Diverging metabolic effects of 2 energy-restricted diets differing in nutrient quality: a 12-week randomized controlled trial in subjects with abdominal obesity

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ABSTRACT

Background: Despite the established relation between energy restriction (ER) and metabolic health, the most beneficial nutrient composition of a weight-loss diet is still a subject of debate.

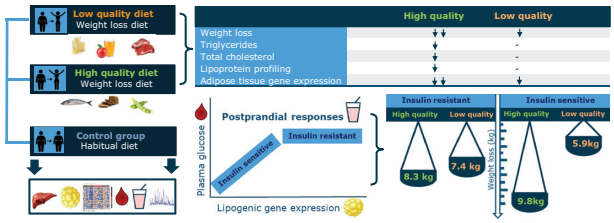
Objectives: The aim of the study was to examine the additional effects of nutrient quality on top of ER.

Methods: A parallel-designed, 12-week 25% ER dietary intervention study was conducted (clinicaltrials.gov: NCT02194504). Participants aged 40–70 years with abdominal obesity were randomized over 3 groups: a 25% ER high-nutrient-quality diet ($n = 40$); a 25% ER low-nutrient-quality diet ($n = 40$); or a habitual diet ($n = 30$). Both ER diets were nutritionally adequate, and the high-nutrient-quality ER diet was enriched in MUFAs, n-3 PUFAs, fiber, and plant protein and reduced in fructose. Before and after the intervention, intrahepatic lipids, body fat distribution, fasting and postprandial responses to a mixed-meal shake challenge test of cardiometabolic risk factors, lipoproteins, vascular measurements, and adipose tissue transcriptome were assessed.

Results: The high-nutrient-quality ER diet (-8.4 ± 3.2) induced 2.1 kg more weight loss ($P = 0.007$) than the low-nutrient-quality ER diet (-6.3 ± 3.9), reduced fasting serum total cholesterol ($P = 0.014$) and plasma triglycerides ($P < 0.001$), promoted an antiatherogenic lipoprotein profile, and induced a more pronounced decrease in adipose tissue gene expression of energy metabolism pathways than the low-quality ER diet. Explorative analyses showed that the difference in weight loss between the two ER diets was specifically present in insulin-sensitive subjects (HOMA-IR ≤ 2.5), in whom the high-nutrient-quality diet induced 3.9 kg more weight loss than the low-nutrient-quality diet.

Conclusions: A high-nutrient-quality 25% ER diet is more beneficial for cardiometabolic health than a low-nutrient-quality 25% ER diet. Overweight, insulin-sensitive subjects may benefit more from a high- than a low-nutrient-quality ER diet with respect to weight loss, due to potential attenuation of glucose-induced lipid synthesis in adipose tissue. *Am J Clin Nutr* 2022;116:132–150.

GRAPHICAL ABSTRACT



Keywords: nutrigenomics, precision nutrition, clinical trial, insulin resistance, adipose tissue, mixed-meal challenge, dietary intervention

Introduction

The increased global burden of obesity stresses the need for dietary strategies that prevent weight gain, induce weight

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Supplemental Figures 1–5 and Supplemental Tables 1–5 is available from the "Supplementary data" link in the online posting of the article and from the same link in the online table of contents at https://academic.oup.com/ajcn/.

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Abbreviations used: CVD, cardiovascular disease; EER, estimated energy requirement; ER, energy restriction; FDR, false discovery rate; FPG, fasting plasma glucose; HbA1c, glycated hemoglobin; MRS, magnetic resonance spectroscopy; PPAR, peroxisome proliferator-activated receptor; QUICKI, quantitative insulin sensitivity check index; RIN, RNA integrity; SAT, subcutaneous adipose tissue; SREBP, sterol regulatory element–binding protein; VAT, visceral adipose tissue.

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loss, and alleviate obesity's comorbidities, thereby reducing the risk of chronic diseases, such as cardiovascular disease (CVD). An evidently effective dietary strategy in this context is the restriction of daily caloric intake: that is, energy restriction (ER), usually targeted at ~10% to 40% of the estimated energy requirement of overweight or obese individuals (1). Studies show that cardiometabolic risk factors, such as blood pressure, dyslipidemia, and measures of insulin sensitivity, often already improve after a 5% loss of initial body weight (2, 3). Magkos et al. (3) recently demonstrated that more progressive weight loss—for example, of 11%–16%—even further improves cardiometabolic risk factors and alters key biological pathways in the adipose tissue.

Despite a better understanding of the relation between the degree of energy restriction, the magnitude of weight loss, and metabolic health, the most beneficial nutrient composition of an ER diet is still a subject of intense debate (4, 5). Numerous trials have been carried out to try to identify the optimal ER diet to augment weight loss or to maximize health improvements. These approaches include emphasizing a specific macronutrient, such as high-protein diets (6, 7); aligning the diet to an a posteriori–defined pattern that is linked to lower risks of chronic diseases, such as the Mediterranean diet (8); or adding specific bioactive compounds, such as fish oil or flavanols (9–13). However, trials that combine multiple high-quality nutrients in one ER diet are, to the best of our knowledge, lacking. In the present study, we enriched an ER diet with nutrients that specifically target the health of important metabolic organs, such as the liver and adipose tissue (14). These nutrients include dietary fiber, soy protein, MUFAs, and n-3 PUFAs. The aim of our study was to investigate the potential additional effects of these high-quality nutrients within an ER diet by comparing the effects of this healthy diet with those of a low-nutrient-quality ER diet, rich in SFAs and monosaccharides such as fructose, and a habitual diet (control group) in subjects with abdominal obesity.

Effects of nutrition on cardiometabolic health are usually subtle, and classical biomarkers assessed in nutritional intervention studies are typically disease markers, while these studies are often executed in relatively healthy populations. To draw conclusions on the effects of diet on an individual's metabolic health state, we need to comprehensively phenotype the participants. In addition to measures on classical markers of metabolic health, such as plasma glucose, insulin, and triglycerides, this also includes plasma metabolomics and an assessment of the static metabolic health status, which include measures of tissue and organ health, such as intrahepatic lipid accumulation and body fat distribution, vascular function, and the underlying molecular adaptation in adipose tissue. It also includes the assessment of the dynamic metabolic health status; that is, phenotypic flexibility, which includes an assessment of changes in the above-mentioned markers during a mixed-meal challenge. Integration of these combined measurements leads to a better understanding of the personal metabolic health status. Therefore, we accurately assessed the effects of two 12-week ER diets differing in nutrient quality on cardiometabolic risk factors by using an array of outcome measures. The primary outcomes were classical biomarkers, such as plasma glucose, insulin and triglycerides, intrahepatic lipid accumulation, body fat distribution, adipose tissue transcriptomics, plasma metabolomics, and vascular measurements, including blood pressure and pulse wave analyses. All measures except body fat distribution and intrahepatic lipid accumulation were determined as both fasting and postprandial measures after a standardized mixed-meal shake challenge test, to assess the participant's flexibility in dealing with a standardized mixed-meal shake test.

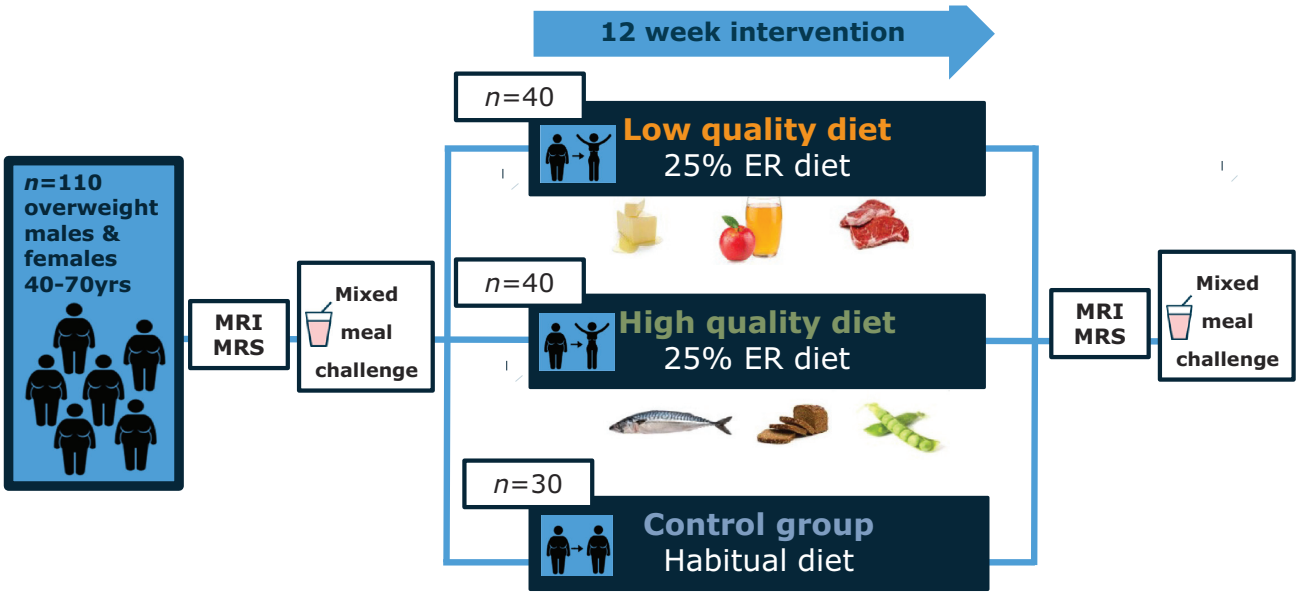
Methods

Study design

This study was a parallel-designed, randomized, 12-week intervention that examined the effects of a 25% ER high-nutrient-quality diet ($n = 40$); a 25% ER low-nutrient-quality diet ($n = 40$); or a habitual diet ($n = 30$) on cardiometabolic risk factors using an array of outcome measures, including whole-body MRI and magnetic resonance spectroscopy (MRS), plasma metabolomics, adipose tissue transcriptomics, and several postprandial measurements, after a standardized mixed-meal shake challenge, both before and after the intervention (**Figure 1**). In total, 151 individuals were assessed for eligibility, of which 41 were excluded from participation, resulting in inclusion of 110 subjects. Of these individuals, 100 participants completed the study, with 39 in the low-nutrient-quality ER diet group (1 dropout), 34 in the high-nutrient-quality ER diet group (7 dropouts, 1 replaced), and 27 (3 dropouts) in the control group (**Supplemental Figure 1**). The study was conducted at Wageningen University, the Netherlands, from 21 July until 21 October 2014.

Energy restricted diets

The intervention was based on dietary advice and the provision of key food products. Participants in the control group were instructed to maintain their habitual diet. Schofield (15) equations using a body mass corresponding with a BMI of 27 kg/m^2 were applied to estimate the basal metabolic rate of each participant. This rate was multiplied by a physical activity level value based on participants' reported activity level to calculate the estimated energy requirement (EER). Participants were instructed to maintain their physical activity habits. An energy restriction of 25% was applied to the EER to allocate participants to an energy group of either 6, 7, 8, or 9 mega joules per day. The 2 diets were isocaloric and matched for alcohol, sodium, and total fat. The differences between the two ER diets were that the high-nutrient-quality ER diet, compared to the low-nutrient-quality ER diet, was enriched in MUFAs (13.4 En% compared with 9.3 En%, respectively), n-3 PUFAs (7.7 En% compared with 4.1 En%, respectively), fiber (3.1 En% compared with 2.2 En%, respectively), and plant protein (10.3 En% compared with 5.7 En%, respectively) and reduced in fructose content (3.4 En% compared with 7.0 En%, respectively). The composition of the diets formed the basis of daily meal plans provided to the participant during the intervention (**Supplemental Table 1**). To increase the intake of n-3 PUFA in the high-nutrient-quality ER diet, a daily capsule providing 400 mg EPA and 300 mg DHA was included in the meal plan. Participants visited the facility weekly for an individual session with a qualified dietician, after which the key food products were provided for the following week. Adherence to the diet was determined based on the collection of leftovers of the supplied products and on the participant's food



Measurements performed before and after the 12-week dietary intervention								
MRS	Intra-hepatic lipids							
MRI	VAT							
	SAT							
		Time after mixed meal challenge (min)						
		0	30	60	120	180	240	360
Blood	Glucose	x	x	x	x	x	x	
	Insulin	x		x	x		x	
	Triglycerides	x			x		x	x
	Free fatty acids	x			x		x	x
	Other clinical chemistry	x						
	Metabolomics	x			x		x	x
Vascular measurement	PWA	x			x		x	
Adipose tissue biology	Transcriptomics	x					x	
	Adipocyte size	x						

FIGURE 1 Study design of the 12-week randomized controlled trial. The 3 intervention arms of the dietary intervention trial are: a 25% ER high-nutrient-quality diet ($n = 40$); a 25% ER low-nutrient-quality diet ($n = 40$); or a habitual diet ($n = 30$). Postprandial measurements per time point during the mixed-meal shake challenge and MRI/MRS were performed before and after the intervention. ER, energy restriction; MRS, magnetic resonance spectroscopy; PWA, pulse wave analyses; SAT, subcutaneous adipose tissue; VAT, visceral adipose tissue.

diary, in which deviations from the protocol were reported. To calculate reported intake of energy and nutrients, the meal plans with reported deviations were used. Additional consumed food items were added to the intake, while leftovers were subtracted. Metabolizable energy values of the two diets were calculated using specific Atwater values (**Supplemental Table 2**) (16).

Participants

The study population consisted of healthy, abdominally obese men and women aged 40–70 years at the time of recruitment. Subjects were recruited from the NQPlus cohort, a database of Wageningen University, and local advertisements. Abdominal obesity was defined as a BMI > 27 kg/m^2 or a waist circumference >88 cm (women) or >102 cm (men). Exclusion criteria were: a diagnosis of diabetes either before or during our screening (oral-glucose-tolerance test; fasting glucose > 7 mmol/L; glucose after 2 hours > 11.1 mmol/L), daily alcohol intake of >20 g (women) or >30g (men), smoking, an unstable body weight (weight gain or loss of >3 kg in the past 3 months), being diagnosed with a long-term medical condition, using medication known to interfere with glucose or lipid homeostasis, being allergic to fish oil, or being restricted to a vegetarian dietary regime. All subjects gave written informed consent. The study was powered to detect changes in intrahepatic lipid accumulation and was based on a study of Lecoultre et al. (17), with an observed difference of 9.5 mmol/kg, an SD of 15.8 mmol/kg, a power of 80%, and a 2-sided significance level of 0.05. Due to the lack of studies describing the effects of whole-diet approaches or a

combination of nutrients on intrahepatic lipids, we extrapolated information from single-nutrient studies on intrahepatic lipids, including studies on fructose and n-3 and n-6 PUFAs (17–19).

Fasting and postprandial measurements

On separate study days at both the start of the study and after the 12-week intervention, measurements on metabolic health were conducted before and during a mixed-meal shake challenge at Wageningen University, the Netherlands. Prior to this day, participants were asked to refrain from alcohol, avoid strenuous exercise, ensure a sufficient night's rest, and consume a low-fat standardized meal before 20:00, after which they had to fast. Fasting blood samples were drawn from the antecubital vein; subsequently, fasting vascular measurements were performed after 10 minutes of rest. Systolic blood pressure, diastolic blood pressure, and heart rate were assessed automatically (DINAMAP PRO100) for 10 minutes with a 3-minute interval. The heart rate–corrected augmentation index, as measure of arterial stiffness (20), was assessed by pulse wave analysis of the radial artery (SphygmoCorCP System, ATcor Medical) as described previously (21). Fasting subcutaneous adipose tissue biopsies were taken caudally from the umbilicus under local anesthesia (1% lidocaine) using a small liposuction cannula. The samples were rinsed with phosphate-buffered saline to eliminate blood and were immediately frozen in liquid nitrogen and stored at −80°C. After completing all fasting measurements, participants consumed a mixed-meal shake within 5 minutes. The mixed meal was a shake consisting of 320 g of water, 20 g of protifar, 83.5 g of dextrose, 60 g of palm olein, and 20 drops of vanilla oil. The mixed-meal shake was 3833 kJ and comprised 76.3 g of carbohydrates, 17.6 g of protein, and 60 g of fat (22). Blood drawings were conducted in the postprandial phase at 30, 60, 120, 180, 240, and 360 minutes, with the values at 120, 240, and 360 minutes used for metabolomics (Figure 1). Vascular measurements were performed at 120 and 240 minutes, and a second adipose tissue biopsy was performed at 240 minutes. Adipose tissue biopsies were taken by trained nurses on sagittal different locations at fasting when compared to 240 minutes to prevent an analysis of acute inflammation and wound healing mediators.

Randomization and masking

Randomization of the participants over the intervention groups was conducted using block randomization in Microsoft Excel by a researcher who was not involved in the study. Stratification among the groups was based on BMI, age, and gender.

Abdominal body fat distribution and intrahepatic lipids

At the start of the study and after 12 weeks of intervention, abdominal body fat distribution and intrahepatic lipids values were determined using a 3T whole-body MRI scanner (Siemens) at hospital Gelderse Vallei, Ede, the Netherlands. Intrahepatic lipid measurements were conducted as described previously (23). For adipose tissue mass measurements, axial T1-weighted spin echo images were acquired and slices were centered at the interspace of L3–L4. A breath-hold technique was applied to avoid breathing-induced artefacts. An analysis of the MRS spectra was performed using jMRUI software v5.2, while adipose tissue depots were quantified based on a single slice centered in the middle of 9 recorded images using the software program HippoFatTM (24).

Clinical chemistry and metabolomics

Clinical chemistry was performed on all samples ($n = 100$); in a small number of cases, samples from some time points were missing (low-nutrient-quality ER diet group, $n = 35$–39 samples; high-nutrient-quality ER diet group, $n = 30$–34 samples; control group, $n = 23$–27 samples). Glycated hemoglobin (HbA1c) was determined in whole-blood samples by hospital Gelderse Vallei, Ede, the Netherlands. Plasma glucose, triglycerides, and insulin, as well as serum total cholesterol, HDL cholesterol, and liver enzymes, were analyzed photometrically (Cobas 8000, Roche Diagnostic Limited) by a center for medical diagnostics (Stichting Huisartsenlaboratorium Oost). Plasma free fatty acids were determined using an enzymatic assay (INstruchemie). Metabolite profiling was performed in EDTA plasma samples using a high-throughput nuclear magnetic resonance metabolomics platform (Nightingale Health Ltd.) (25–27). The HOMA-IR was calculated by dividing the product of fasting glucose (mmol/L) and fasting insulin (mU/L) by 22.5. The quantitative insulin sensitivity check index (QUICKI) was calculated using the equation: 1/[log fasting insulin (mU/L) + log fasting glucose (mg/dL)].

Adipose tissue biopsy

Using adipose tissue biopsy samples, the adipocyte perimeter was determined in a subset of the study population (low-nutrient-quality ER diet group, $n = 22$, high-nutrient-quality ER diet group, $n = 9$; control group, $n = 10$). Of the 100 subjects, 72 subjects had good-quality whole-genome gene expression analyses of the adipose tissue: 27 in the low-quality ER diet group, 27 in the high-quality ER diet group, and 18 in the control group. RNA from frozen adipose tissue samples was isolated using an acid guanidinium thiocyanate-phenol-chloroform extraction (Trizol-chloroform extraction; Thermo Fisher Scientific) and purified using the Qiagen Mini column kit according to the manufacturers' protocol (Qiagen). The RNA yield was quantified on a NanoDrop ND 1000 spectrophotometer (NanoDrop Technologies), while the RNA integrity (RIN) was measured on an Agilent 2100 BioAnalyzer with RNA 6000 Nanochips (Agilent Technologies). Samples with RIN scores of 6.2 and higher were included; the average RIN score was 8.0. Total RNA was labeled using a 1-cycle cDNA labeling kit (MessageAmp II-Biotin Enhanced Kit; Ambion) and hybridized to Genechip Human Gene 2.1 ST arrays (Affymetrix). Sample labeling, hybridization to chips, and image scanning were performed according to the manufacturers' instructions. Raw CEL files were normalized using the Robust Multi-Array Average algorithm (28), as implemented in the affyPLM R package (29). We used a custom annotation based on reorganized oligonucleotide probes that combines all individual probes for a gene (MBNI Brainarray CDF file; ENTREZG v21).

Statistical analysis

The data analysis was executed on a per-protocol basis and was performed blinded. Model residuals were checked for normality, and nonnormally distributed data were transformed prior to the analyses. Changes in fasting parameters (week 12 compared to week 0) were analyzed using a general linear model for univariate analysis in which we included final measures as dependent variables, with baseline values as covariates (ANCOVA), after which least significant difference (LSD) post hoc testing was applied. Changes in postprandial responses to the mixed-meal shake test were analyzed using linear mixed effects modeling with the postprandial time points as repeated measures. The dependent variable in this model was the difference between the response of a certain parameter to the mixed-meal shake test (postprandial value minus fasting value) at each time point, before minus after the intervention. Linear mixed effects modeling was performed using an autoregressive covariance structure and LSD post hoc testing, and using SIDAK post hoc testing if more than 3 means were compared. All statistical analyses were conducted using IBM SPSS Statistics v.22 (IBM Corp), and a 2-tailed P value < 0.05 was considered significant.

For metabolite profiling, all variables were transformed (log2) and analyses were performed on the log2 ratios (difference between baseline value and value after intervention) of the metabolites. Metabolite concentrations that were below the detected threshold were labeled as 0 in the raw data file and subsequently removed from the data set. We performed paired t-tests to assess changes in fasting metabolite levels within each diet group, and an ANOVA with Hochberg's GT2 post hoc testing to assess changes in fasting metabolite levels between diet groups. The P values of the between-group comparisons were corrected for false positives using a false discovery rate (FDR) of 0.05 in the simultaneous analysis of all 153 metabolites. Postprandial metabolite changes were assessed by calculating the response: that is, the concentration of a metabolite in the postprandial phase minus its fasting value. Changes in responses were analyzed using linear mixed effects modeling with the postprandial time points as repeated measures. Linear mixed effects modeling was executed using an unstructured covariance structure and LSD post hoc testing. All analyses were conducted using IBM SPSS Statistics v.22 (IBM Corp), and FDR corrections were performed manually using Microsoft Excel. Visualization of the effects of the diets on lipoproteins levels was performed using Cytoscape v.3.4.0 (30).

For the adipose tissue microarray analysis, significant differences in expression were assessed using Linear Model for Microarray Analysis (31). For all comparisons, genes were defined as significantly different at a P value < 0.05. Analyses of changes in fasting gene expression were conducted by comparing the change (12 weeks − 0 weeks) in gene expression between the 3 different groups (F-test and t-tests), as well as by analyzing the change within an intervention group (paired t-test). Analyses of changes in postprandial gene expression were conducted by comparing the change (12 weeks − 0 weeks) in response (4 hours − 0 hours) between the 3 different groups (F-test and t-tests), as well as by analyzing the change in response within an intervention group (paired t-test). To take into account the paired design of our study, subjects were treated as random effects using the "duplicateCorrelation" function. For the pathways analysis, we performed a preranked gene set enrichment analysis (http://www.broad.mit.edu/gsea) (32). Briefly, genes were ranked based on the t-statistic and analyzed for positive and negative enrichment in predefined gene sets. Gene sets were derived from the Biocarta, KEGG, Reactome, and Wikipathways databases. Gene sets that were either more or less enriched at an FDR Q-value < 0.25 were considered to be statistically significant; these sets were subsequently visualized and clustered using the Enrichment Map (33) feature of Cytoscape v.3.4.0.

Ethical approval

The study was approved by the Medical Ethics Committee of Wageningen University and registered at clinicaltrials.gov as NCT02194504.All procedures were in accordance with the Helsinki Declaration of 1975, as revised in 1983.

Results

A high-nutrient-quality ER diet induced more weight loss and larger adaptations in adipose tissue energy metabolism than a low-nutrient-quality ER diet

The reported intakes resembled the advised composition for both diets, with significantly higher intakes of the key nutrients soy protein, fiber, MUFAs, and n-3 PUFAs ($P < 0.001$) and a significantly lower intake of fructose ($P < 0.001$) in the high- compared to the low-nutrient-quality ER diet (**Table 1**). After 12 weeks, weight loss was significantly changed between the diet groups ($P < 0.001$). Both ER diets induced significant weight loss, of 6.3 ± 3.9 kg in the low-nutrient-quality ER diet group ($P < 0.001$) and 8.4 ± 3.2 kg in the high-nutrient-quality ER diet group ($P < 0.001$), while no weight loss was observed in the control group (+0.8 ± 1.7; $P = 0.018$; **Figure 2**; **Table 2**). Post hoc analyses showed that weight loss was significantly higher (2.1 kg) in the high-quality ER diet group compared to the low-quality ER diet group ($P = 0.007$) and significantly higher in both ER diet groups compared to the control group (P values < 0.001 for both comparisons). In the low-nutrient-quality ER diet group, 75% ($n = 30$) of the participants lost over 5% of their body weight and 21% ($n = 8$) of the participants lost over 10%. In the high-nutrient-quality ER diet, these numbers were 85% ($n = 29$) and 38% ($n = 13$), respectively.

We observed that this progressive weight loss causes alterations in key biological pathways in adipose tissue. Whole transcriptome analyses in subcutaneous adipose tissue (SAT) samples showed that both diets significantly decreased the expression of genes involved in several pathways related to mitochondrial energy production, including oxidative phosphorylation, with a significantly larger reduction in the high-nutrient-quality ER diet when compared to the low-nutrient-quality ER diet (**Figure 3**A). Furthermore, both diets reduced the expression of genes involved in the synthesis of lipids and cholesterol (Figure 3B) and fatty acid β-oxidation. Again, the reductions were significantly larger in the high-nutrient-quality ER diet when compared to the low-nutrient-quality ER diet. PI3/Akt signaling was decreased only in the high-nutrient-quality ER diet group (Figure 3C).

As the high-nutrient-quality ER diet also induced more weight loss, we correlated diet-specific weight loss to the changes in

TABLE 1 Advised and reported dietary intake[1]

	Low-nutrient-quality ER diet		High-nutrient-quality ER diet		*P* value of differences in reported intake between diets
	Advised	Reported	Advised	Reported	
Energy intake, mega joules	6.95 ± 0.78	7.13 ± 0.82	6.99 ± 0.90	7.07 ± 1.00	0.778
ER, %	25.8 ± 5.4	23.9 ± 0.82	26.9 ± 5.6	26.2 ± 5.9	0.775
En% fat	31.5 ± 0.4	31.4 ± 1.6	32.1 ± 0.3	32.5 ± 1.2	0.001
En% SFA	15.5 ± 0.4	14.9 ± 1.5	7.9 ± 0.5	8.3 ± 0.6	<0.001
En% MUFA	9.0 ± 0.4	9.3 ± 1.0	13.9 ± 0.7	13.4 ± 0.9	<0.001
En% PUFA	3.9 ± 0.3	4.1 ± 0.8	7.8 ± 0.2	7.7 ± 0.4	<0.001
En% trans fat	0.4 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.015
ALA, mg	1057 ± 67	1104 ± 278	1795 ± 35	1815 ± 354	<0.001
EPA and DHA, mg	33 ± 5	103 ± 226	1270 ± 0	1224 ± 55	<0.001
Cholesterol, mg	183 ± 23	185 ± 30	124 ± 19	132 ± 20	<0.001
En% protein	15.4 ± 0.2	16.0 ± 1.4	20.4 ± 0.1	20.6 ± 1.7	<0.001
En% animal-based	9.9 ± 0.3	10.3 ± 1.1	9.8 ± 0.2	10.3 ± 1.9	0.886
En% plant-based	5.5 ± 0.1	5.7 ± 1.0	10.5 ± 0.1	10.3 ± 0.5	<0.001
En% carbohydrates	50.5 ± 0.2	49.6 ± 2.0	44.0 ± 0.3	43.7 ± 1.5	<0.001
En% mono- and disaccharides	25.3 ± 0.8	24.8 ± 1.9	19.0 ± 0.5	18.8 ± 1.1	<0.001
En% polysaccharides	25.2 ± 0.9	24.8 ± 1.2	25.0 ± 0.4	24.9 ± 1.1	0.905
En% fructose	7.4 ± 0.1	7.0 ± 0.9	3.5 ± 0.4	3.4 ± 0.4	<0.001
En% fiber	2.2 ± 0.1	2.2 ± 0.2	3.2 ± 0.0	3.1 ± 0.2	<0.001
En% alcohol	0.8 ± 0.3	1.1 ± 0.9	0.8 ± 0.3	1.0 ± 0.7	0.599
Sodium, g	1.6 ± 0.2	2.3 ± 0.6	1.7 ± 0.3	2.3 ± 0.4	0.659

[1]Data are presented as the mean ± 1 SD. Values were calculated based on the Dutch Food Composition Database 2011 (NEVO 2011/3.0, RIVM). Differences in reported intakes between diets were assessed using a paired *t*-test. ALA, α-linolenic acid; En%, energy percentage; ER, energy restriction.

gene expression of these pathways. Weight loss was correlated to 1 gene (*GCKR*, −0.550; $P = 0.003$) in the high-nutrient-quality ER diet and 3 genes [*NDUFA3*, 0.575 ($P = 0.002$); *CYCS*, −0.493 ($P = 0.009$); *ATP6V1A*, −0.493 ($P = 0.009$)] in the low-nutrient-quality ER diet, without FDR correction. Concurrent with the weight loss and gene expression changes, the adipocyte perimeter showed a trend towards a more reduced size in the high-nutrient-quality ER diet group when compared to the low-nutrient-quality ER diet group (group comparison, $P = 0.034$; post hoc analyses: high-nutrient-quality ER diet group compared with control group, $P = 0.010$; high- compared with low-nutrient-quality ER diet groups, $P = 0.098$), as assessed in a subset (low-nutrient-quality ER diet group, $n = 22$; high-nutrient-quality ER diet group, $n = 9$; control group, $n = 10$) of the study population.

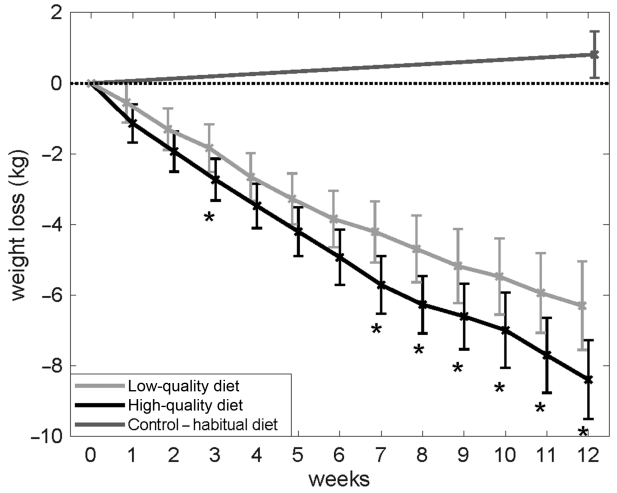


FIGURE 2 Cumulative weight loss in the 3 study groups (high-quality ER diet group, $n = 34$; low-quality ER diet group, $n = 39$; control group, $n = 27$) over 12-weeks of the intervention. The error bars represent 1 SD. *Significant difference in cumulative weight loss between the 2 ER groups at a *P* value <0.05. ER, energy restriction.

Only in insulin-sensitive persons, a high-nutrient-quality ER diet induces more weight loss than a low-nutrient-quality ER diet

Whole-genome gene expression was also assessed 4 hours postprandially before and after the intervention. We observed that the expression of lipogenic genes in adipose tissue was highly induced postprandially before the intervention when analyzing the total population (**Supplemental Figure 3**). Interestingly, the postprandial (240 minutes) increases in the expression of genes involved in cholesterol biosynthesis, the tricarboxylic acid cycle, and lipid metabolism pathways before the intervention were significantly attenuated after consumption of the low-nutrient-quality ER diet (**Supplemental Figure 2**). The high-nutrient-quality ER diet induced significant attenuation of the increase in cholesterol biosynthesis.

We further explored whether meal-induced insulin might be responsible for the postprandial increase in lipogenic gene expression and the attenuated increase after the ER interventions. We used baseline data of the total population to correlate the postprandial changes in insulin and glucose with the postprandial changes in lipogenesis-related gene expression (**Figure 4**). To

TABLE 2 Baseline characteristics and changes in body composition, plasma markers, and vascular measurements upon intervention[1]

	Baseline	Change	Within-group *P* value	Between-group *P* value
Baseline characteristics				
Age, years, median (range)				
Low-nutrient-quality ER diet	60 (28)	—		
High-nutrient-quality ER diet	61 (28)	—		
Control group	60 (27)	—		
Gender, n males, %				
Low-nutrient-quality ER diet	13, 42%	—		
High-nutrient-quality ER diet	13, 45%	—		
Control group	9, 43%	—		
Body composition				
Body weight, kg				
Low-nutrient-quality ER diet	93.1 ± 13.9	−6.3 ± 3.9[a]	<0.001	<0.001
High-nutrient-quality ER diet	94.8 ± 13.2	−8.4 ± 3.2[b]	<0.001	
Control group	92.1 ± 12.6	0.8 ± 1.7[c]	0.018	
BMI, kg/m^2				
Low-nutrient-quality ER diet	31.2 ± 3.3	−2.1 ± 1.3[a]	<0.001	<0.001
High-nutrient-quality ER diet	31.8 ± 3.4	−2.8 ± 1.1[b]	<0.001	
Control group	30.9 ± 3.9	0.3 ± 0.6[c]	0.015	
Waist circumference, cm				
Low-nutrient-quality ER diet	108.7 ± 9.8	−3.1 ± 5.3[a]	<0.001	<0.001
High-nutrient-quality ER diet	109.5 ± 9.8	−4.7 ± 5.7[a]	<0.001	
Control group	106.6 ± 8.1	4.1 ± 5.2[b]	<0.001	
SAT,[2,3] cm^2				
Low-nutrient-quality ER diet	332.5 ± 92.4	−45.3 ± 32.7[a]	<0.001	<0.001
High-nutrient-quality ER diet	348.0 ± 106.5	−54.1 ± 55.3[a]	<0.001	
Control group	355.6 ± 118.8	1.6 ± 36.9[b]	0.628	
VAT,[2,3] cm^2				
Low-nutrient-quality ER diet	149.1 ± 77.0	−44.1 ± 48.2[a]	<0.001	<0.001
High-nutrient-quality ER diet	175.7 ± 94.2	−61.5 ± 51.3[a]	<0.001	
Control group	115.8 ± 26.1	3.7 ± 17.9[b]	0.551	
VAT/SAT ratio[2,3]				
Low-nutrient-quality ER diet	50.6 ± 34.8	−9.4 ± 16.9[a]	0.001	0.044
High-nutrient-quality ER diet	61.2 ± 54.9	−16.0 ± 28.1[a]	0.001	
Control group	36.2 ± 16.0	0.8 ± 6.3[b]	0.775	
Adipocyte size,[2] μm				
Low-nutrient-quality ER diet	2564 ± 551	−164 ± 458	0.051	0.034
High-nutrient-quality ER diet	2548 ± 367	−508 ± 418[a]	0.008	
Control group	2753 ± 514	137 ± 714[b]	0.761	
Intrahepatic lipids,[2,3] % of water peak				
Low-nutrient-quality ER diet	7.3 ± 6.5	−3.4 ± 5.1[a]	<0.001	<0.001
High-nutrient-quality ER diet	7.1 ± 8.7	−3.9 ± 5.7[a]	0.001	
Control group	5.9 ± 5.8	1.4 ± 1.9[b]	0.021	
Plasma markers				
Plasma glucose,[3] mmol/L				
Low-nutrient-quality ER diet	5.6 ± 0.8	−0.3 ± 0.6[a]	0.003	0.013
High-nutrient-quality ER diet	5.7 ± 0.5	−0.3 ± 0.4[a]	<0.001	
Control group	5.6 ± 0.4	0.0 ± 0.4[b]	0.585	
Plasma insulin,[3] mU/L				
Low-nutrient-quality ER diet	14.6 ± 10.3	−3.9 ± 7.5[a]	0.002	0.020
High-nutrient-quality ER diet	13.5 ± 7.8	−3.6 ± 5.3[a]	<0.001	
Control group	12.1 ± 5.3	0.3 ± 6.1[b]	0.782	
HbA1c,[3] mmol/mol				
Low-nutrient-quality ER diet	36.3 ± 2.1	−0.8 ± 2.0[a]	0.013	0.021
High-nutrient-quality ER diet	37.3 ± 2.9	−1.5 ± 2.0[a]	<0.001	
Control group	35.1 ± 2.6	0.4 ± 1.3[b]	0.146	
HOMA-IR				
Low-nutrient-quality ER diet	3.8 ± 3.1	−1.3 ± 2.3[a]	0.001	0.006
High-nutrient-quality ER diet	3.5 ± 2.1	−1.0 ± 1.5[a]	<0.001	
Control group	3.0 ± 1.4	0.1 ± 1.5[b]	0.824	
QUICKI				
Low-nutrient-quality ER diet	0.33 ± 0.04	0.01 ± 0.03[a,b]	0.022	0.014
High-nutrient-quality ER diet	0.33 ± 0.03	0.02 ± 0.03[a]	<0.001	
Control group	0.33 ± 0.02	0.00 ± 0.02[b]	0.845	

(*Continued*)

TABLE 2 (*Continued*)

	Baseline	Change	Within-group *P* value	Between-group *P* value
Serum total cholesterol, mmol/L				
Low-nutrient-quality ER diet	5.7 ± 1.1	−0.2 ± 0.8[a]	0.218	0.013
High-nutrient-quality ER diet	5.5 ± 0.8	−0.5 ± 0.6[b]	<0.001	
Control group	5.4 ± 1.0	0.0 ± 0.5[a]	0.633	
Serum HDL cholesterol, mmol/L				
Low-nutrient-quality ER diet	1.3 ± 0.4	0.0 ± 0.2	0.096	0.878
High-nutrient-quality ER diet	1.4 ± 0.4	−0.1 ± 0.2	0.084	
Control group	1.4 ± 0.4	0.0 ± 0.3	0.439	
Plasma triglycerides,[2,3] mmol/L				
Low-nutrient-quality ER diet	1.8 ± 0.8	−0.2 ± 0.6[a]	0.018	0.001
High-nutrient-quality ER diet	1.6 ± 0.6	−0.4 ± 0.6[b]	0.001	
Control group	1.7 ± 0.7	−0.1 ± 0.5[a]	0.280	
Plasma-free fatty acids, mmol/L				
Low-nutrient-quality ER diet	0.41 ± 0.21	−0.02 ± 0.20	0.522	0.517
High-nutrient-quality ER diet	0.57 ± 0.37	−0.04 ± 0.49	0.471	
Control group	0.42 ± 0.18	0.05 ± 0.24	0.291	
Serum ALT,[2,3] U/L				
Low-nutrient-quality ER diet	26.4 ± 10.1	−2.2 ± 9.8	0.164	0.232
High-nutrient-quality ER diet	27.6 ± 11.2	−3.6 ± 11.3	0.071	
Control group	27.2 ± 10.9	0.0 ± 6.4	1.000	
Serum AST,[2,3] U/L				
Low-nutrient-quality ER diet	24.1 ± 13.3	−2.4 ± 14.1	0.302	0.210
High-nutrient-quality ER diet	22.9 ± 6.1	−1.0 ± 5.5	0.282	
Control group	27.2 ± 13.8	−0.4 ± 7.4	0.778	
Serum GGT,[3] U/L				
Low-nutrient-quality ER diet	31.2 ± 23.6	−9.2 ± 15.7[a]	0.001	<0.001
High-nutrient-quality ER diet	25.0 ± 9.8	−7.3 ± 7.4[a]	<0.001	
Control group	27.4 ± 18.8	−0.2 ± 3.4[b]	0.781	
Vascular measurements				
Systolic blood pressure,[3] mmHg				
Low-nutrient-quality ER diet	125 ± 19	−6 ± 13[a]	0.005	<0.001
High-nutrient-quality ER diet	131 ± 15	−12 ± 13[a]	<0.001	
Control group	127 ± 15	1 ± 9[b]	0.674	
Diastolic blood pressure,[3] mmHg				
Low-nutrient-quality ER diet	71 ± 8	−3 ± 7[a]	0.018	<0.001
High-nutrient-quality ER diet	76 ± 9	−5 ± 7[a]	<0.001	
Control group	75 ± 9	2 ± 5[b]	0.076	
Heart rate, BPM				
Low-nutrient-quality ER diet	59 ± 10	−2 ± 10	0.158	0.125
High-nutrient-quality ER diet	61 ± 7	−4 ± 7	<0.001	
Control group	59 ± 10	1 ± 9	0.733	
Augmentation index,[3] %				
Low-nutrient-quality ER diet	23.5 ± 8.9	0.5 ± 3.1	0.413	0.455
High-nutrient-quality ER diet	25.4 ± 7.9	−1.1 ± 3.1	0.060	
Control group	22.2 ± 6.8	−0.8 ± 4.7	0.372	
Central systolic pressure,[3] mmHg				
Low-nutrient-quality ER diet	122 ± 19	−5 ± 14	0.074	0.418
High-nutrient-quality ER diet	121 ± 15	−8 ± 9	<0.001	
Control group	116 ± 15	−4 ± 13	0.082	

[1]Data are presented as means ± 1 SD. Different letters (a, b, c) indicate significant post hoc differences ($P < 0.05$) between diet groups. Changes within groups were analyzed using paired *t*-tests, and differences in changes between groups were analyzed using a general linear model for univariate analysis with baseline values as covariates (ANCOVA) with least significant difference (LSD) post hoc testing. The addition of age, BMI, and gender as a covariate to the model did not change the results. A Benjamin-Hochberg correction was applied on all primary outcome measures. For SAT and VAT: Western-type diet group, $n = 28$; high-quality diet group, $n = 26$; and control group, $n = 16$. For IHL: Western-type diet group, $n = 34$; high-quality diet group, $n = 29$; and control group, $n = 19$. For adipocyte size: Western-type diet group, $n = 22$; high-quality diet group, $n = 9$; and control group, $n = 10$. For measures from pulse wave analysis (augmentation index and central systolic blood pressure): Western-type diet group, $n = 37$; high-quality diet group, $n = 32$; and control group, $n = 26$. For all other values: Western-type diet group, $n = 39$; high-quality diet group, $n = 34$; and control group $n = 27$. ALT, alanine transaminase; AST, aspartate transaminase; BPM, beats per minute; ER, energy restriction; GGT, gamma-glutamyltransferase; HbA1c, glycated hemoglobin; IHL, intrahepatic lipid; QUICKI, quantitative insulin sensitivity check index; SAT, subcutaneous adipose tissue mass; VAT, visceral adipose tissue mass.

[2]Values are log2 transformed for statistical analyses to improve normality.

[3]Primary outcome measures.

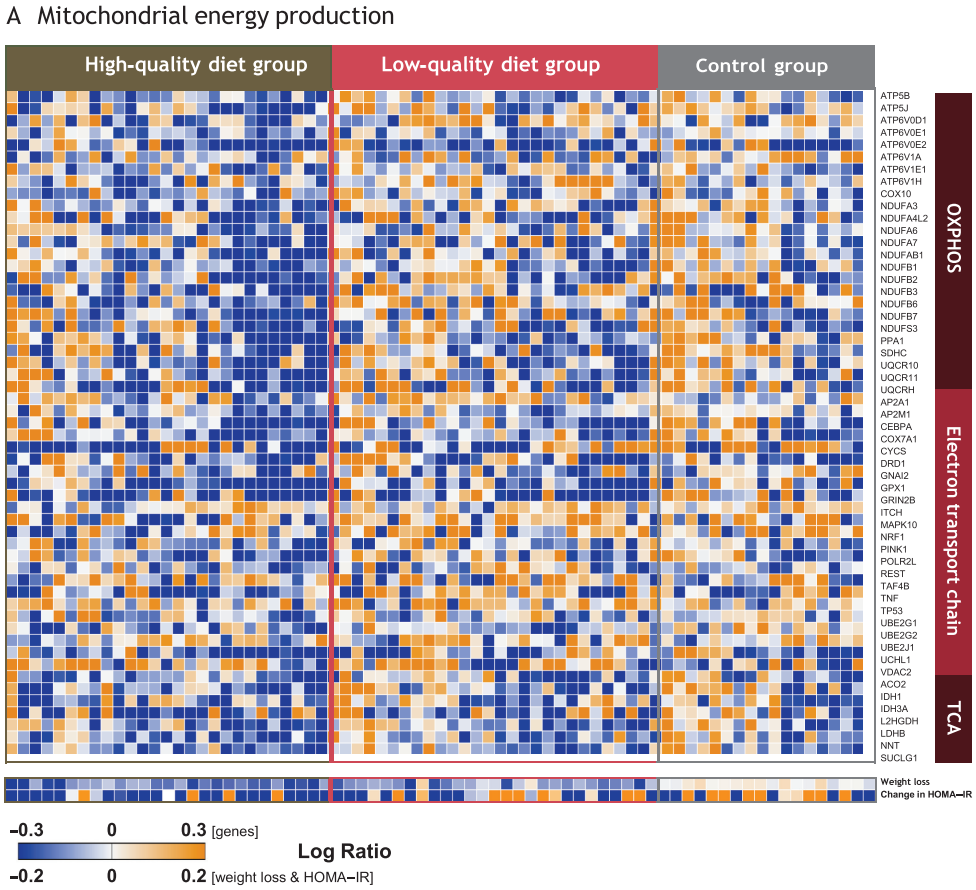


FIGURE 3 Continue.

take into account that plasma insulin- and glucose-induced transcription may depend on the insulin sensitivity state of the tissue, we divided the total study population into insulin-sensitive ($n = 45$) and insulin-resistant ($n = 55$) subjects based on HOMA-IR values ≤ 2.5 and > 2.5. A clear difference in correlation was observed between groups, with significant positive correlations for postprandial glucose and, to a lesser extent, for insulin with expression changes of genes involved in lipogenesis in individuals with a HOMA-IR below 2.5, whereas no significant correlations were observed in individuals with a HOMA-IR above 2.5 (Figure 4).

If a causal relation is present, this may indicate that a reduction in postprandial blood glucose would reduce fat synthesis in the adipose tissue of insulin-sensitive subjects. Based on the content of the intervention diets, one might expect a lower overall glucose response during the intervention in those on the high-nutrient-quality ER diet compared to the low-nutrient-quality ER diet. To test this hypothesis, we performed an explorative analysis to examine whether insulin-sensitive subjects (HOMA-IR $\leq$ 2.5) lost more weight than insulin-resistant subjects (HOMA-IR $>$ 2.5) on the high-nutrient-quality compared to the low-nutrient-quality ER diet. Indeed, linear mixed-model analyses including the control group showed that the diets had significantly different effects on weight loss in insulin-sensitive and insulin-resistant subjects ($P = 0.010$ for the interaction between diet, HOMA-IR, and week). Insulin-sensitive subjects showed significantly ($P < 0.007$) greater weight loss on the high-nutrient-quality ER diet (-9.8 kg $\pm$ 3.2) compared to the low-nutrient-quality ER diet (-5.9 kg $\pm$ 4.0), whereas insulin-resistant subjects lost similar ($P = 0.37$) amounts of weight on both diets: 7.4 kg $\pm$ 4.1 and 8.3 kg $\pm$ 3.6 for the low- and high-nutrient-quality ER diets, respectively (**Figure 5**). Comparable findings were observed when weight loss was calculated as percentage weight loss, with a P value of 0.017 for the interaction between diet, HOMA-IR, and weeks of intervention and a significant greater percentage weight reduction in those on the high-nutrient-quality ER diet compared to the low-nutrient-quality ER diet ($P < 0.015$).

A high-nutrient-quality ER diet has a beneficial effect on fasting blood lipids and lipoproteins, while a low-nutrient-quality ER diet does not

In addition to adipose tissue gene expression, the effects of the intervention on plasma triglycerides and total cholesterol and on lipid profiles both fasting and postprandially were determined. All effects were compared to the control group. After 12 weeks, both plasma triglycerides and total cholesterol values were significantly changed between all diet groups ($P = 0.001$; $P = 0.013$). The high-nutrient-quality ER diet resulted in significantly greater reductions in fasting plasma triglycerides ($P < 0.001$) and serum total cholesterol ($P = 0.014$)

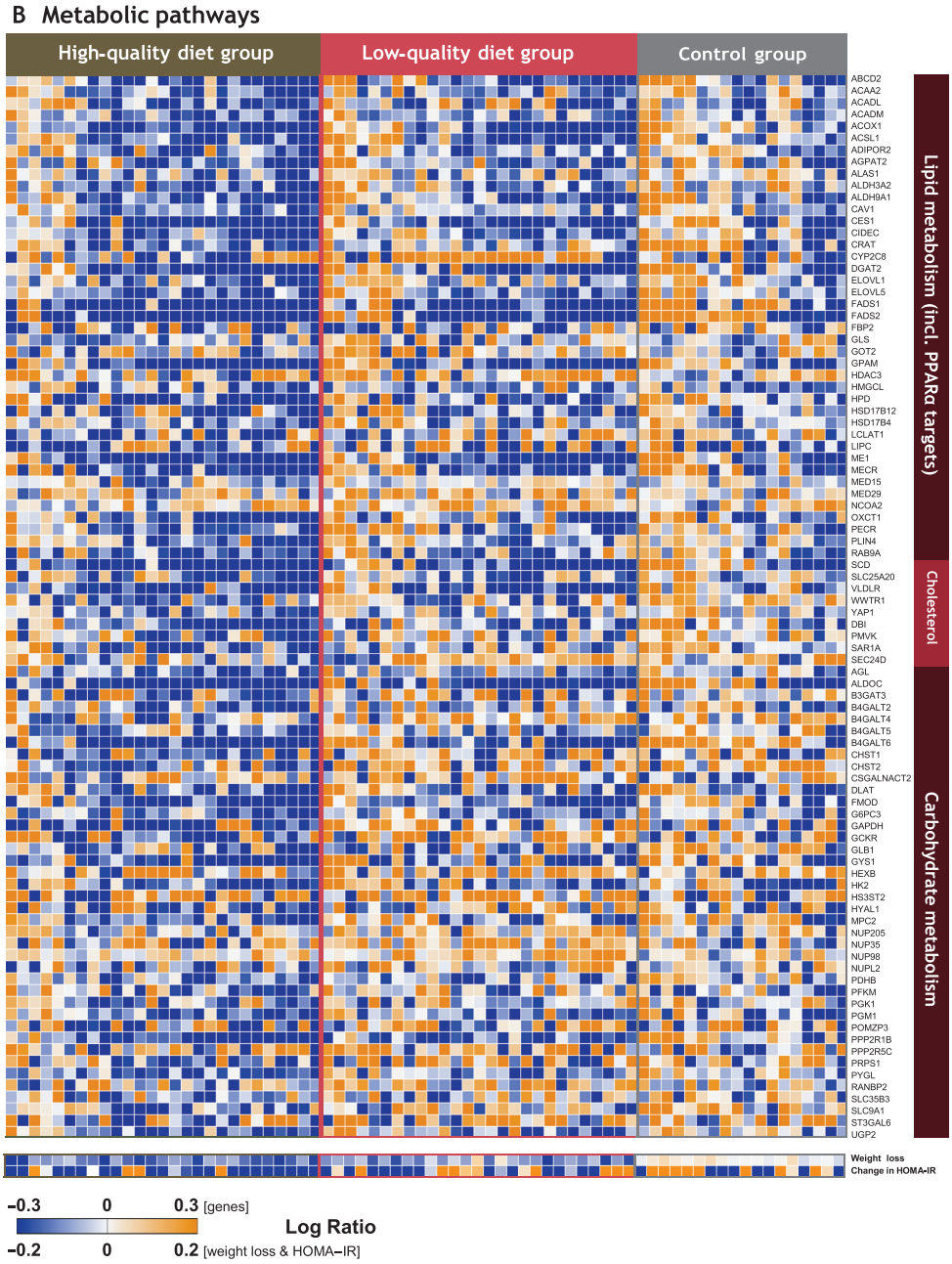


FIGURE 3 Continue.

compared to the low-nutrient-quality ER diet (Table 2). In line with the findings on triglycerides and cholesterol, only the high-nutrient-quality ER diet induced significant changes in fasting lipoproteins (**Figure 6**A; **Supplemental Table 3**). The high-nutrient-quality ER diet significantly decreased fasting concentrations and constituents of VLDL particles, ranging from extremely large (>75.0 nm) to very large (64.0 nm), large (53.6 nm), and medium (44.5 nm). In addition, the high-nutrient-quality ER diet significantly decreased medium (10.9 nm) and small (8.7 nm) HDL particles and increased larger (12.1–14.3 nm) HDL particles, although this latter increase was not significant compared to values from the low-nutrient-quality ER diet or control groups.

The high-nutrient-quality ER diet also significantly decreased the free cholesterol contents of medium (23.0 nm) and small (18.7 nm) LDL particles. The low-nutrient-quality ER diet did not change the fasting blood lipid profile (Figure 6B). For the postprandial analyses, we observed no significant difference in the overall change in postprandial response between the diet groups. Within the groups, both ER diets reduced postprandial levels of IDL and LDL, including apoB, and attenuated the increase of VLDL particles, but the effects were more pronounced in the high-nutrient-quality ER diet (**Supplemental Figure 4**A and B; **Supplemental Table 4**). No effect on either the fasting or postprandial response was observed in the control group (Supplemental Figure 4C).

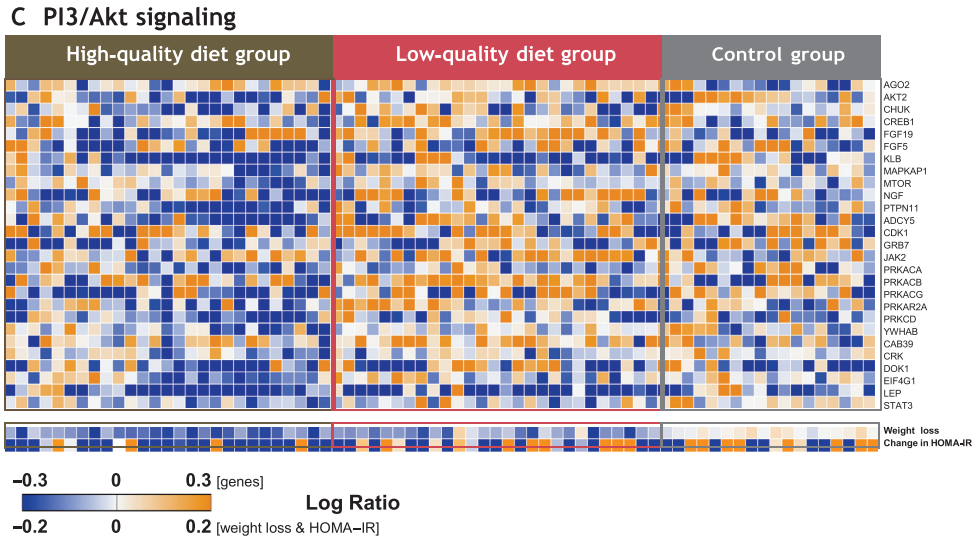


FIGURE 3 Heat maps of individual changes in genes from differentially enriched pathways between the high-nutrient-quality ($n = 27$) and low-nutrient-quality ($n = 27$) ER diets. Individual changes in gene expression, clustered per diet (habitual diet, $n = 18$), of genes contributing to differential enrichment in the 3 clusters between the high-nutrient-quality and low-nutrient-quality ER diets. Genes related to (A) mitochondrial energy production, (B) metabolic pathways, and (C) PI3/Akt signaling. ER, energy restriction; OXPHOS, oxidative phosphorylation; PPAR, peroxisome proliferator–activated receptor; TCA, tricarboxylic acid cycle.

Both 25% ER diets similarly decrease body fat, liver fat, and blood pressure and improve insulin sensitivity

After 12 weeks of the intervention, the SAT mass ($P < 0.001$), visceral adipose tissue (VAT) mass ($P < 0.001$), and VAT/SAT ratio ($P = 0.044$) were significantly changed between the diet groups (Table 2). Weight loss in both diet groups was accompanied by reductions in SAT (high-quality diet: $P < 0.001$; low-quality diet: $P < 0.001$) and VAT (high-quality diet: $P < 0.001$; low-quality diet: $P < 0.001$) masses in the abdominal region compared to the control group, without significant differences between the ER groups and within the control group. The VAT/SAT ratio was significantly reduced in the high-quality diet group compared to the control group ($P = 0.009$), and was not significantly reduced in the low-quality diet group compared to the control group ($P = 0.069$), without significant differences between the ER diets and within the control group. Intrahepatic lipids were also significantly changed between the diet groups ($P < 0.001$) and were strongly reduced in both weight loss groups compared to the control group (high-quality diet, $P < 0.001$; low-quality diet, $P < 0.001$). On average, intrahepatic lipids were reduced by 55% in the high-nutrient-quality ER diet ($P = 0.001$) and 46% in the low-nutrient-quality ER diet ($P < 0.001$). No

	HOMA–IR ≤ 2.5											
Gene expression	**Plasma Insulin (mU/L) *n* = 29**					**Plasma glucose (mmol/L) *n* = 25**						
240 –0 min	**iAUC**	**Baseline**	**60–0 min**	**120–0 min**	**240–0min**	**iAUC**	**Baseline**	**30–0 min**	**60–0 min**	**120–0 min**	**180–0 min**	**240–0 min**
LDLR	***0.547***	−0.084	**0.411**	***0.562***	−0.030	***0.605***	0.056	***0.623***	***0.590***	**0.448**	−0.208	−0.357
INSIG1	**0.377**	−0.072	0.304	**0.370**	−0.056	***0.564***	0.337	***0.641***	***0.539***	0.367	−0.345	**−0.480**
LPIN1	0.343	−0.079	0.140	**0.456**	0.082	**0.435**	0.087	**0.489**	0.355	**0.444**	0.070	−0.292
HMGCS1	0.352	−0.032	0.328	0.298	0.000	**0.465**	0.341	**0.413**	**0.483**	0.304	−0.328	−0.361
SREBF1	0.197	−0.007	0.156	0.194	0.004	***0.535***	0.311	***0.597***	***0.538***	0.244	−0.261	***−0.606***
ANGPTL8	0.286	−0.129	0.182	0.312	0.145	0.388	0.219	**0.448**	0.351	0.292	−0.245	**−0.411**
ACLY	0.230	−0.075	0.283	0.137	−0.040	0.236	0.279	***0.508***	0.327	−0.004	−0.330	−0.376
THRSP	0.29	−0.104	0.181	0.322	0.087	0.574	0.291	0.549	0.557	0.368	−0.264	−0.516
	HOMA–IR > 2.5											
Gene expression	**Plasma Insulin (mU/L) *n* = 42**					**Plasma glucose (mmol/L) *n* = 39**						
240 –0 min	**iAUC**	**Baseline**	**60–0 min**	**120–0 min**	**240–0min**	**iAUC**	**Baseline**	**30–0 min**	**60–0 min**	**120–0 min**	**180–0 min**	**240–0 min**
LDLR	0.011	−0.121	0.101	−0.049	0.032	0.121	**−0.363**	0.053	0.085	0.111	0.300	−0.007
INSIG1	−0.180	−0.300	0.001	−0.271	−0.127	−0.050	−0.302	−0.011	−0.069	−0.071	0.237	0.002
LPIN1	0.098	0.042	0.201	0.009	0.026	0.152	0.042	0.203	0.296	−0.067	−0.089	−0.100
HMGCS1	−0.07	−0.182	0.088	−0.167	0.010	−0.022	−0.309	0.050	−0.065	−0.063	0.288	0.076
SREBF1	−0.024	−0.044	0.086	−0.113	0.095	−0.067	**−0.362**	0.108	−0.067	−0.154	0.109	0.301
ANGPTL8	−0.172	−0.134	−0.063	−0.215	−0.164	−0.031	***−0.420***	−0.031	−0.112	0.001	**0.323**	0.208
ACLY	−0.010	−0.081	0.149	−0.118	−0.084	−0.048	−0.254	0.022	−0.046	−0.176	0.181	0.118
THRSP	−0.037	−0.021	0.070	−0.102	−0.284	−0.021	**−0.348**	0.063	−0.003	−0.131	0.031	0.116

FIGURE 4 Pearson correlation coefficients between incremental area under the curve (iAUC) and changes in postprandial time points of plasma glucose (HOMA-IR ≤ 2.5: $n = 25$; HOMA-IR > 2.5: $n = 39$) and insulin (HOMA-IR ≤ 2.5: $n = 29$; HOMA-IR > 2.5: $n = 42$) after the mixed-meal shake test with postprandial changes in expression of genes involved in lipid synthesis in adipose tissue after 240 minutes. Red indicates a positive correlation and green indicates a negative correlation. Bold numbers indicate a significant Pearson correlation at a P value <0.05.

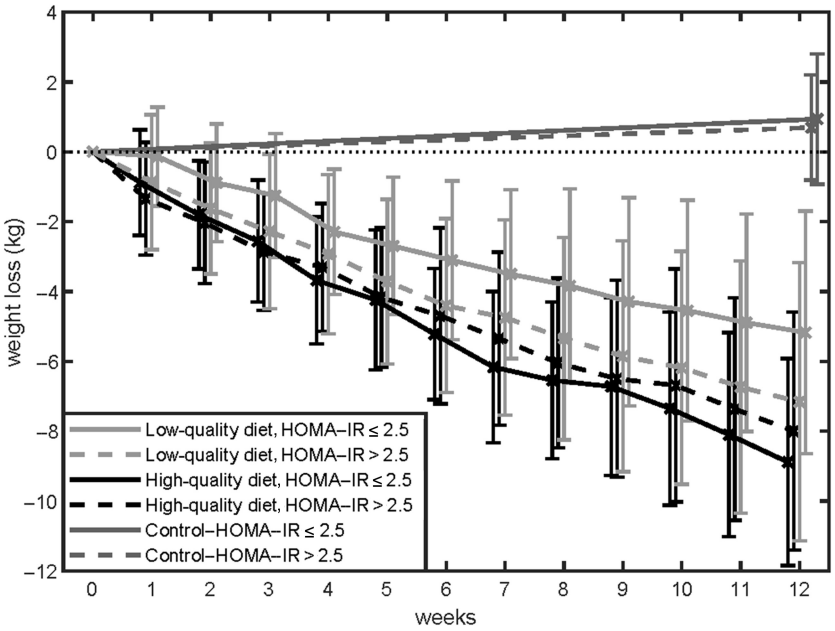


FIGURE 5 Cumulative weight loss in the 3 study groups over 12 weeks of intervention for insulin-sensitive (HOMA-IR $\leq$ 2.5) and insulin-resistant (HOMA-IR $>$ 2.5) subjects. High-nutrient-quality ER diet: $n = 15$ insulin-sensitive and 19 insulin-resistant subjects; low-nutrient-quality ER diet: $n = 17$ insulin-sensitive and 22 insulin-resistant subjects; and control diet: $n = 13$ insulin-sensitive and 14 insulin-resistant subjects. Error bars represent 1 SD. The linear mixed model P value for the interaction of Diet∗HOMA-IR∗week is 0.010. Least significant difference (LSD) post hoc analyses showed significantly ($P < 0.007$) greater weight loss on the high-nutrient-quality ER diet compared to the low-nutrient-quality ER diet within insulin-sensitive subjects. ER, energy restriction.

differences in intrahepatic lipids were observed between the ER diet groups ($P = 0.129$), although an increase in intrahepatic lipids was observed in the control group ($P = 0.021$). Fasting plasma glucose and insulin values were significantly changed between the diets groups ($P = 0.013$ and $P = 0.020$, respectively). Weight loss in both ER diet groups resulted in lower fasting plasma glucose (high-quality diet, $P = 0.018$; low-quality diet, $P = 0.002$) and insulin (high-quality diet, $P = 0.005$; low-quality diet, $P = 0.018$) values compared to the control group, with no differences between the ER diet groups. No effect was observed in the control group for plasma glucose and insulin.

HOMA-IR and QUICKI values were significantly changed between the diets groups ($P = 0.006$ and $P = 0.014$, respectively). Weight loss in both ER diet groups resulted in improved HOMA-IR values (high-quality diet, $P = 0.004$; low-quality diet, $P = 0.004$), and the high-quality diet improved QUICKI values (high-quality diet, $P = 0.004$; low-quality diet, $P = 0.084$) compared to the habitual diet, but not compared to the low-quality diet. No effect was observed in the control group for HOMA-IR and QUICKI values (Table 2). Furthermore, long-term glycemic control, as reflected by a lower HbA1c, was significantly changed between the diet groups ($P = 0.021$), with improvements after both ER interventions (low-quality diet, $P = 0.036$; high-quality diet, $P = 0.004$) compared to the control group. The HbA1c levels were not different between the two ER diets or within the control group.

Postprandially significant differences in responses between diet groups were only observed for the plasma triacylglycerol (TAG) levels ($P = 0.019$) and heart rates ($P = 0.011$; **Supplemental Table 5**). For plasma TAG levels, differences were significant at the 240-minute time point between the high-quality and control groups and at the 360-minute time point between the high- and low-quality diet groups. Within the diet groups, no significant changes were observed. For heart rate, no other significant differences were observed. Both ER diets lowered postprandial glucose levels within the diet group (30, 60, 120, 180, and 240 minutes) and caused a trend towards lower postprandial insulin levels within the group (60, 120, and 240 minutes) after the mixed-meal shake (76.3 g of carbohydrates, 17.6 g of protein, 60.0 g of fat), while no differences were observed in the control group (**Supplemental Figure 5;** Supplemental Table 5). Both ER interventions also significantly reduced fasting branchial systolic and diastolic blood pressure measurements when compared to the control group. Changes in the outcomes of the pulse wave analysis, augmentation index, and central systolic blood pressure measurements did not differ between the 3 groups (Table 2). In addition, no changes in postprandial vascular measurements (120 and 240 minutes) were observed from the intervention (Supplemental Table 5).

Discussion

In this study, we examined the potential additional effects of high-quality nutrients, such as soy protein, fiber, MUFAs, n-3 PUFAs, and reduced fructose, within an ER diet on cardiometabolic risk factors in overweight subjects and explored the underlying mechanisms by comparing the effects of this healthy diet with the effects of a low-nutrient-quality ER diet and a habitual diet (control group). A high-nutrient-quality ER diet induced 2.1 kg more weight loss, reduced fasting cholesterol and triglycerides, promoted an antiatherogenic lipoprotein profile, and induced more pronounced changes in adipose tissue gene

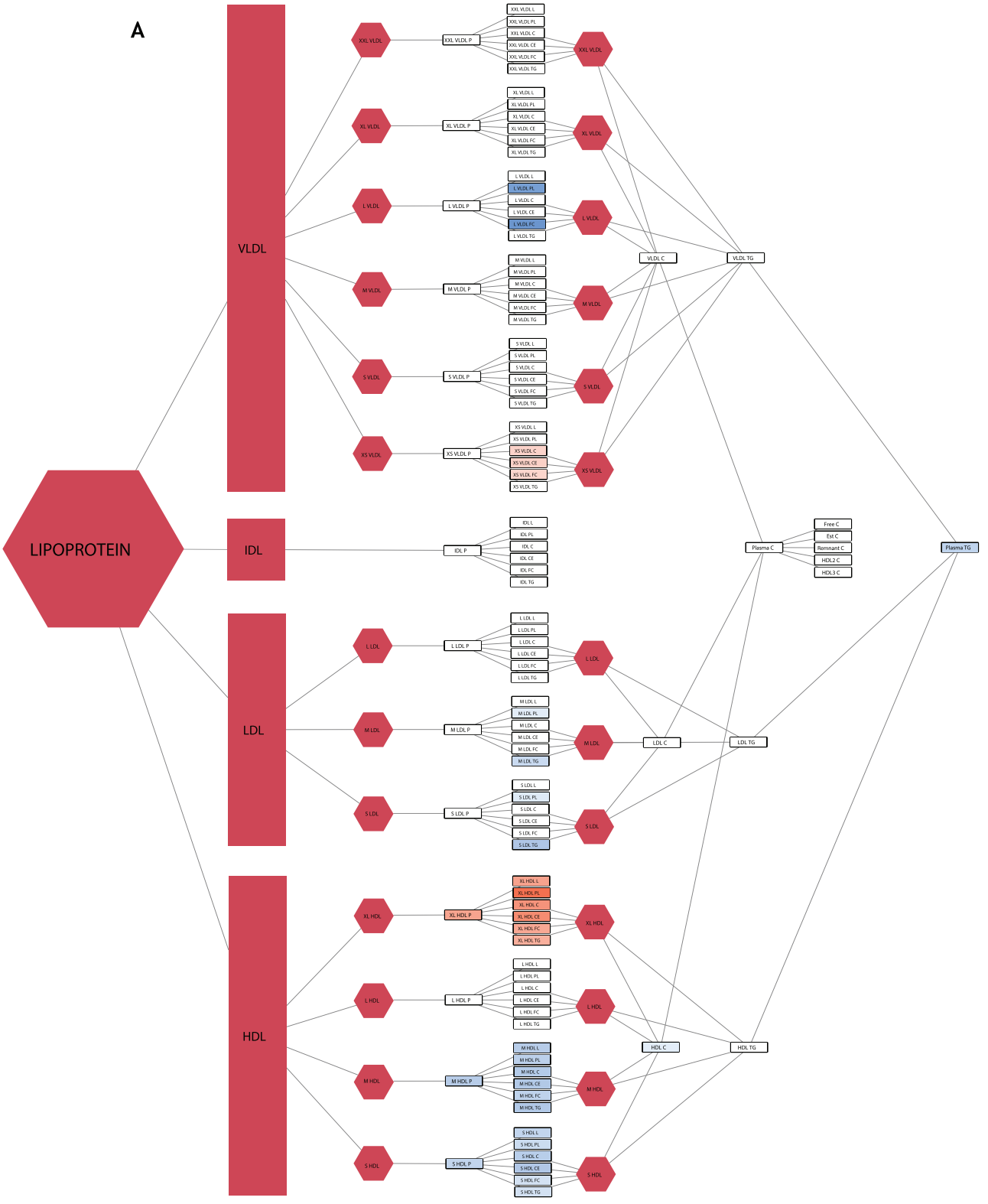


FIGURE 6 Continue.

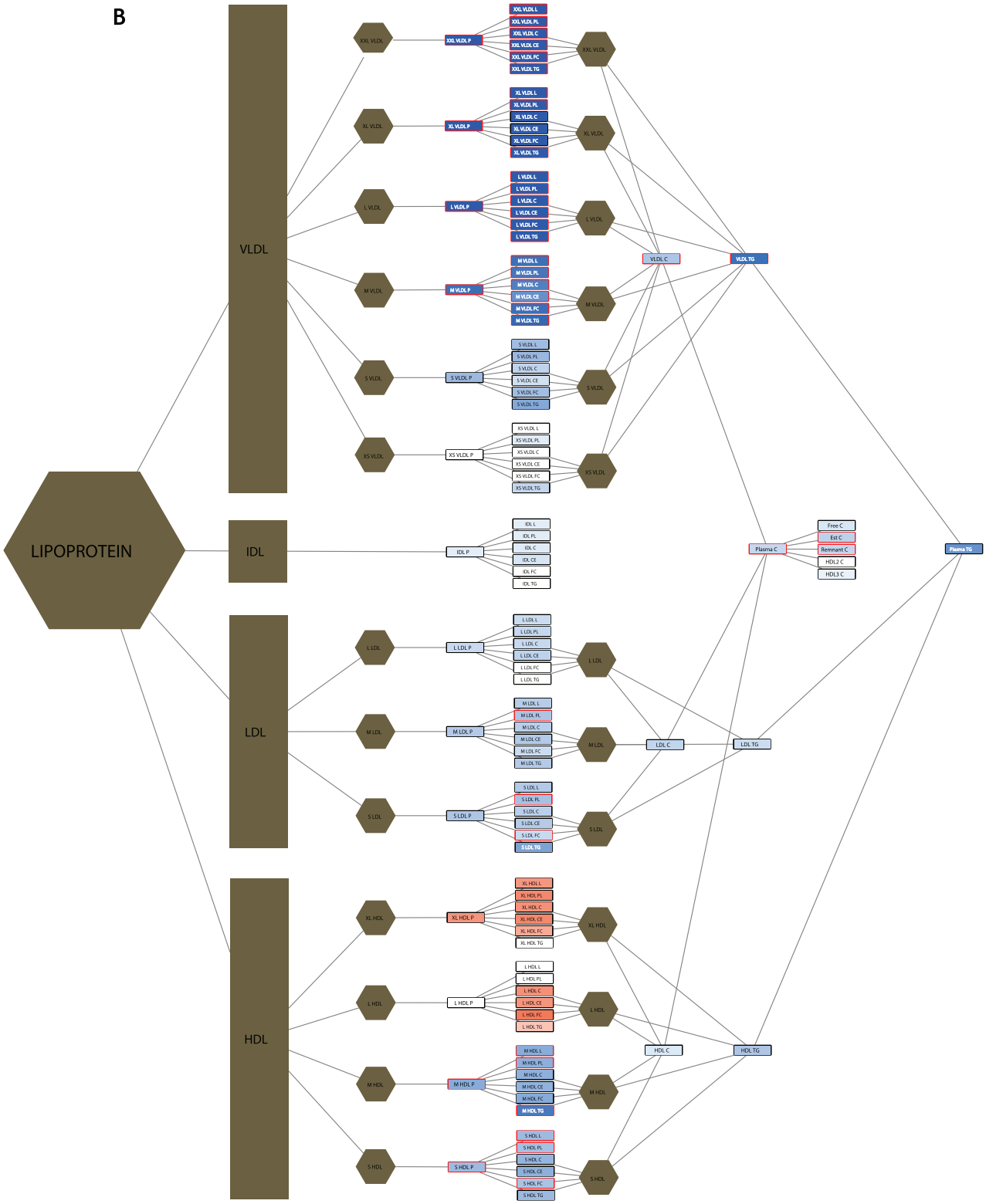


FIGURE 6 Changes in fasting lipoprotein profiles compared to the control group ($n = 27$). Colored boxes indicate significantly changed (log2 ratio) particles within an ER group—(A) the low-nutrient-quality ER diet ($n = 39$) or (B) high-nutrient-quality ER diet ($n = 34$)—as assessed with a paired *t*-test, where the red color indicates an increased level and the blue color indicates a decreased level. The color intensity is related to the size of the effect, where a darker color indicates a larger change. The red outline on the boxes indicates a significant difference between an ER group (low-nutrient-quality or high-nutrient-quality groups) and the control, as analyzed using ANOVA and Hochberg's GT2 post hoc analyses on changes in metabolites in all 3 groups. *P* values were corrected for false positives using a false discovery rate of 0.05 in the simultaneous analysis of all 153 metabolites. C, cholesterol; CE, cholesterol esters; FC, free cholesterol; L, lipids; P, particles; PL, phospholipids; TG, triglycerides.

expression of energy metabolism pathways compared to a low-nutrient-quality ER diet. The postprandial glucose response correlated positively to the postprandial lipid synthesis gene expression response in insulin-sensitive persons only. A further explorative analysis showed that the difference in weight loss between the diets was specifically present among these insulin-sensitive subjects. This subgroup had a 3.9-kg higher weight loss on the high- compared with the low-nutrient-quality ER diet, whereas insulin-resistant subjects lost similar amounts of weight in both ER diet groups. Both ER diets effectively reduced body fat, liver fat, and blood pressure and increased insulin sensitivity.

Although both diets were designed to have an ER of 25%, the total weight loss was, on average, 2.1 kg higher in the high-nutrient-quality ER diet group than the low-nutrient-quality ER diet group. Differences in reported intake were not significant between the groups but could have accounted for a 0.28-kg difference in weight loss. A small part of the inequality in weight loss, 0.43 kg, could be explained by the grouping of participants into discrete energy groups, resulting in average advised ERs of 25.8% $\pm$ 5.4% in the low-nutrient-quality ER diet and 26.9% $\pm$ 5.9% in the high-nutrient-quality ER diet. Furthermore, the differences in metabolizable energy between the two diets, recalculated using specific Atwater values (16), could have accounted for only 0.24 kg. Thus, in total we can explain less than 1 kg of the 2.1 kg difference in weight loss, leaving more than 1 kg unexplained. Theoretically, it is possible that participants in the high-nutrient-quality ER diet group increased their physical activity level. Although this was not measured, it is not very plausible, as participants were randomly allocated and were instructed to maintain their habitual level of physical exercise. In addition, increased satiety driven by higher protein (20.6 En% compared with 16.0 En%, respectively) and/or fiber (3.1 En% compared with 2.2 En%, respectively) intake in the high-quality diet group as compared to the low-quality diet group might have resulted in better adherence to the dietary guidelines, but this was not reflected by the reported intake. Specific nutrients—for example, the larger proportion of protein in the high-nutrient-quality ER diet—might have increased diet-induced thermogenesis.

Together with the observed weight loss, both ER diets induced a reduction in the expression of genes involved in energy metabolism and lipid and cholesterol synthesis in adipose tissue. This has been described previously as a potential adaptive mechanism to the decrease in nutrients (34–36). Despite similar reported caloric intakes, the high-nutrient-quality ER diet induced larger adaptations in adipose tissue energy metabolism pathways compared to the low-nutrient-quality ER diet. The absence of a relationship between weight loss and the expression of genes involved in energy metabolism suggests a role of specific nutrients in the high-nutrient-quality ER diet. Soy isoflavones exert specific actions that can be described as "caloric restriction mimicking" (37–39). We previously showed that 8 weeks of consumption of isoflavone supplements can induce a downregulation of energy metabolism–related genes in the SAT of postmenopausal women (40). In addition, the n-3 PUFAs EPA and DHA—as well as MUFAs, which had high intake in the high-nutrient-quality ER diet—are better agonists of peroxisome proliferator-activated receptors (PPARs) than the SFAs, which had high intake in the low-nutrient-quality ER diet. Activation of PPARγ in murine adipose tissue upregulates the expression of genes involved in fatty acid oxidation and stimulates browning (41). If uncoupled from ATP production, the elevated fatty acid oxidation might have enlarged the energy deficit in the high-nutrient-quality ER diet, which in turn could have been a stimulus for the adipose tissue to go even further into a "standby mode" with regard to the lipid and energy metabolism.

Next to the fasting changes in gene expression, both ER diets reduced postprandial adaptive gene expression responses to a mixed-meal shake challenge, reflected by reduced cholesterol synthesis in both diets and attenuated lipid anabolic processes for the low-nutrient-quality ER diet. Mice studies have described that the postprandial signal, insulin, activates the transcription factors of sterol regulatory element–binding proteins (SREBPs) (42), which activate the gene expression of lipogenic pathways and the cholesterol metabolism (43). Remarkably, the responsiveness in cholesterol biosynthesis in the low-nutrient-quality ER diet was completely abolished, meaning that no postprandial increase in this pathway was observed after the intervention. As the low-nutrient-quality ER diet contained high amounts of SFAs (14.9 En%) and dietary cholesterol, intracellular cholesterol in SAT might have remained high or even increased after the intervention. High cholesterol levels inhibit the cleavage of SREBP precursors into functional proteins, providing a negative feedback loop for reducing the synthesis of more cholesterol, which might have nullified the postprandial stimulatory effects of insulin.

This led us to explore the data further by performing correlation analyses between postprandial changes in insulin and glucose and postprandial changes in lipogenic gene expression in SAT. Insulin-sensitive subjects, as determined by a HOMA-IR $\leq$ 2.5, showed a positive correlation between postprandial changes in plasma glucose and changes in SAT lipid synthesis gene expression, while this correlation was absent in insulin-resistant subjects. Our findings are in line with a small study by Soronen et al. (44) that found a reduced induction of lipogenic gene expression in SAT in insulin-resistant compared with insulin-sensitive subjects during a euglycemic hyperinsulinemia clamp. Although the clamp is an artificial situation and cannot directly be compared to a postprandial physiological situation, both findings point towards a reduced capacity to accurately regulate lipogenic gene expression in SAT in insulin-resistant subjects.

If plasma glucose, directly or indirectly, increases lipid synthesis in adipose tissue, then an increase in plasma glucose levels is expected to stimulate fat accumulation in adipose tissue in insulin-sensitive individuals. Consequently, insulin-sensitive subjects might profit more from a low-glycemic diet than a high-glycemic diet with respect to weight management. As the high-nutrient-quality ER diet in our study had a relatively lower glycemic content than the low-nutrient-quality ER diet, we could explore this hypothesis by comparing weight losses between both diets in insulin-sensitive and insulin-resistant subjects. We indeed found that exposure to our high-nutrient-quality ER diet led to significantly greater weight loss, of 3.9 kg, in insulin-sensitive subjects, while insulin-resistant subjects had similar weight loss on both diets. We have to consider that these analyses were performed in subgroups, and therefore are explorative; the study was not powered on these subgroup analyses.

Differences in weight loss responses to diets differing in macronutrient and fiber contents based upon stratification for

pretreatment plasma glucose and insulin values have been described before and are summarized in a review (45). In these studies, pretreatment fasting glucose and insulin values were used to stratify the analyses using fasting plasma glucose (FPG) levels above and below 5.6 mmol/L and the median fasting plasma insulin value as the cutoff (45). Their hypothesis points towards a benefit of a low-glycemic diet, especially for insulin-resistant subjects, based on a reduced induction of satiety in the brains of insulin-resistant subjects (46). We hypothesize that overweight, insulin-sensitive, healthy subjects may benefit more from a low- than a high-glycemic diet based on a potential glucose-related induction of fat synthesis in the adipose tissue with respect to weight loss. It must be noted that according to the cutoffs Hjorth et al. (45) used, only 4 individuals had a pretreatment FPG level above 5.6 mmol/L in our study, pointing towards a much healthier and different population, which might explain the divergent hypotheses.

In addition to the more pronounced effects on weight loss, the high-nutrient-quality ER diet also induced beneficial effects on fasting plasma triglycerides, total cholesterol, and lipoproteins, which were absent in the low-nutrient-quality ER diet group. The larger triglyceride-loaded VLDL particles, which were reduced in the high-nutrient-quality ER diet group, have been associated with increased risks of both type 2 diabetes and coronary artery disease (47, 48) and are reduced with remission of type 2 diabetes (49), pointing towards a beneficial effect of the high-nutrient-quality ER diet on the cardiometabolic risk. As a smaller HDL size has been associated with an increased CVD risk, the shift from a small to a larger HDL in the high-nutrient-quality ER diet may reduce the CVD risk (50). It is well documented that small LDL particles have a higher atherogenic potential than larger ones (51) and that a high cholesterol content in LDL is related to an increased risk of coronary heart disease (52, 53). Therefore, the high-nutrient-quality ER diet–induced decrease in cholesterol-rich small LDL particles might decrease the CVD risk.

Of the high-quality nutrients in the high-nutrient-quality ER diet, the n-3 PUFAs EPA and DHA have repeatedly been demonstrated to lower plasma triglycerides. Both fatty acids stimulate the degradation of VLDL-related apoB, resulting in a lower hepatic VLDL output (54, 55). The contribution of n-3 PUFAs to cholesterol levels is ambiguous; in particular, DHA is known for promoting LDL production (56). However, MUFAs and soy protein, which also had high intakes in the high-nutrient-quality ER diet, have been shown to lower levels of LDL cholesterol (57–59). A human study using stable-isotope tracers has shown that a MUFA-rich diet also strongly affects both the secretion and clearance of triglyceride-rich lipoproteins, such as VLDL (60). Lastly, soluble fiber, which had high intake in the high-nutrient-quality ER diet, has been shown to have small, but significant, decreasing effects on total and LDL cholesterol levels (61, 62).

While previous ER studies showed that weight loss itself exerts beneficial effects on blood lipid profiles, such as reductions in total and LDL cholesterol (63–65), the low-nutrient-quality ER diet was unable to induce significant improvements in fasting blood lipid profiles. Potential favorable effects of ER could have been counteracted by the relatively large amount (14.9 En%) of SFAs in the low-nutrient-quality ER diet. SFAs, such as lauric, myristic, and palmitic acid, are known to raise total cholesterol, as well as HDL and LDL levels (66). In addition, dietary fructose, which had a higher intake in the low-nutrient-quality ER diet, is able to increase triglyceride and LDL cholesterol levels (67).

In line with other weight loss trials, weight loss in both diet groups resulted in significant improvements in risk factors for type 2 diabetes (1) when compared to the control group, such as improved glucose homeostasis and insulin sensitivity. As substantial weight loss has been shown to help patients achieve long-term remission of type 2 diabetes (68), the larger weight loss in the high-nutrient-quality ER diet group may be more beneficial for the type 2 diabetes risk than the weight loss in the low-nutrient-quality ER diet group. However, the improved glucose homeostasis, as reflected by reduced fasted glucose, insulin, and HbA1c levels, was not larger in the low-nutrient-quality ER diet group. Apparently, larger differences in weight loss than 2.1 kg are needed before differences in glucose homeostasis can be achieved. The observed decreases in the VAT/SAT ratios in both ER diet groups indicated a preferential loss of VAT in both groups. Likewise, many other studies report that VAT is preferentially lost during modest weight loss, possibly due to its highly lipolytic nature (69). Some of the nutrients in the high-nutrient-quality ER diet, including MUFAs and PUFAs, were selected based on their ability to decrease liver fat, simultaneously increasing hepatic β-oxidation through activation of PPARα and decreasing de novo lipogenesis by inhibiting SREBP1 (14). ER is known as a potent driver of ectopic fat loss (70), and likely overruled the potential beneficial effects of nutrient quality on intrahepatic lipid reductions.

Our study had a large sample size and relatively few dropouts. The dietary intervention in our study was implemented in a feasible, real-life fashion, and the weekly visits resulted in high compliance, as indicated by reported intakes and weight loss. Our findings are generalizable to a broad population, as we included both males and females with a common metabolic phenotype since, at least in the Netherlands, more than half of the middle-aged population is overweight. We may have had potential biases associated with not having an intent-to-treat analysis and with the fact that subjects and dieticians could not be blinded. A limitation of our study is that self-reported dietary intake always carries a degree of uncertainty, which may have contributed to the relatively large difference in weight loss between the diets. In addition, many other variables contribute to the complex relation between caloric intake and actual weight loss, including satiety, energy expenditure, metabolic rate, and the amount of metabolizable energy. We tried to take most of these variables into account, but still conclude that our findings on weight loss are remarkable and add fuel to the long-running debate over whether a calorie is a calorie.

In conclusion, our results demonstrate that that the nutrient composition of an ER diet is of great importance for improvements in metabolic health in an overweight, middle-aged population. A high-nutrient-quality ER diet enriched with soy protein, fiber, MUFAs, and n-3 PUFAs and reduced in fructose provided additional health benefits over a low-nutrient-quality ER diet, resulting in greater weight loss and larger adaptations in the adipose tissue and promoting an antiatherogenic blood lipid profile. But more importantly, sensitive high-throughput techniques and postprandial analyses enabled us to further explore potential underlying mechanisms of the beneficial effects of the high-nutrient-quality ER diet, leading to a new hypothesis. There was a reduction in triglyceride-loaded, larger VLDL

particles and a positive correlation between postprandial plasma glucose and lipid synthesis gene expression in the adipose tissue of insulin-sensitive subjects, but not insulin-resistant individuals. The subsequent discovery of greater weight loss in insulin-sensitive subjects upon exposure to the high-nutrient-quality ER diet, which had lower-glycemic foods compared to the low-nutrient-quality ER diet, led us to hypothesize that overweight, insulin-sensitive, middle-aged subjects may benefit more from a high- than a low-nutrient-quality ER diet with respect to weight management, due to the potential attenuation of plasma glucose–induced lipid synthesis in adipose tissue.

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The authors' responsibilities were as follows – SS, MM, LAA: designed the research; ES: was responsible for the dietary intervention; SS, DE: conducted the research; DE, JCM, CJRM, SS, HCB: analyzed data or performed the statistical analysis; SS, MD: wrote the paper; LAA: had primary responsibility for the final content; and all authors: read and approved the final manuscript. The authors report no conflicts of interest.

Data Availability

Transcriptome data are available as raw CEL files and are registered as GSE197285 in the Gene Expression Omnibus.

Data described in the manuscript, code book, and analytic code will be made available upon request pending application and approval.

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