroni meal (30% of energy intake [en%] fat, 49 en% CHO, 21 en% protein; 1,560–2,460 kJ, depending on energy group) the evening before the OGTT, after which they remained fasted until the OGTT.

The primary outcome disposition index was calculated as: [Matsuda index * (AUC30 min insulin/AUC30 min glucose)], where AUC30 min is the area under the curve between baseline and 30 min of the OGTT for insulin (pmol/L) and glucose (mmol/L) as calculated using the trapezoidal method, respectively. The homeostasis model assessment of insulin resistance (HOMA-IR) was calculated as (fasting glucose [mmol/L] × fasting insulin [mU/L])/22.5.[52] HOMA of β-cell function (HOMA- β) was calculated as (20 × fasting insulin [mU/L])/(fasting glucose [mmol/L] – 3.5). Matsuda index was defined as: [10,000 ÷ square root of [fasting plasma glucose (mg/dL) × fasting insulin (mU/L)] × [mean glucose (mg/dL) x mean insulin (mU/L)]], using glucose and insulin values of time points 0, 30, 60, 90, and 120 min.[29] Criteria of the WHO[53] were used define glucose status: normal glucose tolerance (NGT), fasting glucose <6.1 mmol/L and 2-hour glucose <7.8 mmol/L; impaired fasting glucose (IFG), fasting glucose 6.1 – 6.9 mmol/L and 2-hour glucose <7.8 mmol/L; impaired glucose tolerance (IGT), fasting glucose <6.1 mmol/L and 2-hour glucose 7.8 – 11.0 mmol/L; combined IFG/IGT, fasting glucose 6.1 – 6.9 mmol/L and 2-hour glucose 7.8-11.0 mmol/L; T2DM, fasting glucose ≥7.0 mmol/L and/or 2-hour glucose ≥11.1 mmol/L.

High-fat mixed-meal challenge test

A high-fat mixed-meal challenge test was performed at least 4 days after the OGTT, to determine the effects of the diets on postprandial glucose and lipid metabolism after a high-fat challenge. Participants consumed the same standardized low-fat macaroni meal as before the OGTT, after which they fasted 12 hours overnight. The liquid HFMM (350 g containing 2.8 MJ, 49 g [64 en%] fat, 48 g [29 en%] CHO, 12 g [7 en%] protein) was prepared in the university kitchen using whipped cream ice cream, whipped cream, full-fat milk, and sugar. An intravenous cannula was inserted in the antecubital vein for blood sampling. At least 30 min following insertion of the catheter, a fasting blood sample was drawn (t = 0 min). Subsequently, participants were asked to consume the liquid HFMM within 5 min and postprandial blood samples were drawn at t = 30, 60, 90, 120, 180, and 240 min for determination of glucose, insulin, free fatty acids (FFA) and triacylglycerol (TAG). Total cholesterol and HDL cholesterol were determined in fasting serum.

Body composition, fat distribution and ectopic fat deposition

Measurements of body weight and waist and hip circumference were performed according to standardized measurements.[24] Whole-body and regional fat mass, fat percentage, and lean body mass were assessed using dual-energy X-ray absorptiometry (DXA), while participants were fasted for ≥2 h (MUMC+, Discovery A, Hologic; WUR, Lunar Prodigy, GE Healthcare).

Fat distribution and ectopic fat deposition were assessed using magnetic resonance imaging (MRI) and/or magnetic resonance spectroscopy (MRS). At MUMC+, a whole-body scan was made after a ≥2 h fast with a 3T MRI scanner (3T MAGNETOM Prisma fit, Siemens Healthcare), using a radiofrequency transmit/receive body coil at Scannexus, Maastricht, the Netherlands. Analyses were performed using a computational modeling method [AMRA Medical AB, Linköping, Sweden] for quantification of visceral adipose tissue (VAT), intrahepatic lipid content (IHL), and muscle fat infiltration (MFI) in the anterior thighs. At WUR, IHL and abdominal fat were assessed with proton magnetic resonance spectroscopy (^{1}H-MRS) and MRI, respectively, on a 3T whole-body scanner (Siemens, Munich, Germany; Philips Healthcare, Best, the Netherlands from November 2020 onwards). MRI measurements were performed after a ≥2 h fast at hospital Gelderse Vallei, Ede, the Netherlands. Spectra for determination of IHL were obtained from a 30 × 30 × 20 mm voxel placed in the right lobe of the liver, avoiding blood vessels and bile ducts. Participants were instructed to hold their breath when spectra were acquired to reduce respiratory motion artifacts. Spectra were post-processed and analyzed using the AMARES algorithm in jMRUI software.[42] VAT was quantified in single-slice axial T1-weighted spin echo transverse images at the inter-vertebral space L3-L4 using the image analysis software program c (version 5.0, Tomovision).

Continuous glucose monitoring

Participants wore a continuous glucose monitor (CGM) for 6 days during characterization weeks 1 and 2. The CGM device (iPro2 and Enlite Glucose Sensor; Medtronic, Tolochenaz, Switzerland) was worn lateral to the umbilicus and recorded subcutaneous interstitial glucose values every 5 minutes. Participants were asked to perform four daily capillary glucose self-measurements (SMBG) via Contour XT (Ascensia Diabetes Care, Mijdrecht, the Netherlands) while wearing the CGM device. The CGM data files were then calibrated retrospectively using the SMBG values in CareLink (Medtronic, Tolochenaz, Switzerland) according to manufacturer's instructions. To avoid insufficient calibration, sensor glucose readings outside the time interval of the first and last SMBG measurements were excluded from the analysis. Participants were blinded to the CGM recording, but not to the SMBG values. In addition, CGM data files with irregular measurement frequencies (i.e. other than 5 minute) were excluded from the analysis (n = 3). The iglu package[40] (version 3.3.0) in R (version 4.0.2) was used to calculate mean glucose, standard deviation (SD), coefficient of variation (CV), time in range (between 3.9 and 7.8 mmol/L; TIR) and mean amplitude of glycemic excursions (MAGE).

Blood pressure

Systolic and diastolic pressure were measured in triplicate on the non-dominant arm with an automated sphygmomanometer after a 5-minute rest. The first measurement was used to acclimatize the subject to the measurements, and therefore omitted from the data.

Physical activity monitoring

Physical activity was assessed with the activPAL3 micro triaxial accelerometer (PAL Technologies Ltd., Glasgow, UK). The monitor was worn continuously attached to the anterior thigh, in the middle between the knee and the greater trochanter for ~14 days during both the characterization weeks, of which ~7 days in free-living conditions. Parameters of physical activity were quantified with a modified version of a home-written script,[41] using sleeping and waking times recorded by the participants as input. We distinguished light-intensity physical activity (LPA) and moderate-to-vigorous physical activity (MVPA). LPA includes standing and stepping times with Metabolic Equivalent of Task (MET) values <3.[41] MVPA includes activities with MET values ≥3. Both measures were determined in hours per day. In the present study, only LPA and MVPA during the free-living days were used because physical activity during the characterization weeks with university visits and measurements is not reflective of regular physical activity level.

Self-reported sleep, well-being, and physical (in-)activity

General perceived health was assessed by the Physical and Mental Component Summary (PCS and MCS) scores obtained from the RAND-36.[54] Perceived stress was assessed with the 10-item Perceived Stress Scale (PSS-10).[55] Physical and mental fatigue were assessed using the 14-item Chalder fatigue scale.[56] Sleep quality was assessed with the 10-item Pittsburgh Sleep Quality Index.[57] Daytime sleepiness was assessed with the 8-item Epworth Sleepiness scale.[58] Self-reported habitual physical activity and sedentary behavior were assessed using the Baecke questionnaire.[59]

Adjusted COVID-19 protocol

Due to strict Dutch COVID-19 restrictions from March to June 2020, post-intervention measurements of 22 individuals were performed according to an adjusted protocol. The protocol included CGM measurements, anthropometric measurements and questionnaires as described above. The participants performed the measurements at home under guidance of the researcher via video connection. All other measurements were not performed during this period. The dietary intervention part of the study was completed according to the original protocol. The COVID-19 protocol was approved by the Medical Ethical Committee of the MUMC+ and participants gave their written informed consent.

Biochemical analyses of blood samples and biobanking

Venous blood was collected in EDTA tubes (Becton Dickinson, Eysins, Switzerland), which were centrifuged at 1,200 g, 4°C for 10 min and plasma was aliquoted subsequently. Serum tubes were left at room temperature for at least 30 min to allow clotting after sampling and centrifuged at 1,200 g, 20°C for 10 min before aliquoting of serum. All biological samples were snap-frozen in liquid nitrogen and stored at −80°C until analysis. Samples from both centers were analyzed at central laboratories. Plasma glucose, insulin, and FFA were measured on a Cobas Pentra C400 using ABX Pentra Glucose HK CP reagens (Horiba ABX Diagnostics, Montpellier, France), ELISA (Meso Scale Discovery, Gaithersburg, USA), and NEFA HR reagens (Wako chemicals, Neuss, Germany), respectively. Serum TAG, total cholesterol, and HDL cholesterol were measured on a Cobas Pentra C400 using ABX Pentra Triglycerides HK CP reagens, ABX Pentra Cholesterol CP reagens, and ABX Pentra HDL Direct, respectively. A fasting blood sample was drawn for determination of glycated hemoglobin (HbA1c) by the hospital laboratories of MUMC+ and Ziekenhuis Gelderse Vallei, Ede, the Netherlands. The inflammatory marker C-reactive protein (CRP) was measured in fasting plasma using a Luminex immunoassay performed by DSM Nutritional Products (Kaiseraugst, Switzerland).

QUANTIFICATION AND STATISTICAL ANALYSIS

Power and sample size

A total sample size of 202 was previously calculated to be required to detect a standardized effect size of 0.46 with a power of 90%.[24] Due to practical issues related to the unforeseen COVID-19 pandemic, 199 individuals completed the measures related to the primary outcome the disposition index. Main reasons for dropout were related to personal reasons and being unhappy with the diet (Figure S1). Participants for whom data that was missing due to the adjusted COVID-19 protocol were not considered dropouts but were excluded from the analyses related to these missing data to limit interference with study outcomes.

Statistical analyses

The number of dropouts between the two intervention groups was not significantly different ($p = 0.11$), and baseline characteristics did not differ between dropouts and completers (all $p > 0.05$) (Table S3). An intention to treat (ITT) analysis, which assumes that data was missing at random, was performed using a mixed model with repeated measures to test intervention effects on primary and secondary parameters comparing PhenoDiet groups A and B. The model included age, sex, and study center as covariates, and time (baseline and week 12) as repeated measure. *Post-hoc* analyses with stratification for IR phenotype were performed in case of a significant group *x* time interaction. Estimated marginal means with 95% confidence intervals adjusted for the covariates are reported. For OGTT and high-fat mixed-meal responses, the AUC was calculated using the trapezoid method. Baseline characteristics were compared between the MIR and LIR phenotype, and between the diet groups within MIR and LIR groups using independent samples T-test for numerical data (mean ± SD) and using Fisher's exact test for categorical data (%).

Model assumptions were tested by plotting residual and predicted values and by visually inspecting residual Q-Q plots, to test homogeneity of variances and normality of residuals, respectively. Skewed variables were log-transformed ($\log_{10}$) to improve normality. Two-tailed $p < 0.05$ was considered statistically significant. Analyses were performed using IBM SPSS Statistics software version 28.

ADDITIONAL RESOURCES

The trial was registered on: https://clinicaltrials.gov/ct2/show/NCT03708419, and the design paper was published previously: Gijbels et al.[24]