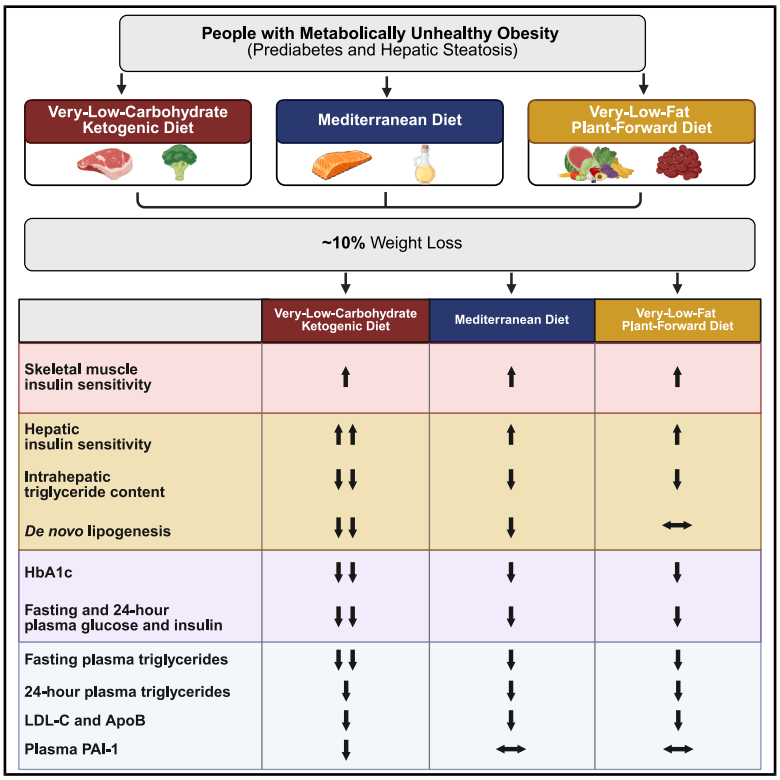
Cell Metabolism
Clinical and Translational Report

Effect of diet macronutrient content on the cardiometabolic response to weight loss: A randomized clinical trial

Graphical abstract



Highlights

- Diet macronutrient content affects the cardiometabolic benefits of weight loss
- Ketogenic diet weight loss decreases liver fat more than higher-carbohydrate diets
- Ketogenic diet improves liver metabolism more than higher-carbohydrate diets

Authors

Max C. Petersen, Gordon I. Smith, Sarah S. Farabi, ..., Marc K. Hellerstein, Bruce W. Patterson, Samuel Klein

Correspondence

sklein@wustl.edu

In brief

Petersen et al. report that 10% weight loss induced by consuming a ketogenic diet causes greater improvements in hepatic metabolic function and intrahepatic triglyceride content than the same weight loss induced by consuming a Mediterranean or very-low-fat plant-forward diet in people with metabolically unhealthy obesity.

Petersen et al., 2026, Cell Metabolism *38*, 1–12
October 6, 2026
https://doi.org/10.1016/j.cmet.2026.07.020

CellPress

Cell Metabolism

CellPress
OPEN ACCESS

Clinical and Translational Report

Effect of diet macronutrient content on the cardiometabolic response to weight loss: A randomized clinical trial

Max C. Petersen,[1,2] Gordon I. Smith,[1] Sarah S. Farabi,[1,3] Hector H. Palacios,[1] Mahalakshmi Shankaran,[4] Marc K. Hellerstein,[4] Bruce W. Patterson,[1] and Samuel Klein[1,5,*]

[1]Center for Human Nutrition, St. Louis, MO, USA
[2]Division of Endocrinology, Metabolism, and Lipid Research, Washington University School of Medicine, St. Louis, MO, USA
[3]Goldfarb School of Nursing at Barnes-Jewish College, St. Louis, MO, USA
[4]University of California, Berkeley, Berkeley, CA, USA
[5]Lead contact
*Correspondence: sklein@wustl.edu
https://doi.org/10.1016/j.cmet.2026.07.020

SUMMARY

The influence of diet macronutrient content on the cardiometabolic effects of weight loss in people with metabolically unhealthy obesity (prediabetes and hepatic steatosis) has important clinical implications. We evaluated the cardiometabolic effects of moderate (~10%) weight loss induced by three popular diets with markedly different macronutrient composition (very-low-carbohydrate ketogenic, Mediterranean, and very-low-fat plant-forward). Weight loss increased muscle insulin sensitivity by ~50% in all groups but caused a two-to-three-fold greater increase in hepatic insulin sensitivity in the very-low-carbohydrate group than the other groups ($p < 0.001$). Intrahepatic triglyceride content, hepatic *de novo* lipogenesis, glycated hemoglobin, and 24-h serial plasma glucose and insulin decreased most in the very-low-carbohydrate group. There were no differences among groups in LDL-cholesterol, apolipoprotein B, or 24-h plasma triglyceride concentrations. These results demonstrate that a very-low-carbohydrate diet has greater cardiometabolic benefits, particularly in hepatic metabolic function, than matched 10% weight loss induced by a Mediterranean or very-low-fat diet in people with metabolically unhealthy obesity.

INTRODUCTION

Obesity causes a constellation of metabolic diseases, including atherogenic dyslipidemia, prediabetes, and metabolic dysfunction-associated steatotic liver disease (MASLD), which are risk factors for type 2 diabetes and cardiovascular disease.[1,2] Insulin-resistant glucose metabolism in both skeletal muscle and liver is the key pathogenic driver of obesity-related metabolic diseases.[3] Weight loss induced by decreasing energy intake relative to energy expenditure is the cornerstone of therapy for people with metabolically unhealthy obesity because it improves multi-organ insulin action and ameliorates, or even completely resolves, obesity-related metabolic disease.[4–6] However, the macronutrient composition of a diet can also affect metabolic health, independent of weight loss per se.[7–11] Three popular diets that differ markedly in macronutrient content, namely very-low-carbohydrate ketogenic, Mediterranean, and very-low-fat plant-forward diets, are often recommended for treating people with obesity.[12] Data from randomized clinical trials have shown that these types of diets differ slightly in weight loss efficacy; very-low-carbohydrate diets often display a modest weight loss advantage at 6 months that diminishes with longer duration of follow-up.[13–17] However, it is not known whether these three popular diets confer specific metabolic benefits beyond those achieved by weight loss alone because of limitations common to many diet trials, which include questionable dietary adherence and unequal weight loss among treatment groups.

We conducted a randomized clinical trial to determine whether three popular diets with marked differences in macronutrient content have diet-specific cardiometabolic effects in people with metabolically unhealthy obesity (defined as obesity, prediabetes, and hepatic steatosis) after matched moderate (~10%) weight loss. We assessed skeletal muscle and liver insulin sensitivity, and a comprehensive battery of cardiometabolic outcomes, including body composition, intrahepatic triglyceride content, hepatic *de novo* lipogenesis, blood pressure, plasma lipids, plasma cytokine concentrations, 24-h serial plasma substrate (glucose, nonesterified fatty acid [NEFA], β-hydroxybutyrate and triglyceride) and hormone (insulin, C-peptide, and glucagon) concentrations, oral glucose tolerance, and beta-cell function, before and after moderate weight loss induced by a very-low-carbohydrate ketogenic, Mediterranean, or very-low-fat plant-forward diet. To help ensure dietary compliance, all food was provided as

Please cite this article in press as: Petersen et al., Effect of diet macronutrient content on the cardiometabolic response to weight loss: A randomized clinical trial, Cell Metabolism (2026), https://doi.org/10.1016/j.cmet.2026.07.020

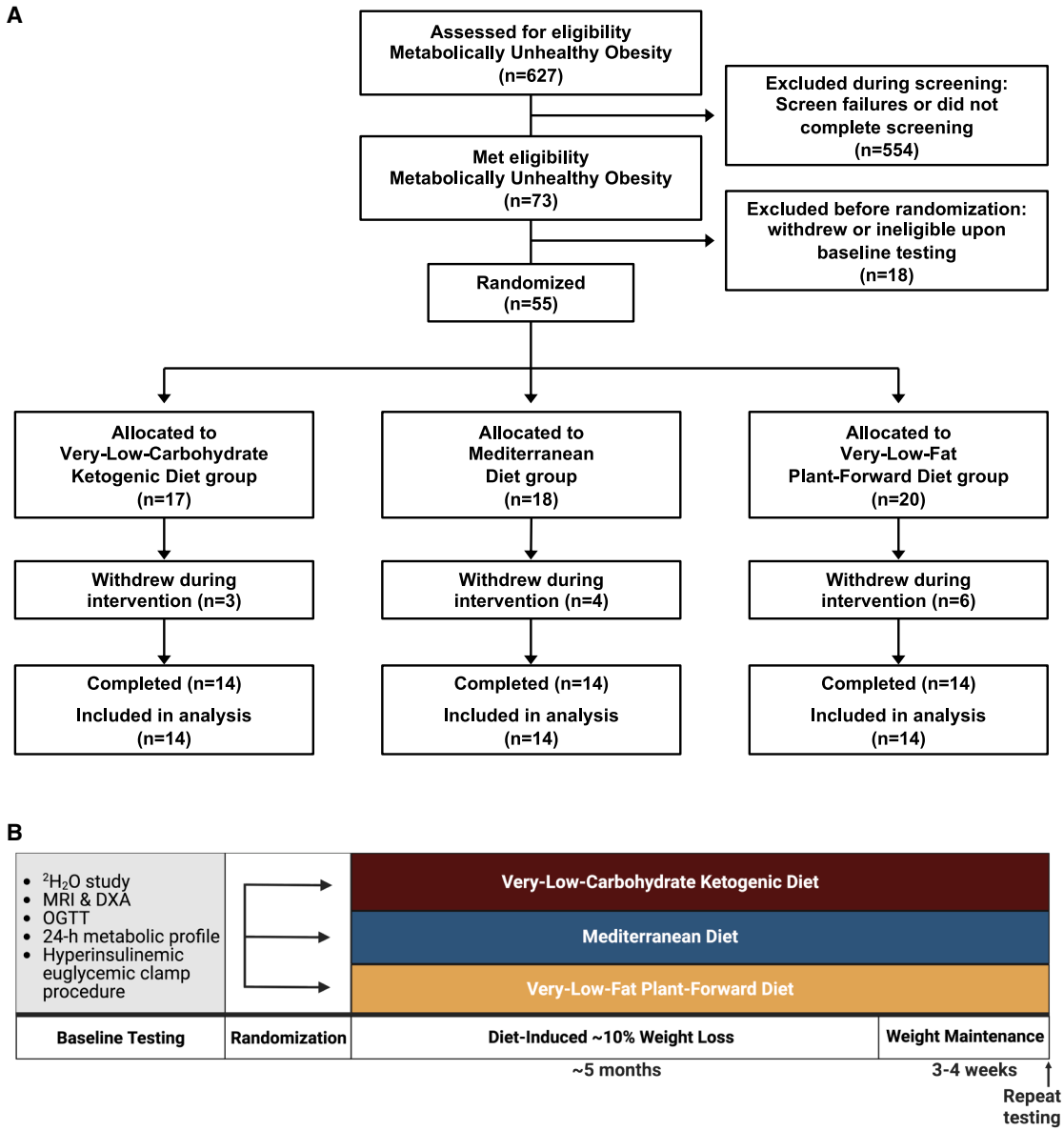


Figure 1. Subject flow and study design

(A) CONSORT study flow diagram.

(B) Overview of study design.

MRI, magnetic resonance imaging; DXA, dual-energy x-ray absorptiometry; OGTT, oral glucose tolerance test.

packed-out meals and participants met weekly with the study dietitian to support adherence to the assigned diet.

RESULTS

Participants and weight loss intervention

A total of 55 participants with metabolically unhealthy obesity completed baseline testing and were randomized to very-low-carbohydrate ketogenic (n = 17), Mediterranean (n = 18), or very-low-fat plant-forward (n = 20) diet therapy (Figure 1A; Table S1). Forty-two participants (9 women and 5 men in each group) 43.2 ± 7.5 years old with initial body mass index 38.9 ± 4.9 kg/m^2 (mean ± SD) completed the study and were analyzed. Baseline characteristics were similar among the three groups (Tables 1 and S1). Metabolic testing was conducted before and after 10.4% ± 2.3%, 10.2% ± 1.6%, and 10.1% ± 2.2% weight loss in the very-low-carbohydrate ketogenic, Mediterranean, and very-low-fat plant-forward diet groups, respectively, which occurred at 4.6 ± 1.8, 5.4 ± 2.1, and 5.1 ± 1.4 months after initiating therapy. An overview of the study design is shown in Figure 1B. Dietary adherence, assessed as the percentage of study meals and snacks that were logged as consumed, was greater than 95% in all diet groups (Table S2). The macronutrient composition of the diets is shown in Table 2.

Please cite this article in press as: Petersen et al., Effect of diet macronutrient content on the cardiometabolic response to weight loss: A randomized clinical trial, Cell Metabolism (2026), https://doi.org/10.1016/j.cmet.2026.07.020

CellPress
OPEN ACCESS

Table 1. Body composition and cardiometabolic variables before and after weight loss

–	Very-low-carbohydrate ketogenic			Mediterranean			Very-low-fat plant-forward			–
–	Before	After	Difference (95% CI)	Before	After	Difference (95% CI)	Before	After	Difference (95% CI)	ANCOVA *p* value
Body weight, kg	114.3 (17.0)	102.4 (15.6)	−11.9 (−13.7, −10.1)	112.1 (20.2)	100.7 (18.3)	−11.4 (−12.8, −9.9)	114.1 (21.0)	102.7 (20.0)	−11.5 (−13.0, −9.9)	0.877
Body mass index, kg/m^2	39.1 (4.9)	35.0 (4.5)	−4.1 (−4.7, −3.4)	38.2 (4.9)	34.3 (4.6)	−3.9 (−4.3, −3.5)	39.3 (5.3)	35.3 (5.0)	−4.0 (−4.5, −3.5)	0.928
Fat mass, %	46.7 (3.7)	44.0 (4.0)	−2.7 (−3.4, −2.0)	45.3 (6.4)	42.0 (6.3)	−3.3 (−4.3, −2.2)	45.9 (7.2)	42.5 (8.2)	−3.4 (−4.4, −2.3)	0.576
Fat-free mass, kg	59.8 (11.3)	56.6 (10.8)	−3.2 (−4.0, −2.5)	60.0 (9.8)	57.3 (9.9)	−2.6 (−3.7, −1.6)	60.3 (10.6)	57.3 (10.9)	−3.0 (−3.9, −2.0)	0.623
Intrahepatic triglyceride, %	19.4 (9.0)	6.2 (3.0)	−13.2 (−17.3, −9.1)	18.1 (9.1)	10.3 (6.9)	−7.8 (−10.8, −4.8)[a]	17.7 (10.3)	9.6 (7.3)	−8.1 (−12.3, −3.9)[c]	**0.011**
Systolic blood pressure, mmHg	123 (16)	117 (9)	−5 (−15, 5)	126 (12)	115 (10)	−11 (−17, −5)	126 (9)	118 (10)	−8 (−17, 1)	0.707
Diastolic blood pressure, mmHg	75 (11)	72 (7)	−3 (−9, 4)	76 (9)	70 (6)	−6 (−12, −1)	77 (7)	76 (10)	−2 (−9, 6)	0.214
Glucose, mg/dL	97 (8)	85 (7)	−12 (−15, −8)	102 (11)	97 (10)	−6 (−9, −2)[b]	96 (9)	90 (6)	−6 (−10, −2)[c]	**0.001**
2-hr OGTT plasma glucose, mg/dL	166 (25)	136 (37)	−30 (−57, −2)	177 (35)	141 (24)	−35 (−55, −15)	175 (30)	153 (17)	−23 (−39, −6)	0.342
HbA1c, %	5.6 (0.4)	5.0 (0.3)	−0.6 (−0.8, −0.4)	5.4 (0.5)	5.3 (0.3)	−0.1 (−0.4, 0.1)[b]	5.7 (0.7)	5.3 (0.3)	−0.4 (−0.7, −0.1)[c]	**0.001**
Insulin, μU/mL	27.7 (9.5)	13.1 (4.5)	−14.6 (−18.4, −10.8)	27.1 (10.4)	17.0 (8.1)	−10.1 (−14.1, −6.1)[a]	28.2 (12.8)	17.9 (7.9)	−10.2 (−14.5, −6.0)[c]	**0.010**
HOMA-IR	6.7 (2.5)	2.8 (1.0)	−3.9 (−4.9, −2.8)	6.8 (2.6)	4.1 (2.3)	−2.7 (−3.7, −1.7)[a]	6.8 (3.4)	4.0 (1.7)	−2.8 (−4.0, −1.6)[c]	**0.008**
Matsuda Index	1.5 (0.8)	2.8 (1.5)	1.3 (0.7, 1.9)	1.5 (0.7)	2.1 (1.1)	0.6 (0.1, 1.0)[a]	1.5 (0.5)	2.1 (0.5)	0.6 (0.2, 1.0)	**0.033**
Nonesterified fatty acids, mmol/L	0.5 (0.1)	0.4 (0.1)	0.0 (−0.1, 0.1)	0.4 (0.1)	0.4 (0.1)	0.0 (−0.1, 0.1)	0.4 (0.1)	0.4 (0.1)	0.0 (−0.1, 0.0)	0.432
Triglycerides, mg/dL	180 (70)	99 (38)	−82 (−112, −52)	173 (37)	131 (30)	−41 (−60, −23)[b]	171 (90)	147 (71)	−24 (−43, −4)[d]	**<0.001**
Total cholesterol, mg/dL	184 (35)	166 (32)	−18 (−32, −4)	196 (20)	171 (20)	−26 (−36, −16)	170 (34)	152 (27)	−17 (−30, −4)	0.818
LDL-cholesterol, mg/dL	111 (26)	101 (26)	−10 (−22, 2)	125 (16)	103 (19)	−22 (−29, −14)	100 (29)	92 (22)	−8 (−18, 1)	0.444
HDL-cholesterol, mg/dL	36 (6)	38 (8)	2 (0, 4)	40 (9)	38 (8)	−2 (−4, 0)	36 (9)	32 (9)	−4 (−7, −1)[d]	**0.005**
Apolipoprotein B, mg/dL	96 (20)	90 (17)	−6 (−15, 3)	103 (13)	88 (15)	−15 (−20, −10)	90 (24)	81 (19)	−9 (−16, −3)	0.243
Large VLDL particles, nmol/L	9.0 (3.8)	3.9 (2.8)	−5.1 (−6.9, −3.2)	7.4 (2.6)	6.3 (2.6)	−1.1 (−3.2, 0.9)[a]	8.3 (5.0)	7.6 (4.0)	−0.7 (−2.2, 0.8)[d]	**<0.001**
Mean VLDL, size in nm	61 (7)	48 (6)	−13 (−18, −9)	59 (7)	54 (7)	−5 (−9, −2)[a]	60 (8)	56 (8)	−4 (−7, −1)[d]	**<0.001**
LP-IR index	71 (11)	50 (17)	−21 (−28, −15)	68 (5)	61 (8)	−7 (−12, −2)[b]	67 (13)	65 (14)	−3 (−7, 1)[d]	**<0.001**
PAI-1, ng/mL	30.3 (16.5)	11.0 (5.3)	−19.3 (−28.4, −10.1)	24.1 (12.3)	20.1 (9.7)	−4.0 (−11.6, 3.7)[a]	21.1 (9.0)	18.2 (13.4)	−3.0 (−12.8, 6.9)	**0.045**
IL-6, pg/mL	1.1 (0.6)	1.3 (0.9)	0.2 (−0.5, 0.9)	1.5 (0.5)	1.9 (0.9)	0.4 (−0.2, 1.0)	2.2 (1.6)	2.3 (1.5)	0.1 (−0.6, 0.8)	0.469
MCP-1, pg/mL	173 (37)	161 (37)	−12 (−27, 3)	179 (36)	170 (32)	−9 (−24, 7)	199 (27)	185 (27)	−14 (−29, 1)	0.740
TNF-α, pg/mL	1.1 (0.4)	0.8 (0.2)	−0.3 (−0.5, −0.2)	0.9 (0.1)	0.9 (0.2)	0.0 (−0.1, 0.1)[a]	0.9 (0.3)	0.9 (0.3)	0.0 (−0.1, 0.1)[d]	**0.002**

Data are means (SD) and mean difference (95% CI). Plasma concentrations are from samples collected after participants fasted for ∼12 h overnight unless otherwise specified. OGTT, oral glucose tolerance test; HOMA-IR; homeostasis model assessment of insulin resistance; HDL, high-density lipoprotein; LDL, low-density lipoprotein; VLDL, very-low-density lipoprotein; LP-IR, lipoprotein-insulin resistance; PAI-1, plasminogen activator inhibitor-1; IL-6, interleukin-6; MCP-1, monocyte chemoattractant protein-1; TNF-α, tumor necrosis factor-α. Sample size is $n = 14$ per group for all outcomes except fat mass and fat-free mass ($n = 13$ very-low-carbohydrate ketogenic, 14 Mediterranean, and 14 very-low-fat plant-forward); fasting glucose, insulin, and HOMA-IR ($n = 14$ very-low-carbohydrate ketogenic, 12 Mediterranean, and 14 very-low-fat plant-forward); 2-h OGTT plasma glucose, Matsuda Index, and MCP-1 ($n = 14$ very-low-carbohydrate ketogenic, 14 Mediterranean, and 13 very-low-fat plant-forward); HbA1c ($n = 14$ very-low-carbohydrate ketogenic, 13 Mediterranean, and 14 very-low-fat plant-forward); and nonesterified fatty acids ($n = 14$ very-low-carbohydrate ketogenic, 12 Mediterranean, and 14 very-low-fat plant-forward). For outcomes with a significant effect of diet by ANCOVA:

[a] $p < 0.05$ for very-low-carbohydrate ketogenic vs. Mediterranean

[b] $p < 0.001$ for very-low-carbohydrate ketogenic vs. Mediterranean

[c] $p < 0.05$ for very-low-carbohydrate ketogenic vs. very-low-fat plant-forward

[d] $p < 0.001$ for very-low-carbohydrate ketogenic vs. very-low-fat plant-forward by using estimated marginal means with Tukey's honest significant difference (HSD) correction for multiple comparisons. See also Table S1.

Please cite this article in press as: Petersen et al., Effect of diet macronutrient content on the cardiometabolic response to weight loss: A randomized clinical trial, Cell Metabolism (2026), https://doi.org/10.1016/j.cmet.2026.07.020

Table 2. Macronutrient compositions of the study diets per 2,000 kcal

Nutrient	Very-low-carbohydrate ketogenic	Mediterranean	Very-low-fat plant-forward
Carbohydrate, g	20	254	352
Energy from carbohydrate, %	4	50	70
Protein, g	112	83	90
Energy from protein, %	23	15	15
Fat, g	164	80	35
Energy from fat, %	73	35	15
Saturated fatty acids, g	52	19	7
Energy from saturated fatty acids, %	23	8	3
Cholesterol, mg	749	160	16

Values are means of the 500 kcal entrees of each diet multiplied by 4. Participants consumed 2 to 4 entrees per day during the weight loss intervention; the number of entrees was determined by the participant's estimated total daily energy expenditure and weight loss trajectory. Data were compiled by using the Nutrition Data System for Research (University of Minnesota). See also Tables S2 and S4.

No serious adverse events occurred and no participant withdrew because of an adverse event (Table S3). Estimated glomerular filtration rate (GFR) increased in the very-low-carbohydrate ketogenic diet group and did not change in the other groups ($p = 0.002$) (Table S4). The increase in GFR reflected a decrease in cystatin C, but not creatinine, in the very-low-carbohydrate ketogenic diet group (Table S4). Urinary nitrogen excretion increased in the very-low-carbohydrate ketogenic diet group and decreased in the other groups ($p < 0.001$), reflecting dietary protein intake (Tables 2 and S4). Urinary 8-isoprostane concentration, an index of lipid peroxidation/oxidative stress,[18] decreased in the very-low-carbohydrate ketogenic diet group and did not change in the other groups ($p = 0.005$) (Table S4).

Insulin sensitivity and liver-related metabolic function

Skeletal muscle insulin sensitivity was assessed by using the hyperinsulinemic-euglycemic clamp procedure in conjunction with stable isotopically labeled glucose tracer infusion.[19] Weight loss increased skeletal muscle insulin sensitivity by ~50% in all groups, without a difference among groups ($p = 0.617$) (Figure 2A). Hepatic insulin sensitivity was assessed by determining the hepatic insulin sensitivity index,[20,21] which is calculated as the reciprocal of the product of basal endogenous glucose production rate and plasma insulin concentration. Weight loss increased the hepatic insulin sensitivity index in all groups, but the increase was two-to-three-fold greater in the very-low-carbohydrate ketogenic diet group than the Mediterranean and very-low-fat plant-forward diet groups ($p < 0.001$) (Figure 2A). A sensitivity analysis conducted by using a linear mixed model to test for a diet x time interaction in all 55 randomized participants yielded similar results as the primary analysis of covariance (ANCOVA) analysis for both skeletal muscle ($p = 0.560$) and hepatic insulin sensitivity ($p < 0.001$). Weight loss decreased intrahepatic triglyceride content in all groups, but the decrease was greater in the very-low-carbohydrate ketogenic (−67%) than the Mediterranean (−45%) and very-low-fat plant-forward (−45%) diet groups ($p < 0.05$) (Figure 2B; Table 1). Basal endogenous glucose production rate and hepatic *de novo* lipogenesis decreased in the very-low-carbohydrate ketogenic and Mediterranean diet groups but did not change in the very-low-fat plant-forward diet group; the decreases in the very-low-carbohydrate ketogenic diet group were greater than the changes in the Mediterranean and very-low-fat plant-forward groups ($p < 0.001$) (Figure 2B). The change in hepatic *de novo* lipogenesis after weight loss correlated with the change in 24-h plasma insulin area under the curve ($r = 0.725$, $p < 0.001$) (Figure S1).

Body composition and cardiometabolic variables

Body fat and fat-free masses decreased after weight loss in all diet groups, without differences among groups (Table 1); the proportion of weight loss from fat was 71% ± 11%, 74% ± 17%, and 75% ± 14% in the very-low-carbohydrate ketogenic, Mediterranean, and very-low-fat plant-forward diet groups, respectively. Fasting plasma glucose, fasting plasma insulin, and the homeostasis model assessment of insulin resistance (HOMA-IR)[22] decreased after weight loss in all groups; the decrease was greater in the very-low-carbohydrate ketogenic diet group than in the Mediterranean and very-low-fat plant-forward diet groups ($p < 0.05$) (Table 1). Plasma glucose at 2 h after 75-g glucose ingestion decreased after weight loss in all groups, without an effect of diet (Table 1). Glycated hemoglobin decreased after weight loss in the very-low-carbohydrate ketogenic and very-low-fat plant-forward diet groups; the decrease was greater in the very-low-carbohydrate ketogenic diet group than in the Mediterranean and very-low-fat plant-forward diet groups ($p < 0.05$) (Table 1). The Matsuda index of whole-body insulin sensitivity increased after weight loss in all groups; the increase was greater in the very-low-carbohydrate ketogenic diet group than in the Mediterranean diet group ($p < 0.05$) (Table 1). Beta-cell function (insulin secretion rate in relation to plasma glucose concentration during the first 30 min after glucose ingestion)[23] was not different after weight loss than before weight loss in all groups (Figure 3). Weight loss resulted in a greater remission of prediabetes in participants in the very-low-carbohydrate ketogenic diet group (50%) than the Mediterranean (29%) and very-low-fat plant-forward (7%) diet groups ($p < 0.05$).

Systolic and diastolic blood pressure tended to decrease after weight loss, without a diet effect (Table 1). Fasting plasma NEFAs were not different before and after weight loss in all groups (Table 1). Fasting plasma triglycerides and mean very-low-density lipoprotein (VLDL) particle size decreased after weight loss in all diet groups, and the decrease was greater in the very-low-carbohydrate ketogenic group than the Mediterranean and very-low-fat plant-forward groups ($p < 0.005$) (Table 1). Fasting large VLDL particle concentration only decreased in the very-low-carbohydrate ketogenic group, and the decrease was greater in the very-low-carbohydrate group than the Mediterranean and very-low-fat plant-forward groups ($p < 0.01$) (Table 1). Plasma HDL-cholesterol decreased after weight loss

Please cite this article in press as: Petersen et al., Effect of diet macronutrient content on the cardiometabolic response to weight loss: A randomized clinical trial, Cell Metabolism (2026), https://doi.org/10.1016/j.cmet.2026.07.020

CellPress
OPEN ACCESS

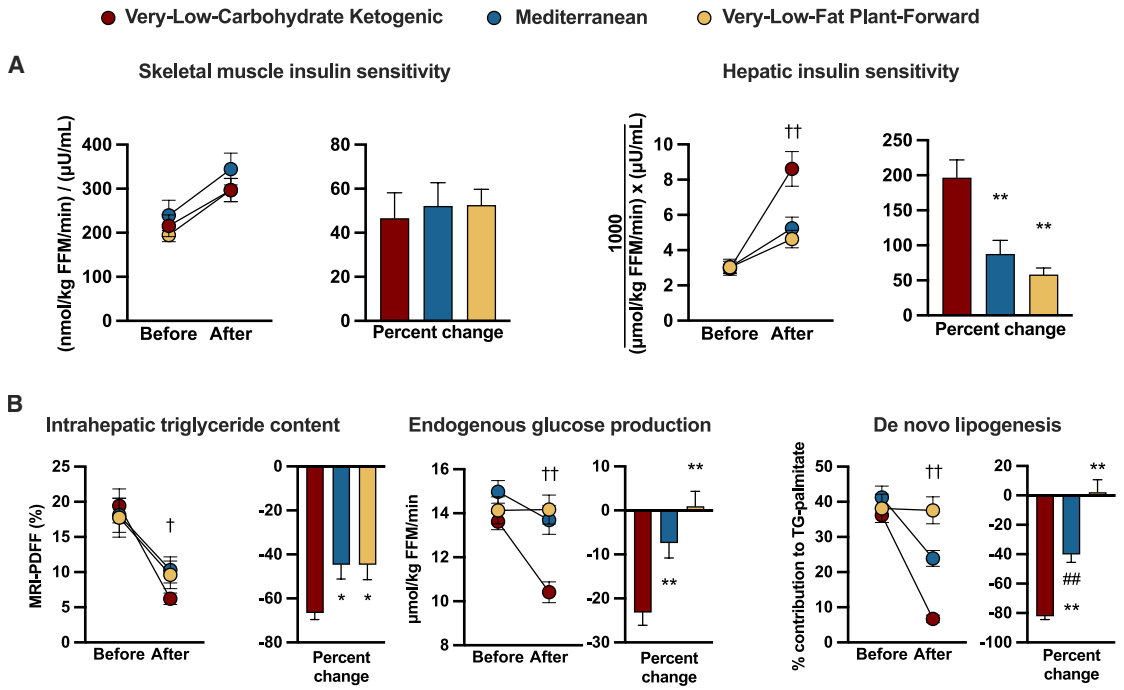


Figure 2. Skeletal muscle and hepatic metabolic function

(A and B) Skeletal muscle insulin sensitivity assessed as glucose disposal rate (nmol/kg fat-free mass/min divided by plasma insulin concentration) (left) and hepatic insulin sensitivity index (1,000 divided by the product of basal endogenous glucose production rate and plasma insulin concentration) (right) during the hyperinsulinemic-euglycemic clamp procedure before and after ~10% weight loss.

(C) Hepatic metabolic function: intrahepatic triglyceride content (left), basal endogenous glucose production rate (center), and hepatic *de novo* lipogenesis (right). For skeletal muscle insulin sensitivity, endogenous glucose production, and hepatic insulin sensitivity index, data from 14 very-low-carbohydrate ketogenic, 12 Mediterranean, and 14 very-low-fat plant-forward participants are shown. For intrahepatic triglyceride content, data from 14 participants are shown for each diet group. For *de novo* lipogenesis, data for 13 very-low-carbohydrate ketogenic, 11 Mediterranean, and 10 very-low-fat plant-forward participants are shown. For each outcome, data on the left are means ± SEM before and after weight loss and data on the right are percent change as means ± SEM. $^{\dagger}p < 0.05$, $^{\dagger\dagger}p < 0.001$ for effect of diet by using ANCOVA. For pairwise diet group comparisons on the right, $^{*}p < 0.05$, $^{**}p < 0.001$ vs. very-low-carbohydrate ketogenic; $^{\#\#}p < 0.001$ vs. very-low-fat plant-forward by using one-way ANOVA or Kruskal-Wallis test with Tukey's or Dunn's post hoc tests, respectively, to identify group differences. MRI-PDFF, magnetic resonance imaging-proton density fat fraction; DXA, dual-energy x-ray absorptiometry; FFM, fat-free mass; TG, triglyceride.
See also Figure S1.

in the Mediterranean and very-low-fat plant-forward diet groups but tended to increase in the very-low-carbohydrate ketogenic diet group ($p = 0.005$) (Table 1). Total plasma cholesterol, LDL-cholesterol, and apolipoprotein B concentrations tended to decrease after weight loss, without differences among diet groups (Table 1). The lipoprotein-insulin resistance (LP-IR) index[24] decreased or tended to decrease after weight loss in all groups, and the decrease was greater in the very-low-carbohydrate group than the Mediterranean and very-low-fat plant-forward groups ($p < 0.001$) (Table 1). Weight loss decreased plasma plasminogen activator inhibitor-1 (PAI-1) and tumor necrosis factor-α (TNF-α) concentrations in the very-low-carbohydrate ketogenic diet group but not in the Mediterranean and very-low-fat plant-forward diet groups ($p < 0.05$), tended to decrease plasma monocyte chemoattractant protein-1 (MCP-1) concentration without an effect of diet, and did not affect plasma interleukin-6 (IL-6) concentration (Table 1).

24-h metabolic profiles

The effects of weight loss with each diet on 24-h plasma substrate and hormone concentration areas under the curve (Figures 4 and 5; Table S5) are summarized as follows: (1) plasma glucose concentration decreased in all groups, but the decrease was greater in the very-low-carbohydrate ketogenic diet group (−20%) than the Mediterranean (−8%) and very-low-fat plant-forward (−8%) diet groups ($p < 0.001$); (2) plasma triglyceride concentration decreased in the very-low-carbohydrate ketogenic (−23%), Mediterranean (−23%), and very-low-fat plant-forward (−17%) diet groups, without a difference among groups; (3) plasma NEFA concentration increased by 63% in the very-low-carbohydrate ketogenic diet group, did not change in the Mediterranean group, and decreased by 29% in the very-low-fat plant-forward diet group ($p < 0.001$); (4) plasma β-hydroxybutyrate concentration increased more than 20-fold in the very-low-carbohydrate ketogenic diet group, did not change in the Mediterranean group, and decreased by 35% in the very-low-fat plant-forward diet group ($p < 0.001$); (5) plasma insulin concentration decreased in all groups, but the decrease was greater in the very-low-carbohydrate ketogenic diet group (−74%) than the Mediterranean (−44%) and very-low-fat plant-forward (−27%) diet groups ($p < 0.001$) and was greater in the Mediterranean diet group than the very-low-fat plant-forward

Please cite this article in press as: Petersen et al., Effect of diet macronutrient content on the cardiometabolic response to weight loss: A randomized clinical trial, Cell Metabolism (2026), https://doi.org/10.1016/j.cmet.2026.07.020

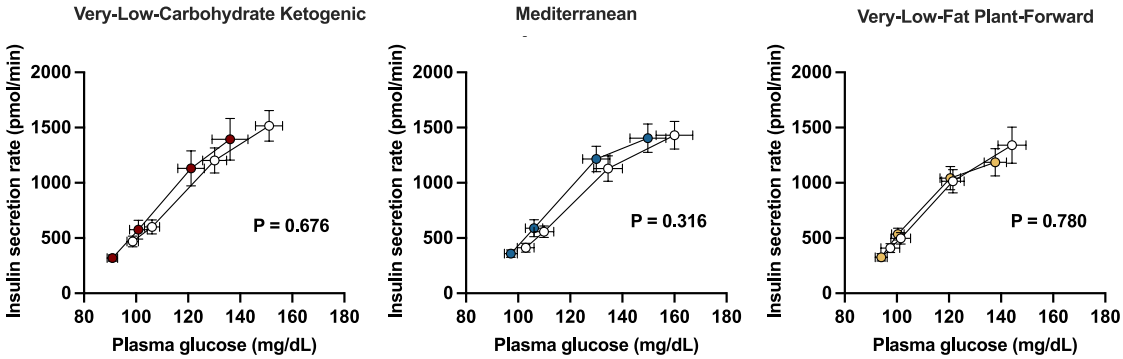


Figure 3. Effects of weight loss induced by a very-low-carbohydrate ketogenic, Mediterranean, or very-low-fat plant-forward diet on beta-cell function

The relationship between insulin secretion rate and plasma glucose concentration during the first 30 min of a 75-g OGTT (blood samples obtained before and at 10, 20, and 30 min after glucose ingestion), before (open circles) and after (filled circles) moderate (~10%) weight loss induced by consumption of a very-low-carbohydrate ketogenic diet (n = 14), Mediterranean diet (n = 14), and very-low-fat plant-forward diet (n = 13). Data are means ± SEM. p values test the null hypothesis that one second-order polynomial curve fits both before and after datasets by using the extra-sum-of-squares F test.

diet group ($p < 0.05$); (6) the insulin secretion rate decreased and the insulin clearance rate increased in all groups, but the changes were greater in the very-low-carbohydrate ketogenic diet group than in the other two groups ($p < 0.001$); (7) plasma glucagon concentration increased by 52% in the very-low-carbohydrate ketogenic diet group but decreased by 32% and 41% in the Mediterranean and very-low-fat plant-forward diet groups, respectively ($p < 0.001$); and (8) the plasma glucagon:insulin ratio increased more than three-fold in the very-low-carbohydrate ketogenic diet group but did not change in the Mediterranean and very-low-fat plant-forward diet groups ($p < 0.001$).

DISCUSSION

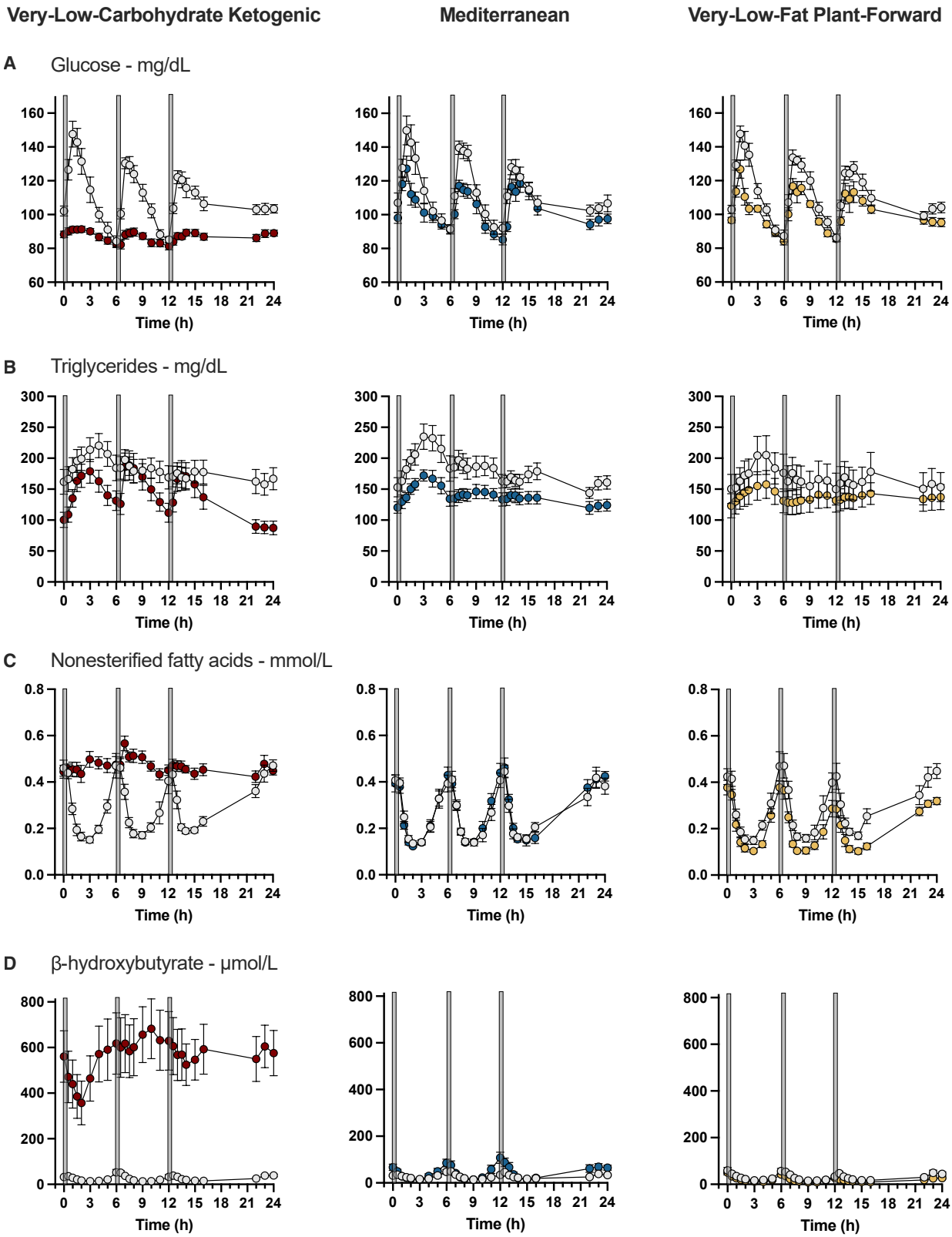
We evaluated whether marked differences in macronutrient composition of three popular weight loss diets (very-low-carbohydrate ketogenic, Mediterranean, and very-low-fat plant-forward), influence the therapeutic cardiometabolic effects of moderate 10% weight loss in people with metabolically unhealthy obesity, which we defined as obesity with prediabetes and hepatic steatosis. All food was provided to participants to help ensure compliance with the dietary intervention. Matched moderate weight loss induced by all three diets increased skeletal muscle and hepatic insulin sensitivity and decreased other cardiovascular disease risk factors. However, the increase in hepatic insulin sensitivity and prediabetes remission rate, and the decreases in glycated hemoglobin, 24-h plasma glucose and insulin concentrations, intrahepatic triglyceride content, fasting plasma triglyceride and large VLDL particle concentrations, mean VLDL particle size, and PAI-1 concentration were greater in the very-low-carbohydrate ketogenic diet group than the other groups. Oral glucose-stimulated insulin secretion did not increase after very-low-carbohydrate ketogenic diet-induced weight loss, thus demonstrating that "resting" the beta-cell for a prolonged period by restricting carbohydrate intake for months does not improve beta-cell function in people with prediabetes, in contrast with the beneficial effect of beta-cell rest in people with type 2 diabetes,[25] which is consistent with and extends the results of a previous study.[26] These results underscore the importance of dietary macronutrient composition, in addition to calorie restriction, in the clinical management of people with metabolically unhealthy obesity. Moreover, our study demonstrates the superiority of a very-low-carbohydrate ketogenic diet in improving hepatic metabolic function, reducing intrahepatic triglyceride content, and lowering other risk factors for diabetes and cardiovascular disease.

Hepatic steatosis is a common obesity-related comorbidity and an important risk factor for cardiovascular disease mortality.[27,28] Weight loss induced by a very-low-carbohydrate ketogenic diet caused a greater reduction in intrahepatic triglyceride content than the same weight loss induced by a Mediterranean or very-low-fat plant-forward diet. This difference presumably reflects the marked decrease in 24-h plasma glucose and insulin and increase in 24-h plasma glucagon and glucagon:insulin ratio in the very-low-carbohydrate group. This is because hepatic *de novo* lipogenesis is stimulated by plasma glucose and insulin[29,30] and inhibited by glucagon,[31] lipolysis of intrahepatic triglyceride is stimulated by glucagon[32] and inhibited by insulin,[33] and glucagon stimulates hepatic fatty acid oxidation and ketogenesis.[32] Additionally, decreasing *de novo* lipogenesis decreases intrahepatic triglyceride by lowering both fatty acid synthesis and malonyl-CoA concentrations, which increases fatty acid oxidation.[34–37] Our results suggest that the marked changes in circulating glucose, insulin, and glucagon induced by a very-low-carbohydrate ketogenic diet have profound effects on intrahepatic lipid metabolism and triglyceride content by decreasing hepatic lipogenesis and increasing intrahepatic triglyceride lipolysis, fatty acid oxidation, and ketogenesis.

There is concern that a high-saturated-fat diet has adverse effects on plasma lipids and cardiovascular disease risk.[38,39] However, weight loss induced by a very-low-carbohydrate ketogenic diet—containing 23% of its energy from saturated fat—resulted in similar effects on total and LDL-cholesterol, apolipoprotein B, 24-h triglycerides, and blood pressure as the Mediterranean and very-low-fat plant-forward diets. Additionally, the very-low-carbohydrate ketogenic diet was associated with greater improvements in plasma HDL-cholesterol, large

Please cite this article in press as: Petersen et al., Effect of diet macronutrient content on the cardiometabolic response to weight loss: A randomized clinical trial, Cell Metabolism (2026), https://doi.org/10.1016/j.cmet.2026.07.020

CellPress
OPEN ACCESS



(legend on next page)

Please cite this article in press as: Petersen et al., Effect of diet macronutrient content on the cardiometabolic response to weight loss: A randomized clinical trial, Cell Metabolism (2026), https://doi.org/10.1016/j.cmet.2026.07.020

VLDL particle concentration, the LP-IR index, lipid peroxidation/oxidative stress, and plasma PAI-1, which are risk factors for cardiovascular disease.[40] These results indicate that weight loss induced by a very-low-carbohydrate ketogenic diet has beneficial effects on cardiovascular disease risk factors in people with metabolically unhealthy obesity.

Our assessment of 24-h plasma substrate and hormone concentrations provides unique insights into the metabolic effects of weight loss induced by the different diets during postabsorptive and postprandial conditions throughout the day. Weight loss induced by a very-low-carbohydrate ketogenic diet caused a greater decrease in 24-h plasma glucose and insulin concentrations than weight loss induced by the Mediterranean and very-low-fat plant-forward diets; the greater decrease in plasma glucose was caused by a marked reduction in glucose ingestion and endogenous glucose production, and the greater decrease in plasma insulin was caused by a greater decrease in insulin secretion and an increase in insulin clearance. The increase in insulin clearance could have been caused by an upregulation of insulin receptor cell surface expression that occurs when plasma insulin concentrations are decreased.[41] Consistent with the results of previous studies,[42] the decrease in fasting plasma triglycerides was greatest in the very-low-carbohydrate ketogenic diet group. However, 24-h plasma triglyceride concentrations were not different among groups because of higher postprandial triglycerides in the very-low-carbohydrate ketogenic diet group. Plasma NEFAs decreased rapidly after meal ingestion in the Mediterranean and very-low-fat plant-forward diet groups because of insulin-mediated suppression of adipose tissue lipolysis, but they remained at basal concentrations throughout the day in the very-low-carbohydrate ketogenic diet group presumably because of increased NEFAs released during lipolysis of postprandial plasma chylomicrons and attenuated suppression of adipose tissue lipolysis.[43] These data demonstrate that the sustained exposure to basal plasma NEFA concentrations associated with a very-low-carbohydrate ketogenic diet does not worsen skeletal muscle insulin resistance. This finding contrasts with the skeletal muscle insulin resistance that has been observed when plasma NEFA concentrations are increased to higher than basal concentrations by infusing a lipid emulsion.[44–46] However, it is possible that a deleterious effect of increased circulating NEFA exposure on skeletal muscle insulin sensitivity[44] was more than offset by the insulin-sensitizing effect of lower 24-h plasma insulin concentrations, such that the net effect of weight loss induced by consuming the very-low-carbohydrate ketogenic diet was an increase in skeletal muscle insulin sensitivity. Plasma β-hydroxybutyrate concentration increased in the very-low-carbohydrate ketogenic diet group, but not the Mediterranean or very-low-fat plant-forward diet groups; however, plasma β-hydroxybutyrate concentrations were much lower than those achieved after several days of starvation[47] and therefore unlikely to affect endogenous glucose production or insulin sensitivity.[48] Plasma glucagon and the glucagon:insulin ratio increased after weight loss in the very-low-carbohydrate ketogenic diet group but decreased after weight loss in the other groups. The increase in plasma glucagon was greatest after meals because the stimulatory effect of dietary amino acids was not offset by insulin-mediated suppression of glucagon secretion.[49]

Our study demonstrates the important influence of dietary macronutrient composition in enhancing the effect of moderate weight loss on hepatic metabolic function, 24-h plasma substrate and hormone concentrations, and cardiometabolic health. Moderate weight loss while consuming a very-low-carbohydrate ketogenic diet has greater cardiometabolic benefits, particularly in hepatic metabolic function, than moderate weight loss while consuming higher-carbohydrate diets in people with metabolically unhealthy obesity.

Limitations of the study

Our study has strengths and limitations. We achieved matched moderate weight loss in all diet groups and dietary adherence was very high because we provided all food to the participants. This enabled a robust assessment of the modifying influence of diet macronutrient content on the beneficial metabolic effects of weight loss in people with metabolically unhealthy obesity. In addition, we conducted a comprehensive and rigorous assessment of clinically relevant metabolic outcomes. However, our study's sample size was small, so it is possible that some differences among diet groups were missed because of inadequate statistical power. Nonetheless, we identified significant differences among diet groups in one primary outcome and many secondary outcomes. We cannot determine whether a less-severe reduction in carbohydrate intake without ketosis would have the same therapeutic effects that were observed in the very-low-carbohydrate ketogenic diet group. Finally, our study cannot determine whether the beneficial hepatic effects of the very-low-carbohydrate ketogenic diet require that the diet is consumed during weight loss or whether similar benefits might be observed if the diet is initiated for weight loss maintenance.[50]

RESOURCE AVAILABILITY

Lead contact

Requests for further information and resources should be directed to and will be fulfilled by the lead contact, Samuel Klein (sklein@wustl.edu).

Materials availability

This study did not generate any new, unique reagents.

Data availability

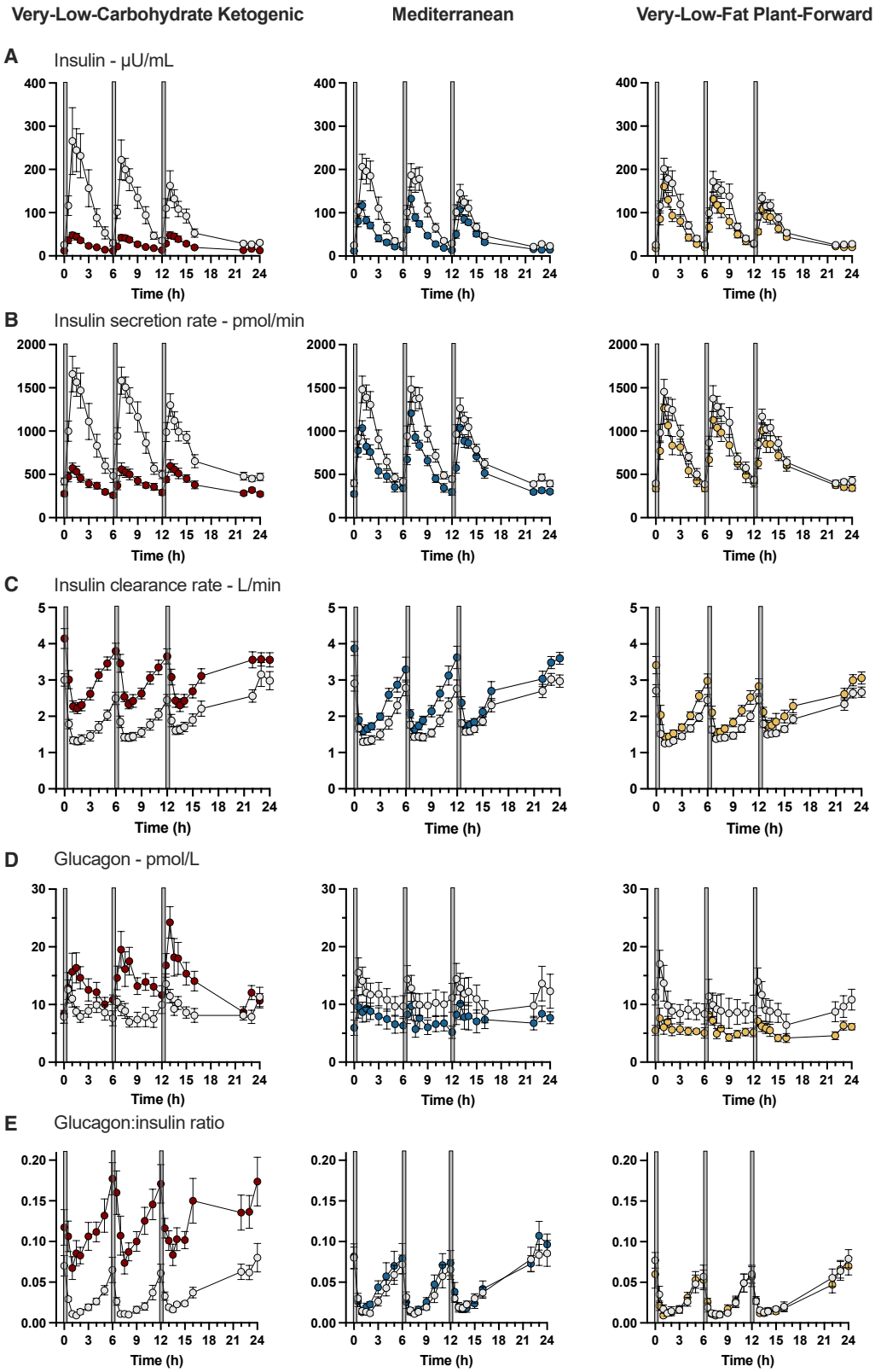
- Raw data used to generate the descriptive statistics presented in this manuscript are provided in Data S1.
- This paper does not report original code.

Figure 4. Effect of weight loss induced by very-low-carbohydrate ketogenic, Mediterranean, or very-low-fat plant-forward diets on 24-h substrate profiles

(A–D) 24-h serial plasma glucose (A), triglycerides (B), nonesterified fatty acids (C), and β-hydroxybutyrate (D) concentrations before the intervention while consuming a standard Western diet (gray circles), and after ~10% weight loss while consuming the assigned very-low-carbohydrate ketogenic (n = 14), Mediterranean (n = 12), or very-low-fat plant-forward (n = 14) diet (colored circles). Meals were consumed at times 0 (07:00 h), 6 (13:00 h), and 12 (19:00 h) h (gray bars). Data are means ± SEM.
See also Figure S2; Table S5.

Please cite this article in press as: Petersen et al., Effect of diet macronutrient content on the cardiometabolic response to weight loss: A randomized clinical trial, Cell Metabolism (2026), https://doi.org/10.1016/j.cmet.2026.07.020

CellPress
OPEN ACCESS



(legend on next page)

- Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

Received: June 3, 2026
Revised: July 6, 2026
Accepted: July 22, 2026

ACKNOWLEDGMENTS

We thank Jennifer Shew, Freida Custodio, Ashwin Mathew, and Chunyan Wang for their technical assistance in processing the study samples and performing gas chromatography-mass spectrometry analyses; Nikki Plassmeyer, Tara Wilmot, Jessica Britt, and the Clinical Translational Research Unit staff for their assistance in conducting the studies; Ken Schechtman for statistical guidance; and the study participants for their participation. The graphical abstract and Figure 1B were created in Biorender. This study was supported by grants P30 DK56341, P30 DK20579, UL1 TR002345 (subaward KL2 TR002346), and S10 OD027006 from the National Institutes of Health and support from the Foundation for Barnes-Jewish Hospital.

AUTHOR CONTRIBUTIONS

All authors contributed to the acquisition, analysis, or interpretation of data for this work. All authors critically reviewed the work for important intellectual content. All authors approved the final version, and all authors vouch for the fidelity of the trial as well as the report of this study. G.I.S. and S.K. conceived and/or designed the study. M.C.P. and G.I.S. analyzed data and/or performed the statistical analysis. M.C.P. and S.K. drafted the manuscript.

DECLARATION OF INTERESTS

S.K. reports receiving scientific advisory board fees from AbbVie, 89Bio, and Boehringer Ingelheim; an investigator-initiated grant from Merck; and support for an industry-initiated multi-center clinical trial from Viking Therapeutics.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

- KEY RESOURCES TABLE
- EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS
  - Study design and oversight
- METHOD DETAILS
  - Diet intervention
  - Study outcomes
  - Body composition analyses
  - Serial 24-hour blood sampling and hyperinsulinemic-euglycemic clamp procedure
  - Deuterated water supplementation
  - Sample analyses
- QUANTIFICATION AND STATISTICAL ANALYSIS
  - Calculations
  - Statistical analysis
- ADDITIONAL RESOURCES

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.cmet.2026.07.020.

REFERENCES

1. Klein, S., Wadden, T., and Sugerman, H.J. (2002). AGA technical review on obesity. Gastroenterology *123*, 882–932. https://doi.org/10.1053/gast.2002.35514.
2. DeFronzo, R.A., Ferrannini, E., Groop, L., Henry, R.R., Herman, W.H., Holst, J.J., Hu, F.B., Kahn, C.R., Raz, I., Shulman, G.I., et al. (2015). Type 2 diabetes mellitus. Nat. Rev. Dis. Primers *1*, 15019. https://doi.org/10.1038/nrdp.2015.19.
3. Klein, S., Gastaldelli, A., Yki-Järvinen, H., and Scherer, P.E. (2022). Why does obesity cause diabetes? Cell Metab. *34*, 11–20. https://doi.org/10.1016/j.cmet.2021.12.012.
4. Yoshino, M., Kayser, B.D., Yoshino, J., Stein, R.I., Reeds, D., Eagon, J.C., Eckhouse, S.R., Watrous, J.D., Jain, M., Knight, R., et al. (2020). Effects of Diet versus Gastric Bypass on Metabolic Function in Diabetes. N. Engl. J. Med. *383*, 721–732. https://doi.org/10.1056/NEJMoa2003697.
5. Mittendorfer, B., Kayser, B.D., Yoshino, M., Yoshino, J., Watrous, J.D., Jain, M., Eagon, J.C., Patterson, B.W., and Klein, S. (2023). Heterogeneity in the effect of marked weight loss on metabolic function in women with obesity. JCI Insight *8*, e169541. https://doi.org/10.1172/jci.insight.169541.
6. Samovski, D., Smith, G.I., Palacios, H., Pietka, T., Fuchs, A., Patti, G.J., Nawaz, A., Kahn, C.R., and Klein, S. (2025). Effect of Marked Weight Loss on Adipose Tissue Biology in People With Obesity and Type 2 Diabetes. Diabetes Care *48*, 1342–1351. https://doi.org/10.2337/dc24-2739.
7. Salas-Salvadó, J., Bulló, M., Estruch, R., Ros, E., Covas, M.I., Ibarrola-Jurado, N., Corella, D., Arós, F., Gómez-Gracia, E., Ruiz-Gutiérrez, V., et al. (2014). Prevention of diabetes with Mediterranean diets: a subgroup analysis of a randomized trial. Ann. Intern. Med. *160*, 1–10. https://doi.org/10.7326/M13-1725.
8. Nuttall, F.Q., Schweim, K., Hoover, H., and Gannon, M.C. (2008). Effect of the LoBAG30 diet on blood glucose control in people with type 2 diabetes. Br. J. Nutr. *99*, 511–519. https://doi.org/10.1017/S0007114507819155.
9. Krauss, R.M., Blanche, P.J., Rawlings, R.S., Fernstrom, H.S., and Williams, P.T. (2006). Separate effects of reduced carbohydrate intake and weight loss on atherogenic dyslipidemia. Am. J. Clin. Nutr. *83*, 1025–1205. https://doi.org/10.1093/ajcn/83.5.1025.
10. Tay, J., Luscombe-Marsh, N.D., Thompson, C.H., Noakes, M., Buckley, J.D., Wittert, G.A., Yancy, W.S., Jr., and Brinkworth, G.D. (2014). A very low-carbohydrate, low-saturated fat diet for type 2 diabetes management: a randomized trial. Diabetes Care *37*, 2909–2918. https://doi.org/10.2337/dc14-0845.
11. Ebbeling, C.B., Feldman, H.A., Klein, G.L., Wong, J.M.W., Bielak, L., Steltz, S.K., Luoto, P.K., Wolfe, R.R., Wong, W.W., and Ludwig, D.S. (2018). Effects of a low carbohydrate diet on energy expenditure during weight loss maintenance: randomized trial. BMJ *363*, k4583. https://doi.org/10.1136/bmj.k4583.

Figure 5. Effects of weight loss induced by very-low-carbohydrate ketogenic, Mediterranean, or very-low-fat plant-forward diets on 24-h insulin kinetics and plasma glucagon

(A–E) 24-h serial plasma insulin (A), insulin secretion rate (B), insulin clearance rate (C), plasma glucagon (D), and plasma glucagon:insulin ratio (pmol/L:pmol/L) (E) before the intervention while consuming a standard Western diet (gray circles), and after ~10% weight loss while consuming the assigned very-low-carbohydrate ketogenic, Mediterranean, or very-low-fat plant-forward diet (colored circles). Meals were consumed at times 0 (07:00 h), 6 (13:00 h), and 12 (19:00 h) h (gray bars). The number of participants in (A)–(C) are 14 in the very-low-carbohydrate ketogenic, 12 in the Mediterranean, and 14 in the very-low-fat plant-forward diet groups. The number of participants in (D) and (E) are 11 in the very-low-carbohydrate ketogenic, 11 in the Mediterranean, and 10 in the very-low-fat plant-forward diet groups. Data are means ± SEM.
See also Figures S1 and S2; Table S5.

12. Malik, V.S., and Hu, F.B. (2007). Popular weight-loss diets: from evidence to practice. Nat. Clin. Pract., Cardiovasc. Med. *4*, 34–41. https://doi.org/10.1038/ncpcardio0726.

13. Nadolsky, K., Garvey, W.T., Agarwal, M., Bonnecaze, A., Burguera, B., Chaplin, M.D., Griebeler, M.L., Harris, S.R., Schellinger, J.N., Simonetti, J., et al. (2025). American Association of Clinical Endocrinology Consensus Statement: Algorithm for the Evaluation and Treatment of Adults with Obesity/Adiposity-Based Chronic Disease - 2025 Update. Endocr. Pract. *31*, 1351–1394. https://doi.org/10.1016/j.eprac.2025.07.017.

14. Shai, I., Schwarzfuchs, D., Henkin, Y., Shahar, D.R., Witkow, S., Greenberg, I., Golan, R., Fraser, D., Bolotin, A., Vardi, H., et al. (2008). Weight loss with a low-carbohydrate, Mediterranean, or low-fat diet. N. Engl. J. Med. *359*, 229–241. https://doi.org/10.1056/NEJMoa0708681.

15. Foster, G.D., Wyatt, H.R., Hill, J.O., McGuckin, B.G., Brill, C., Mohammed, B.S., Szapary, P.O., Rader, D.J., Edman, J.S., and Klein, S. (2003). A randomized trial of a low-carbohydrate diet for obesity. N. Engl. J. Med. *348*, 2082–2090. https://doi.org/10.1056/NEJMoa022207.

16. Gardner, C.D., Trepanowski, J.F., Del Gobbo, L.C., Hauser, M.E., Rigdon, J., Ioannidis, J.P.A., Desai, M., and King, A.C. (2018). Effect of Low-Fat vs Low-Carbohydrate Diet on 12-Month Weight Loss in Overweight Adults and the Association With Genotype Pattern or Insulin Secretion: The DIETFITS Randomized Clinical Trial. JAMA *319*, 667–679. https://doi.org/10.1001/jama.2018.0245.

17. Johnston, B.C., Kanters, S., Bandayrel, K., Wu, P., Naji, F., Siemieniuk, R.A., Ball, G.D.C., Busse, J.W., Thorlund, K., Guyatt, G., et al. (2014). Comparison of weight loss among named diet programs in overweight and obese adults: a meta-analysis. JAMA *312*, 923–933. https://doi.org/10.1001/jama.2014.10397.

18. Graille, M., Wild, P., Sauvain, J.J., Hemmendinger, M., Guseva Canu, I., and Hopf, N.B. (2020). Urinary 8-isoprostane as a biomarker for oxidative stress. A systematic review and meta-analysis. Toxicol. Lett. *328*, 19–27. https://doi.org/10.1016/j.toxlet.2020.04.006.

19. Koh, H.E., van Vliet, S., Meyer, G.A., Laforest, R., Gropler, R.J., Klein, S., and Mittendorfer, B. (2021). Heterogeneity in insulin-stimulated glucose uptake among different muscle groups in healthy lean people and people with obesity. Diabetologia *64*, 1158–1168. https://doi.org/10.1007/s00125-021-05383-w.

20. Korenblat, K.M., Fabbrini, E., Mohammed, B.S., and Klein, S. (2008). Liver, muscle, and adipose tissue insulin action is directly related to intrahepatic triglyceride content in obese subjects. Gastroenterology *134*, 1369–1375. https://doi.org/10.1053/j.gastro.2008.01.075.

21. Matsuda, M., and DeFronzo, R.A. (1999). Insulin sensitivity indices obtained from oral glucose tolerance testing: comparison with the euglycemic insulin clamp. Diabetes Care *22*, 1462–1470. https://doi.org/10.2337/diacare.22.9.1462.

22. Matthews, D.R., Hosker, J.P., Rudenski, A.S., Naylor, B.A., Treacher, D.F., and Turner, R.C. (1985). Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia *28*, 412–419. https://doi.org/10.1007/BF00280883.

23. Cao, C., Koh, H.E., Reeds, D.N., Patterson, B.W., Klein, S., and Mittendorfer, B. (2024). Critical Evaluation of Indices Used to Assess beta-Cell Function. Diabetes *73*, 391–400. https://doi.org/10.2337/db23-0613.

24. Shalaurova, I., Connelly, M.A., Garvey, W.T., and Otvos, J.D. (2014). Lipoprotein insulin resistance index: a lipoprotein particle-derived measure of insulin resistance. Metab. Syndr. Relat. Disord. *12*, 422–429. https://doi.org/10.1089/met.2014.0050.

25. Brown, R.J., and Rother, K.I. (2008). Effects of beta-cell rest on beta-cell function: a review of clinical and preclinical data. Pediatr. Diabetes *9*, 14–22. https://doi.org/10.1111/j.1399-5448.2007.00272.x.

26. Jansen, L.T., Yang, N., Wong, J.M.W., Mehta, T., Allison, D.B., Ludwig, D.S., and Ebbeling, C.B. (2022). Prolonged Glycemic Adaptation Following Transition From a Low- to High-Carbohydrate Diet: A Randomized Controlled Feeding Trial. Diabetes Care *45*, 576–584. https://doi.org/10.2337/dc21-1970.

27. Adams, L.A., Lymp, J.F., St Sauver, J., Sanderson, S.O., Lindor, K.D., Feldstein, A., and Angulo, P. (2005). The natural history of nonalcoholic fatty liver disease: a population-based cohort study. Gastroenterology *129*, 113–121. https://doi.org/10.1053/j.gastro.2005.04.014.

28. Rinella, M.E., Neuschwander-Tetri, B.A., Siddiqui, M.S., Abdelmalek, M.F., Caldwell, S., Barb, D., Kleiner, D.E., and Loomba, R. (2023). AASLD Practice Guidance on the clinical assessment and management of nonalcoholic fatty liver disease. Hepatology *77*, 1797–1835. https://doi.org/10.1097/HEP.0000000000000323.

29. Smith, G.I., Shankaran, M., Yoshino, M., Schweitzer, G.G., Chondronikola, M., Beals, J.W., Okunade, A.L., Patterson, B.W., Nyangau, E., Field, T., et al. (2020). Insulin resistance drives hepatic de novo lipogenesis in nonalcoholic fatty liver disease. J. Clin. Investig. *130*, 1453–1460. https://doi.org/10.1172/JCI134165.

30. ter Horst, K.W., Vatner, D.F., Zhang, D., Cline, G.W., Ackermans, M.T., Nederveen, A.J., Verheij, J., Demirkiran, A., van Wagensveld, B.A., Dallinga-Thie, G.M., et al. (2021). Hepatic Insulin Resistance Is Not Pathway Selective in Humans With Nonalcoholic Fatty Liver Disease. Diabetes Care *44*, 489–498. https://doi.org/10.2337/dc20-1644.

31. Han, S., Akiyama, T.E., Previs, S.F., Herath, K., Roddy, T.P., Jensen, K.K., Guan, H.P., Murphy, B.A., McNamara, L.A., Shen, X., et al. (2013). Effects of small interfering RNA-mediated hepatic glucagon receptor inhibition on lipid metabolism in db/db mice. J. Lipid Res. *54*, 2615–2622. https://doi.org/10.1194/jlr.M035592.

32. Perry, R.J., Zhang, D., Guerra, M.T., Brill, A.L., Goedeke, L., Nasiri, A.R., Rabin-Court, A., Wang, Y., Peng, L., Dufour, S., et al. (2020). Glucagon stimulates gluconeogenesis by INSP3R1-mediated hepatic lipolysis. Nature *579*, 279–283. https://doi.org/10.1038/s41586-020-2074-6.

33. Zhang, W., Bu, S.Y., Mashek, M.T., O-Sullivan, I., Sibai, Z., Khan, S.A., Ilkayeva, O., Newgard, C.B., Mashek, D.G., and Unterman, T.G. (2016). Integrated Regulation of Hepatic Lipid and Glucose Metabolism by Adipose Triacylglycerol Lipase and FoxO Proteins. Cell Rep. *15*, 349–359. https://doi.org/10.1016/j.celrep.2016.03.021.

34. Queathem, E.D., Stagg, D.B., Nelson, A.B., Chaves, A.B., Crown, S.B., Fulghum, K., d'Avignon, D.A., Ryder, J.R., Bolan, P.J., Hayir, A., et al. (2025). Ketogenesis mitigates metabolic dysfunction-associated steatotic liver disease through mechanisms that extend beyond fat oxidation. J. Clin. Investig. *135*, e191021. https://doi.org/10.1172/JCI191021.

35. Luukkonen, P.K., Dufour, S., Lyu, K., Zhang, X.M., Hakkarainen, A., Lehtimäki, T.E., Cline, G.W., Petersen, K.F., Shulman, G.I., and Yki-Järvinen, H. (2020). Effect of a ketogenic diet on hepatic steatosis and hepatic mitochondrial metabolism in nonalcoholic fatty liver disease. Proc. Natl. Acad. Sci. USA *117*, 7347–7354. https://doi.org/10.1073/pnas.1922344117.

36. Kim, C.W., Addy, C., Kusunoki, J., Anderson, N.N., Deja, S., Fu, X., Burgess, S.C., Li, C., Ruddy, M., Chakravarthy, M., et al. (2017). Acetyl CoA Carboxylase Inhibition Reduces Hepatic Steatosis but Elevates Plasma Triglycerides in Mice and Humans: A Bedside to Bench Investigation. Cell Metab. *26*, 394–406.e6. https://doi.org/10.1016/j.cmet.2017.07.009.

37. Qadri, S.F., Porthan, K., Dufour, S., Lehtimäki, T.E., Petersen, K.F., Shulman, G.I., Yki-Järvinen, H., and Luukkonen, P.K. (2026). Distinct effects of ketogenic and non-ketogenic weight-loss diets on hepatic steatosis and mitochondrial metabolism in MASLD. J. Hepatol. *85*, 26–36. https://doi.org/10.1016/j.jhep.2026.02.001.

38. Sacks, F.M., Lichtenstein, A.H., Wu, J.H.Y., Appel, L.J., Creager, M.A., Kris-Etherton, P.M., Miller, M., Rimm, E.B., Rudel, L.L., Robinson, J.G., et al. (2017). Dietary Fats and Cardiovascular Disease: A Presidential Advisory From the American Heart Association. Circulation *136*, e1–e23. https://doi.org/10.1161/CIR.0000000000000510.

39. Chiu, S., Williams, P.T., and Krauss, R.M. (2017). Effects of a very high saturated fat diet on LDL particles in adults with atherogenic dyslipidemia: A randomized controlled trial. PLOS One *12*, e0170664. https://doi.org/10.1371/journal.pone.0170664.

40. Song, C., Burgess, S., Eicher, J.D., O'Donnell, C.J., and Johnson, A.D. (2017). Causal Effect of Plasminogen Activator Inhibitor Type 1 on Coronary Heart Disease. J. Am. Heart Assoc. *6*, e004918. https://doi.org/10.1161/JAHA.116.004918.

41. Lee, W.H., Najjar, S.M., Kahn, C.R., and Hinds, T.D., Jr. (2023). Hepatic insulin receptor: new views on the mechanisms of liver disease. Metabolism *145*, 155607. https://doi.org/10.1016/j.metabol.2023.155607.

42. Kirkpatrick, C.F., Bolick, J.P., Kris-Etherton, P.M., Sikand, G., Aspry, K.E., Soffer, D.E., Willard, K.E., and Maki, K.C. (2019). Review of current evidence and clinical recommendations on the effects of low-carbohydrate and very-low-carbohydrate (including ketogenic) diets for the management of body weight and other cardiometabolic risk factors: A scientific statement from the National Lipid Association Nutrition and Lifestyle Task Force. J. Clin. Lipidol. *13*, 689–711.e1. https://doi.org/10.1016/j.jacl.2019.08.003.

43. Karpe, F., Dickmann, J.R., and Frayn, K.N. (2011). Fatty acids, obesity, and insulin resistance: time for a reevaluation. Diabetes *60*, 2441–2449. https://doi.org/10.2337/db11-0425.

44. Petersen, M.C., and Shulman, G.I. (2018). Mechanisms of Insulin Action and Insulin Resistance. Physiol. Rev. *98*, 2133–2223. https://doi.org/10.1152/physrev.00063.2017.

45. Boden, G., Chen, X., Ruiz, J., White, J.V., and Rossetti, L. (1994). Mechanisms of fatty acid-induced inhibition of glucose uptake. J. Clin. Investig. *93*, 2438–2446. https://doi.org/10.1172/JCI117252.

46. Boden, G., Cheung, P., Stein, T.P., Kresge, K., and Mozzoli, M. (2002). FFA cause hepatic insulin resistance by inhibiting insulin suppression of glycogenolysis. Am. J. Physiol., Endocrinol. Metab. *283*, E12–E19. https://doi.org/10.1152/ajpendo.00429.2001.

47. Klein, S., Holland, O.B., and Wolfe, R.R. (1990). Importance of blood glucose concentration in regulating lipolysis during fasting in humans. Am. J. Physiol. *258*, E32–E39. https://doi.org/10.1152/ajpendo.1990.258.1.E32.

48. Merovci, A., Finley, B., Hansis-Diarte, A., Neppala, S., Abdul-Ghani, M.A., Cersosimo, E., Triplitt, C., and DeFronzo, R.A. (2024). Effect of weight-maintaining ketogenic diet on glycemic control and insulin sensitivity in obese T2D subjects. BMJ Open Diabetes Res. Care *12*, e004199. https://doi.org/10.1136/bmjdrc-2024-004199.

49. Capozzi, M.E., D'Alessio, D.A., and Campbell, J.E. (2022). The past, present, and future physiology and pharmacology of glucagon. Cell Metab. *34*, 1654–1674. https://doi.org/10.1016/j.cmet.2022.10.001.

50. Ebbeling, C.B., Swain, J.F., Feldman, H.A., Wong, W.W., Hachey, D.L., Garcia-Lago, E., and Ludwig, D.S. (2012). Effects of dietary composition on energy expenditure during weight-loss maintenance. JAMA *307*, 2627–2634. https://doi.org/10.1001/jama.2012.6607.

51. Hall, K.D., Sacks, G., Chandramohan, D., Chow, C.C., Wang, Y.C., Gortmaker, S.L., and Swinburn, B.A. (2011). Quantification of the effect of energy imbalance on bodyweight. Lancet *378*, 826–837. https://doi.org/10.1016/S0140-6736(11)60812-X.

52. Mifflin, M.D., St Jeor, S.T., Hill, L.A., Scott, B.J., Daugherty, S.A., and Koh, Y.O. (1990). A new predictive equation for resting energy expenditure in healthy individuals. Am. J. Clin. Nutr. *51*, 241–247. https://doi.org/10.1093/ajcn/51.2.241.

53. Petersen, M.C., Smith, G.I., Palacios, H.H., Farabi, S.S., Yoshino, M., Yoshino, J., Cho, K., Davila-Roman, V.G., Shankaran, M., Barve, R.A., et al. (2024). Cardiometabolic characteristics of people with metabolically healthy and unhealthy obesity. Cell Metab. *36*, 745–761.e5. https://doi.org/10.1016/j.cmet.2024.03.002.

54. Patterson, B.W., Zhao, G., Elias, N., Hachey, D.L., and Klein, S. (1999). Validation of a new procedure to determine plasma fatty acid concentration and isotopic enrichment. J. Lipid Res. *40*, 2118–2124. https://doi.org/10.1016/S0022-2275(20)32435-4.

55. Ward, C.P., Peng, L., Yuen, S., Chang, M., Karapetyan, R., Nyangau, E., Mohammed, H., Palacios, H., Ziari, N., Joe, L.K., et al. (2022). ER Unfolded Protein Response in Liver In Vivo Is Characterized by Reduced, Not Increased, De Novo Lipogenesis and Cholesterol Synthesis Rates with Uptake of Fatty Acids from Adipose Tissue: Integrated Gene Expression, Translation Rates and Metabolic Fluxes. Int. J. Mol. Sci. *23*, 1073. https://doi.org/10.3390/ijms23031073.

56. Van Cauter, E., Mestrez, F., Sturis, J., and Polonsky, K.S. (1992). Estimation of insulin secretion rates from C-peptide levels. Comparison of individual and standard kinetic parameters for C-peptide clearance. Diabetes *41*, 368–377. https://doi.org/10.2337/diab.41.3.368.

57. Mittendorfer, B., Patterson, B.W., Smith, G.I., Yoshino, M., and Klein, S. (2022). beta Cell function and plasma insulin clearance in people with obesity and different glycemic status. J. Clin. Investig. *132*, e154068. https://doi.org/10.1172/JCI154068.

58. Inker, L.A., Eneanya, N.D., Coresh, J., Tighiouart, H., Wang, D., Sang, Y., Crews, D.C., Doria, A., Estrella, M.M., Froissart, M., et al. (2021). New Creatinine- and Cystatin C-Based Equations to Estimate GFR without Race. N. Engl. J. Med. *385*, 1737–1749. https://doi.org/10.1056/NEJMoa2102953.

Please cite this article in press as: Petersen et al., Effect of diet macronutrient content on the cardiometabolic response to weight loss: A randomized clinical trial, Cell Metabolism (2026), https://doi.org/10.1016/j.cmet.2026.07.020

CellPress
OPEN ACCESS

STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Biological samples		
Human plasma from study participants	Clinical study in this paper sponsored by Washington University School of Medicine	Research ethics committee reference: 201512086
Chemicals, peptides, and recombinant proteins		
[U-^{13}C]glucose	Cambridge Isotope Laboratories	Cat# CLM-1396-MPT-PK
Regular insulin (human recombinant)	Lilly USA	Humulin R U-100
Dextrose 20% IV solution	Hospira	Cat# 793519
75-g glucose solution	ThermoFisher Scientific	Cat# TGP401223PA
70% $^{2}H_2O$	Sigma	Cat# 613428
3-hydroxybutyrate-d_4	Cayman Chemical	Cat# 14158
Heptadecanoic acid	Sigma	Cat# H3500
8-isoprostane-d_4	Cayman Chemical	Cat# 316350
8-isoprostane	Cayman Chemical	Cat# 16350
Critical commercial assays		
Plasma glucose concentration	Yellow Springs Instruments	YSI 2900 Biochemistry Analyzer
Insulin and C-peptide concentrations	Roche	Elecsys 2010 immunoassays
Glucagon concentration	Mercodia	Cat# 10-1271-01
Triglyceride concentration	Wako	Cat# 994-02891
High density lipoprotein concentration	Roche	Cat# 07528566190
HbA1c – hemolyzing agent	Roche	Cat# 04528182190
HbA1c – assay	Roche	Cat# 05336163190
TNF-α concentration	R&D Systems	Cat# DTA00D
MCP-1 concentration	R&D Systems	Cat# DCP00
PAI-1 concentration	R&D Systems	Cat# DSE100
IL-6 concentration	MilliporeSigma	Cat# 03-0155-00
Deposited data		
Software and algorithms		
Excel	Microsoft	N/A
Prism 11	GraphPad	N/A
R version 4.5.2	R-Project	N/A
SAAM II Simulation Analysis and Modeling	Nanomath	N/A

EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS

Study design and oversight

This study was conducted between February 2016 and April 2025 at Washington University School of Medicine. Eligible participants had metabolically unhealthy obesity defined as body mass index (BMI) ≥30.0 and ≤50.0 kg/m^2, prediabetes (fasting plasma glucose ≥100 mg/dL, glycated hemoglobin ≥5.7%, and/or plasma glucose concentration 2 hours after a 75-gram oral glucose load ≥140 mg/dL), and hepatic steatosis (intrahepatic triglyceride content ≥5% assessed by magnetic resonance imaging). No subject had a history of diabetes, serious illnesses other than MASLD, or was taking medications that could affect the study outcome measures.

Inclusion criteria were:

1. Age ≥18 and ≤55 years
2. Body mass index ≥30.0 and ≤50.0 kg/m^2
3. Intrahepatic triglyceride content ≥5%

Please cite this article in press as: Petersen et al., Effect of diet macronutrient content on the cardiometabolic response to weight loss: A randomized clinical trial, Cell Metabolism (2026), https://doi.org/10.1016/j.cmet.2026.07.020

4. Glycated hemoglobin (HbA1c) ≥5.7%, or fasting plasma glucose concentration ≥100 mg/dL, or 2-hour 75-gram oral glucose tolerance test plasma glucose concentration ≥140 mg/dL

Exclusion criteria were:

1. Medical, surgical, or biological menopause
2. Previous bariatric surgery involving reconstruction of the gastrointestinal tract (e.g., Roux-en-Y gastric bypass, sleeve gastrectomy, biliopancreatic diversion)
3. Laparoscopic adjustable gastric banding surgery within the last 3 years
4. Structured exercise ≥250 minutes per week (e.g., brisk walking)
5. Unstable weight (>4% change during the last 2 months)
6. Significant organ system dysfunction, including but not limited to diabetes requiring medications; severe pulmonary, renal, or cardiovascular disease; polycystic ovarian syndrome; or liver disease other than metabolic dysfunction-associated steatotic liver disease
7. Cancer or cancer that has been in remission for <5 years
8. Major psychiatric illness
9. Unable to complete all testing procedures (e.g., severe ambulatory impairments, limb amputations, or metal implants incompatible with imaging procedures)
10. Coagulation disorders or abnormal coagulation profile (i.e., elevated prothrombin time, activated partial thromboplastin time, and/or international normalized ratio)
11. Use of medications that are known to affect the study outcomes, including but not limited to corticosteroids or non-statin lipid lowering medications
12. Use of medications that increase the risk of study procedures (e.g., anticoagulants) that cannot be temporarily discontinued for the study
13. Use of antibiotics in last 60 days
14. Cigarette smoking >10 cigarettes per week
15. Marijuana use more than twice per week
16. Any use of illegal drugs
17. Excessive alcohol consumption (>21 standard units of alcohol per week for men or >14 units per week for women)
18. Pregnant or lactating
19. Vegan diet or other dietary restriction precluding randomization to any of the study diets
20. Unable to grant voluntary informed consent
21. Unable or unwilling to follow the study protocol

After completing baseline testing, participants were randomized 1:1:1 to treatment with a very-low-carbohydrate ketogenic, Mediterranean or very-low-fat plant-forward diet by using a random allocation sequence stratified by sex. Participants were assigned to each group sequentially according to the pregenerated list, which was generated by G.I.S. by using a permuted block design. The study team responsible for enrolling participants did not have access to the random allocation sequence. Each of the three diet groups included nine female and five male participants who completed the study. In the very-low-carbohydrate ketogenic diet group, 12 participants self-identified as White and 2 participants self-identified as Black or African-American. In the Mediterranean diet group, 13 participants self-identified as White and 1 participant self-identified as Black or African-American. In the very-low-fat plant-forward diet group, 11 participants self-identified as White, 2 participants self-identified as Black or African-American, and 1 participant self-identified as Native Hawaiian or Pacific Islander. Study testing was conducted at baseline and after participants achieved a targeted ∼10% weight loss. Participants provided written informed consent and the study was approved by the Washington University Institutional Review Board.

METHOD DETAILS

Diet intervention

After completing baseline testing, participants were randomized to treatment with a very-low-carbohydrate ketogenic, Mediterranean, or very-low-fat plant-forward diet, provided to the participants weekly. The very-low-carbohydrate ketogenic, Mediterranean, and very-low-fat plant-forward diets provided 4%, 50%, and 70% of energy from carbohydrate, 73%, 35%, and 15% from fat, and 23%, 15%, and 15% from protein, respectively. The diets were composed by the Washington University Metabolic Kitchen and were comprised of frozen packed-out entrées (500 kcal each) and snacks (78 to 300 kcal each). Each entrée had a macronutrient composition approximately equal to the macronutrient composition of its overall diet (Table 2). Standardized menus containing 2 to 4 entrees and 0 to 3 snacks per day were generated by the study dietitian and modified as needed during the intervention to accommodate participant preferences and energy targets.

The entrees of the very-low-carbohydrate ketogenic diet included but were not limited to: egg salad, omelet with bacon and sausage, almond flour French toast muffin, cheesy beef, pulled pork, Italian chicken with spinach, pecan-encrusted tilapia, salmon

Please cite this article in press as: Petersen et al., Effect of diet macronutrient content on the cardiometabolic response to weight loss: A randomized clinical trial, Cell Metabolism (2026), https://doi.org/10.1016/j.cmet.2026.07.020

with cauliflower mash, pot roast, salmon with broccoli, and chicken with asparagus. The entrees of the Mediterranean diet included but were not limited to: oatmeal with almonds and blueberries, vegetable breakfast hash, mixed berry smoothie, minestrone, spicy tilapia, salmon succotash, quinoa with black beans and corn, chicken pesto pasta, arancini, vegetable burger with roasted sweet potato, and vegetable flatbread. The entrees of the very-low-fat plant-forward diet included but were not limited to: apple banana oatmeal, breakfast potato bake, fruit smoothie, red beans and rice, vegetable tortilla wrap, mixed grains with salmon, vegetable shepherd's pie, sweet potato and black bean soup, and vegetable chili.

Participants were given electronic scales for remote weight monitoring. Participants were asked to consume no other food or caloric beverages other than the study food and to log all meals and snacks in a secure web application. Participants met weekly with the study dietitian to review food logs, barriers to full adherence, adverse events, and any medication changes or intercurrent medical events. After an initial weight-maintenance period of approximately 4 weeks, the energy content of the diet was reduced to achieve a predicted weight loss of 10% over 16 weeks, calculated by using the NIDDK Body Weight Planner[51] and modified as needed to maintain this trajectory. After participants lost ~10% of their initial body weight, the diet energy content was increased to maintain a constant body weight for 3-4 weeks (coefficient of variation in weekly weight was 0.2% ± 1.4%) before studies conducted at baseline were repeated.

Study outcomes

The primary outcomes were the effect of weight loss on skeletal muscle insulin sensitivity, assessed as glucose disposal rate per kilogram fat-free mass divided by plasma insulin concentration during a hyperinsulinemic-euglycemic-clamp procedure, and liver insulin sensitivity, assessed as the hepatic insulin sensitivity index (1000 divided by the product of basal endogenous glucose production rate and plasma insulin).[20,21] Secondary outcomes were the effect of weight loss on intrahepatic triglyceride content, basal endogenous glucose production rate, hepatic *de novo* lipogenesis, body fat and fat-free masses, fasting plasma glucose and insulin, oral glucose tolerance, glycated hemoglobin, beta-cell function, blood pressure, plasma lipids, plasma cytokines, and 24-hour serial plasma substrate and hormone concentrations. Safety outcomes included participant-reported adverse events, and indices of renal function and lipid peroxidation/oxidative stress.

Body composition analyses

Total body fat and fat-free mass (FFM) were determined by using dual-energy X-ray absorptiometry (DXA, Lunar iDXA, GE Healthcare). Intrahepatic triglyceride content was assessed as magnetic resonance imaging-proton density fat fraction (MRI-PDFF) on a 3T MAGNETOM Vida MRI scanner (Siemens, Erlangen, Germany).

Serial 24-hour blood sampling and hyperinsulinemic-euglycemic clamp procedure

Participants were admitted to the Clinical Translational Research Unit (CTRU) at 1700 h on day 1, provided with a standardized meal from the Washington University Metabolic Kitchen, and then consumed nothing by mouth except for water overnight. At approximately 0600 h on day 2, a 24-hour urine collection was started and an intravenous catheter was inserted into an antecubital or forearm vein for serial blood sampling during the 24-hour period from 0700 h on day 2 until 0700 h on day 3. Participants consumed three equicaloric meals on day 2 at 0700 h, 1300 h, and 1900 h. The energy content of the meals was determined by using the Mifflin-St. Jeor equation[52] with an activity factor of 1.25. Each meal provided one-third of the participant's estimated total daily energy expenditure. For baseline studies before the weight loss intervention, all meals were provided as a standard Western diet. The standard Western diet provided 50% of energy from carbohydrate, 35% from fat, and 15% from protein. For post-testing studies conducted after the weight loss intervention, all meals were provided according to the participant's assigned intervention diet. Blood samples were collected hourly from 0700 h to 2300 h on day 2 and from 0500 h to 0700 h on day 3, and additional samples were collected at 30 minutes and 90 minutes after each meal for a total of 26 samples (Figure S2).

At 0700 h on day 3, a primed (8.0 μmol/kg) continuous (0.08 μmol/kg/min) infusion of [U-^{13}C]glucose (Cambridge Isotope Laboratories, Andover, MA) was started and continued for a basal period of 210 minutes. A radial arterial catheter was inserted for arterial blood sampling during the hyperinsulinemic-euglycemic clamp procedure. After completion of the basal period, insulin was infused at a rate of 200 mU/m^2 body surface area (BSA)/min for 5 minutes, followed by 100 mU/m^2 BSA/min for 5 minutes, followed by 50 mU/m^2 BSA/min for 200 minutes. Upon starting the insulin infusion, the [U-^{13}C]glucose infusion was stopped and an infusion of 20% dextrose, enriched to ~1% with [U-^{13}C]glucose, was initiated. The 20% dextrose infusion rate was adjusted as needed every 10 minutes to achieve and maintain a plasma glucose concentration of 100 mg/dL. During the final 20 minutes of the basal period and insulin infusion, four plasma samples were collected at 6-7 minute intervals to determine plasma glucose and insulin concentrations and glucose kinetics.

Deuterated water supplementation

Deuterium enrichment of the total body water pool was achieved by ingesting 70% ^2H_2O (Sigma, St. Louis, MO) for 3 to 5 weeks. Participants consumed four 50-mL aliquots per day for the first 5 days and two 50-mL aliquots per day thereafter. The final dose of ^2H_2O was ingested at 1900 h the day before the hyperinsulinemic-euglycemic clamp procedure, and a blood sample was collected the following morning at 0700 h to determine body water ^2H_2O enrichment and hepatic *de novo* lipogenesis (DNL). Adherence was assessed by the use of paper logs, counting the number of empty containers returned, and interviews. In addition, compliance was

confirmed by measuring total body water deuterium enrichment in blood samples collected every 7 days and saliva samples collected at days 2, 4, 11 and weekly thereafter during the ^2H_2O protocol.

Sample analyses

Plasma glucose concentration was measured by using an automated glucose analyzer (Yellow Springs Instruments, Yellow Springs, OH). Plasma insulin and C-peptide concentrations were measured by electrochemiluminescent assays (Elecsys 2010, Roche Diagnostics, Indianapolis, IN) in the Washington University Core Laboratory for Clinical Studies (CLCS). Plasma glucagon concentrations were measured by ELISA (Mercodia, Uppsala, Sweden). Twenty-four-hour plasma triglyceride concentrations were measured by using a colorimetric assay (Wako Fujifilm, Lexington, MA). Plasma creatinine, cystatin-C, glycated hemoglobin, lipid panel, apolipoprotein B, and urinary nitrogen excretion were analyzed on the COBAS 6000 automated chemistry analyzer (Roche Diagnostics, Indianapolis, IN) in the CLCS. Plasma large VLDL particle concentration and mean VLDL size were determined by nuclear magnetic resonance (LabCorp, Burlington, NC). Plasma tumor necrosis factor-α, monocyte chemoattractant protein-1, and plasminogen activator inhibitor-1 were measured by ELISA (R&D Systems). Plasma interleukin-6 was measured by single molecule counting immunoassay (Millipore Sigma). Urinary 8-isoprostane was measured from 24-hour urine collection samples by ultra-high-performance liquid chromatography-tandem mass spectrometry as described.[53] Nonesterified fatty acids were extracted and derivatized as fatty acid methyl esters from plasma as described[54] after the addition of heptadecanoic acid internal standard (Sigma) and measured by gas chromatography-mass spectrometry (GC-MS) on an Agilent 6890N Network Series GC coupled to an Agilent 5973 Inert MSD Perform Turbo EI MS system with an Omega-Wax column (Supelco, 30 m, 0.25 mm, 0.25 μm). To prepare samples for plasma β-hydroxybutyrate measurement, 120 μL of an 80 μM d_4-3-hydroxybutyrate internal standard solution (Cayman Chemical) was added to 100 μL of plasma. Proteins were precipitated with 1.5 mL acetone. The supernatant was combined with 1.5 mL heptane and 1.5 mL water and the aqueous phase was collected, dried in a vacuum concentrator, and derivatized with 110 μL of 1:1 acetonitrile:*N*-tert-Butyldimethylsilyl-*N*-methyltrifluoroacetamide. To prepare samples for plasma glucose enrichment, the aqueous phase was derivatized with heptafluorobutyric anhydride. Both β-hydroxybutyrate concentration and plasma glucose tracer-to-tracee ratio (TTR) were measured by GC-MS on an Agilent 7890B Series GC coupled to an Agilent 5977B Inert Plus MSD Turbo EI MS system with a DB-5 column (Agilent J&W, 30 m, 0.25 mm, 0.25 μm). Deuterium enrichment in total body water and enrichment and labeling pattern in palmitate from triglyceride-rich lipoprotein triglycerides (TRL-TG) were determined by using GC-MS, as previously described.[29,55]

QUANTIFICATION AND STATISTICAL ANALYSIS

Calculations

Plasma substrate and hormone concentration areas-under-the-curve for 24-hour studies were calculated by using the trapezoidal method. Insulin secretion rate (ISR) was calculated by using a two-compartment model to fit plasma C-peptide concentration time courses.[53,56] Beta-cell function was assessed as the relationship between ISR and plasma glucose concentration at 0, 10, 20 and 30 min of the oral glucose tolerance test, when plasma glucose concentrations were rapidly increasing after glucose ingestion.[53] The lipoprotein-insulin resistance (LP-IR) index was calculated by Labcorp as described.[24] Homeostasis model assessment of insulin resistance (HOMA-IR) was calculated as the product of fasting plasma insulin (in μU/mL) and glucose (in mmol/L) concentrations divided by 22.5.[22] The Matsuda index of whole-body insulin sensitivity was calculated as 10,000 divided by the square root of the product of fasting plasma insulin (in μU/mL), fasting plasma glucose (in mg/dL), mean plasma insulin, and mean plasma glucose concentrations during the first 120 minutes of a 75-g oral glucose tolerance test.[21]

Basal insulin clearance rate (ICR) was calculated as the ISR divided by the plasma insulin concentration: ICR after glucose ingestion was calculated by dividing the ISR by the insulin concentration after taking into account the amount of insulin that was secreted but not cleared or cleared in addition to the insulin that had been secreted between time points.[57] Endogenous glucose rate of appearance (R_a) during the basal period of the clamp was calculated as the [U-^{13}C]glucose infusion rate divided by the mean glucose TTR. The hepatic insulin sensitivity index was calculated as 1000 divided by the product of the endogenous glucose R_a (in μmol/kg fat-free mass/min) and the mean plasma insulin concentration (in μU/mL) during the basal period of the clamp procedure. Glucose rate of disappearance (R_d) during the steady-state period of the clamp procedure was assumed to be equal to the sum of endogenous glucose R_a and the exogenous glucose infusion rate, and skeletal muscle insulin sensitivity (glucose R_d/I) was calculated as glucose R_d divided by the mean plasma insulin concentration during the final 20 minutes of the clamp procedure. For assessment of hepatic DNL, the theoretical maximum enrichment was calculated by using mass isotopomer distribution analysis (MIDA) and total body water enrichment.[29,53] Hepatic DNL was calculated as the percent contribution of DNL to TRL-TG palmitate.[29] Estimated glomerular filtration rate was calculated using the 2021 CKD-EPI creatinine-cystatin C equation.[58]

Statistical analysis

Participants were included in the analysis of an outcome variable only if values were available both before and after weight loss. Continuous outcomes were summarized by diet group using means (SD) or mean (SEM) as indicated in legends at baseline (before weight loss) and post-intervention (after ~10% weight loss). Mean within-group changes from baseline to post-intervention are reported with 95% confidence intervals. Primary inferential analyses were performed by using ANCOVA models with post-intervention value as the dependent variable, diet group as the independent variable, and baseline value of the outcome as a covariate. For outcomes with a significant overall diet effect, adjusted marginal means were estimated and pairwise diet-group comparisons were

Please cite this article in press as: Petersen et al., Effect of diet macronutrient content on the cardiometabolic response to weight loss: A randomized clinical trial, Cell Metabolism (2026), https://doi.org/10.1016/j.cmet.2026.07.020

performed by using Tukey adjustment. Two outcomes (skeletal muscle insulin sensitivity assessed as glucose R_d/I during the steady state period of a hyperinsulinemic-euglycemic clamp procedure and the hepatic insulin sensitivity index) were specified as primary endpoints. Familywise error was controlled using Bonferroni adjustment, with statistical significance defined as $\alpha = 0.025$ for each primary endpoint. All other outcomes were considered exploratory and evaluated without multiplicity adjustment. A two-sided P value < 0.05 was considered statistically significant for secondary outcomes. To compare percent changes among diet groups for the co-primary and key secondary outcomes, one-way ANOVA with Tukey's correction for multiple comparisons or Kruskal-Wallis test with Dunn's correction for multiple comparisons was used as appropriate after assessing the data for normality and equal variance. Rates of prediabetes remission among groups (defined as fasting plasma glucose <100 mg/dL, 2-hour oral glucose tolerance test plasma glucose <140 mg/dL, and HbA1c <5.7%) were compared by using the chi-square test. Linear regression was used to assess the relationship between change in hepatic DNL and change in 24-hour plasma insulin area under the curve. In addition, a sensitivity analysis was conducted to evaluate whether drop-outs impacted the primary outcomes of skeletal muscle and hepatic insulin sensitivity. The sensitivity analysis included all available data from all randomized participants and was performed by using linear mixed-effects models with fixed effects for time, diet group, and the time x diet interaction, and a random intercept for participant. The primary sensitivity test was the time x diet interaction. Statistical analyses were performed in GraphPad Prism version 11 and R version 4.5.2 by using the broom, tidyverse, car, lme4, lmertest, and emmeans packages.

The determination of the number of participants needed to be studied to obtain adequate statistical power for our two primary endpoints (i.e. skeletal muscle and liver insulin sensitivity) was based on the results from a series of studies we have performed during the last decade in people with obesity and insulin resistance. These studies found (means ± SD) skeletal muscle insulin sensitivity (assessed as insulin-mediated glucose R_d/I during a hyperinsulinemic-euglycemic clamp procedure) was 221 ± 89 (nmol/kg FFM/min)/(μU/mL) and hepatic insulin sensitivity (assessed as 1000 divided by the product of endogenous glucose production rate and plasma insulin concentration during the basal period of a hyperinsulinemic-euglycemic clamp procedure) was 3.1 ± 1.3 [1000/[(μmol/kg FFM/min)*(μU/mL)]]. Using a baseline value of 221 ± 89 (nmol/kg FFM/min)/(μU/mL) and within-person correlation of 0.9, for skeletal muscle insulin sensitivity, we estimated that the power to detect a significant diet effect was >90% with n=15 per diet group if weight loss increased skeletal muscle insulin sensitivity by an average of 50% and the difference between groups exceeded 25%. Using a baseline value of 3.1 ± 1.3 [1000/[(μmol/kg FFM/min)*(μU/mL)]] and within-person correlation of 0.9 for hepatic insulin sensitivity, we estimated that the power to detect a significant diet effect was >90% with n=15 per diet group if moderate weight loss increased hepatic insulin sensitivity by an average of 50% and the difference between groups exceeded 25%. Power was estimated by using Monte Carlo simulation. For each candidate sample size, 1,000 datasets were generated and analyzed by ANCOVA, and power was defined as the proportion of simulations in which the effect of diet was statistically significant at a two-sided $\alpha = 0.025$. We planned to enroll an estimated n=20 participants per diet group to allow for an estimated 25% dropout rate based on our previous experience with dietary intervention studies.

ADDITIONAL RESOURCES

ClinicalTrials.gov registration: NCT02706262.