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RESEARCH ARTICLE | *Obesity, Diabetes and Energy Homeostasis*

The effect of a short-term low-carbohydrate, high-fat diet with or without postmeal walks on glycemic control and inflammation in type 2 diabetes: a randomized trial

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Myette-Côté É, Durrer C, Neudorf H, Bammert TD, Botezelli JD, Johnson JD, DeSouza CA, Little JP. The effect of a short-term low-carbohydrate, high-fat diet with or without postmeal walks on glycemic control and inflammation in type 2 diabetes: a randomized trial. *Am J Physiol Regul Integr Comp Physiol* 315: R1210–R1219, 2018. First published October 10, 2018; doi: 10.1152/ajpregu.00240.2018.—Lowering carbohydrate consumption effectively lowers glucose, but impacts on inflammation are unclear. The objectives of this study were to: *1*) determine whether reducing hyperglycemia by following a low-carbohydrate, high-fat (LC) diet could lower markers of innate immune cell activation in type 2 diabetes (T2D) and *2*) examine if the combination of an LC diet with strategically timed postmeal walking was superior to an LC diet alone. Participants with T2D ($n = 11$) completed a randomized crossover study involving three 4-day diet interventions: *1*) low-fat low-glycemic index (GL), *2*) and *3*) LC with 15-min postmeal walks (LC+Ex). Four-day mean glucose was significantly lower in the LC+Ex group as compared with LC (−5%, $P < 0.05$), whereas both LC+Ex (−16%, $P < 0.001$) and LC (−12%, $P < 0.001$) conditions were lower than GL. A significant main effect of time was observed for peripheral blood mononuclear cells phosphorylated c-Jun N-terminal kinase ($P < 0.001$), with decreases in all three conditions (GL: −32%, LC: −45%, and LC+Ex: −44%). A significant condition by time interaction was observed for monocyte microparticles ($P = 0.040$) with a significant decrease in GL (−76%, $P = 0.035$) and a tendency for a reduction in LC (−70%, $P = 0.064$), whereas there was no significant change in LC+Ex (0.5%, $P = 0.990$). Both LC (−27%,
$P = 0.001$) and LC+Ex (−35%, $P = 0.005$) also led to significant reductions in circulating proinsulin. An LC diet improved 4-day glycemic control and fasting proinsulin levels when compared with GL, with added glucose-lowering benefits when LC was combined with postmeal walking.

cytokines; exercise; glucose; ketogenic diet

INTRODUCTION

Inflammation is associated with the pathogenesis of insulin resistance, type 2 diabetes (T2D), and related complications. Immune cells become activated and infiltrate various tissues, contributing to a state of chronic low-grade inflammation (8). High glucose promotes proinflammatory cytokine release, elevates surface protein expression of the key innate immune cell activator toll-like receptor (TLR) 4, potentiates proinflammatory signaling pathways [e.g., c-Jun NH_2-terminal kinase (JNK)], and causes the release of characteristic proinflammatory microparticles (MPs) in monocytes/macrophages (10, 20, 44). In T2D, elevated levels of circulating proinflammatory cytokines (52), increased monocyte TLR4 expression (9), overactivation of the JNK pathway in peripheral blood mononuclear cells (PBMCs) (58), and elevated circulating monocyte-derived MPs (MMPs) (35) have been repeatedly demonstrated, linking mechanistic work with clinical features of T2D. Thus, high glucose appears to trigger the activation of innate immune cells, suggesting that hyperglycemia itself drives a vicious cycle of inflammation and insulin resistance in T2D.

Lifestyle therapy is a frontline treatment for improving glucose control in people with T2D. Most current dietary guidelines advocate a "healthy" diet low in saturated fat and sugar, with emphasis on low glycemic index (GI) foods. However, accumulating evidence shows that consuming a low-carbohydrate high-fat (LCHF) diet is more effective for lowering glucose (60). In a companion paper, we demonstrated that a short-term LCHF diet quickly stabilizes glucose in patients with T2D (17). However, whether the rapid improvement in glucose control is accompanied by lowered inflammation has, to our knowledge, not been tested. It is possible that factors related to T2D pathophysiology, other than hyperglycemia, are more important in driving chronic inflammation. It is therefore unknown whether lowering glucose by following an LCHF diet can reduce innate immune cell activation and inflammation in T2D.

Consuming an LCHF diet with less than 130 g of carbohydrates is contraindicated by the American Diabetes Association, partly because of the perceived negative impacts of consuming high amounts of fat (1a). Acute feeding studies indicate that an isolated "high-fat meal" increases inflammation and triggers proinflammatory activation of immune cells, particularly in individuals with insulin resistance or T2D (5, 48). Thus, attempting to lower hyperglycemia using an LCHF diet may have unintended consequences. Strategically timed exercise may be one way to counteract this. Padilla et al. (47) have shown that exercising in the postprandial period reversed

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the detrimental impacts of a high-fat meal on endothelial function. Additionally, several recent studies have highlighted the benefits of postmeal walking exercise on glycemic control (55). Combining an LCHF diet with postmeal exercise may be an optimal combination for improving glucose control and reducing inflammation.

The objective of the present study was to determine whether reducing hyperglycemia with an LCHF diet could lower markers of innate immune cell activation and systemic inflammation in people with T2D. A secondary objective was to examine if the combination of an LCHF diet with strategically timed postmeal walking was superior to an LCHF diet alone. To reduce the confounding influence of both weight loss and long-term adaptation to an LCHF diet (59) we employed a 4-day controlled feeding protocol in attempts to isolate the direct effects of lowering glucose using a whole food LCHF diet, with or without exercise. A control condition involving low GI, low-fat whole foods was employed to directly compare the LCHF conditions to "gold-standard" dietary guidelines (10a). The primary outcome was glucose control, defined as average glucose assessed by continuous glucose monitoring (CGM). Secondary outcomes included a comprehensive array of systemic, cellular, and molecular indices of inflammation from plasma and PBMCs, along with standard markers of metabolic control.

METHODS

Ethical approval. The study was approved by the University of British Columbia Clinical Research Ethics Board (ID H15-01952) and was registered on clinicaltrials.gov (NCT02683135). The study conformed to the standards set by the Declaration of Helsinki. Participants provided written informed consent before study commencement during the initial screening visit.

Overview. Individuals with physician-diagnosed T2D [glycated hemoglobin (HbA1c) >6.5%, fasting plasma glucose (FPG) >7.0 mmol/l, or 2-h glucose oral glucose tolerance test >11.1 mmol/l (32)] were recruited to complete three short-term controlled-intervention periods in a randomized crossover design: *1*) low-fat low-GI diet (GL), *2*) low-carbohydrate high-fat diet (LC), and *3*) LC with 15-min postmeal walks (LC+Ex). The randomization sequence was generated by a computer random number generator and was revealed to study staff from a master list after the participant provided informed consent. Neither participants nor staff were blinded. All food was provided to participants using a local service (https://mealprepforyou.ca/), and diets were matched for energy and protein content. Fasting blood samples following an overnight fast were collected before and after each experimental intervention. Accelerometers (Actigraph wGTX3+) were worn to monitor activity for all conditions and confirm the completion of postmeal walks.

Participants. Participants (n = 16; 8 men and 8 women) aged between 48 and 72 yr, not on exogenous insulin, and without diagnosed cardiovascular, kidney, or any other diabetes complications were recruited from the local community in Kelowna, BC, Canada from November 2015 to March 2017. Individuals currently involved in a regular exercise routine (>3 days of structured exercise per week), following an LCHF diet, or unwilling to consume the provided meat-containing diets were excluded. Five of the sixteen participants did not complete all three conditions because of family reasons (n = 1), inability or unwillingness to follow study diets (n = 3), and change of medications (n = 1, addition of sodium-glucose cotransporter-2 inhibitor after completing one condition) (Fig. 1). Participants were instructed to take their medications as usual during the three experimental conditions, and their compliance was assessed with a medication log.

This study was designed as a pilot trial to generate effect sizes to guide a larger randomized controlled trial. Nygaard et al. (46) previously demonstrated an ~15% reduction in postprandial glucose area under the curve with postmeal walking. With the use of means and SD for CGM from the literature and our own T2D studies (15), a sample size of 11 would be needed to detect a 15% difference in mean glucose with 80% power at an alpha of 0.05, assuming a correlation among repeated measures of r = 0.7 (G*Power v3.1.9.3). To account for drop-outs and/or missing data, we aimed to recruit 16 patients with T2D.

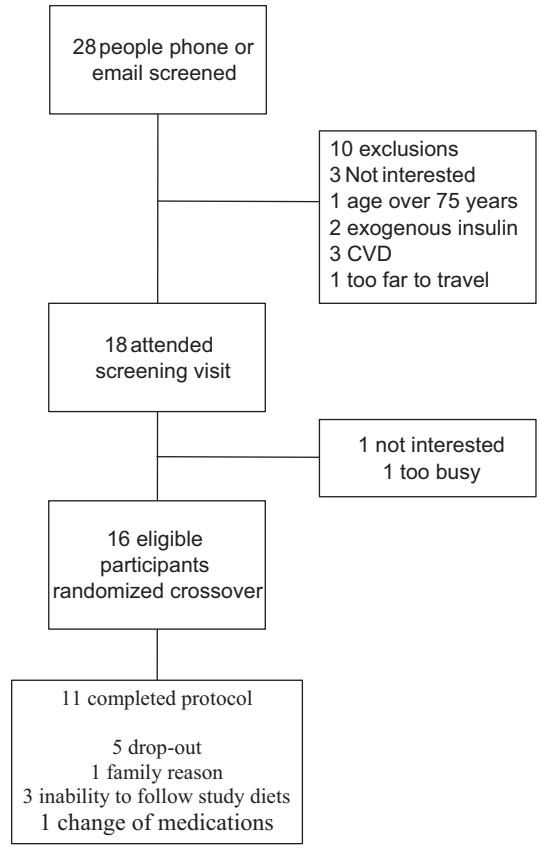

Fig. 1. Consolidated standards of reporting trials flow diagram. CVD, cardiovascular disease.

Experimental protocol. During the initial screening visit, anthropometric measurements (height, body weight, body mass index, and waist circumference) were taken, and both a physical activity readiness (PARQ+) and Godin leisure-time exercise questionnaires were filled out, followed by the three randomized 4-day diet interventions. The 24-h period preceding each diet intervention was standardized for physical activity and included a standardized mixed meal in the evening followed by a ≤10-h fast. A washout period of 9–14 days was introduced between each intervention where participants were asked to return to their regular diet and physical activity habits. Fasting blood samples were collected at the same time in the morning before and after each 4-day intervention for future analysis. During each intervention, a CGM (iPro2 professional CGM, Medtronic, Northridge, CA and Enlite sensor) was worn to measure glycemic control. Average blood glucose across 4 days was the primary outcome. Area under the curves were calculated using the trapezoid method (34), and the mean amplitude of glycemic excursion (MAGE) and continuous overall net glycemic action (CONGA) were computed using EasyGV (version 9.0.R2., University of Oxford). All intervention diets were isoenergetic, with calories estimated using the Harris Benedict equation (27) and habitual intake (i.e., matched from first trial). An example meal plan for 1 day of each diet is provided in Table 1.

Table 1. *Composition of study diets*

Diet	GL	LC	LC+Ex
Carbohydrate/protein/fat, %	55/25/20	10/25/65	10/25/65
Sat./poly./mono. fat, %	5/5/10	15/11/39	15/11/39
Mean glycemic index	40	N/A	N/A
	Breakfast		
		150 g whole egg	150 g whole egg
	95 g oats	110 g egg whites	110 g egg whites
	25 g whey	55 g avocado	55 g avocado
	30 g blueberries	30 g peppers	30 g peppers
	30 g raspberries	40 g onions	40 g onions
		40 g carrots	40 g carrots
		10 g almonds	10 g almonds
	Lunch		
		105 g ground turkey	105 g ground turkey
	105 g chicken breast	38 g cashews	38 g cashew
	230 g yams	15 g olive oil	15 g olive oil
	40 g green beans	35 g spinach	35 g spinach
	17 g cashews	30 g carrots	30 g carrots
		30 g cucumber	30 g cucumber
	Dinner		
		100 g steak (rib eye)	100 g steak (rib eye)
	100 g turkey	30 g cashews	30 g cashews
	85 g brown rice	13 g olive oil	13 g olive oil
	14 g cashews	30 g apple	30 g apple
	40 g broccoli	30 g spinach	30 g spinach
		50 g cucumber	50 g cucumber
	Snack		
	1 × Solo bar (Solo GI Nutrition)	50 g cheddar cheese	50 g cheddar cheese 20 g almonds

Menu shown is an individual example based on a daily energy intake of ~1,700 kcal, including 3 meals of 500 kcal each and 200 kcal snacks for a 68-yr-old female participant with an estimated energy expenditure of 1,700 kcal per day (body mass of 68.2 kg, height 1.51 m, physical activity level of 1.3). In the low-carbohydrate high-fat diet (LC) and exercise (LC+Ex) condition, 135 kcal were added to compensate for the daily postmeal walks. GL, low-fat low-glycemic index guidelines diet; Mono., monounsaturated fat; Poly., polyunsaturated fat; Sat., saturated fat.

GL diet. The GL diet followed the current dietary guidelines for adults with T2D, comprising low-fat, low-GI whole foods (12). Each meal comprised ~55% of total energy from carbohydrates (predominately from low-GI and high-fiber carbohydrate sources), 20% energy from fat (aiming for <7% saturated fatty acids), and 25% protein (primarily from lean meats).

LC diet. The LC diet provided the same energy content as the GL diet but with carbohydrates limited to ~10% of total energy. The percent protein was matched at ~25%, with the remainder of the energy coming from fat (~65% of total kcal).

LC+Ex diet. Participants performed 15 min of walking beginning ~30 min after breakfast, lunch, and dinner. The exercise intensity of the postmeal walking was light-to-moderate, which was confirmed on *day 1* of the intervention by having participants walk on a horizontal treadmill in the laboratory at a comfortable pace that elicited a rating of perceived exertion (CR-10 scale) of 3 ± 1 (equating to ~60% of maximal heart rate, 93 ± 13 beats/min). Participants were instructed to replicate this pace at home for each postmeal walk. Accelerometers were worn to confirm compliance and intensity. Participants consumed the same diet as the LC intervention but with the addition of the estimated individualized calories expended over the 3 daily 15-min postmeal walks [(2.5 METs × 3.5 × weight (kg) × 45 (min)/200)] (1).

Hormones and metabolites. Morning fasting blood samples were collected by venipuncture using a 21-gauge needle into EDTA tubes and were centrifuged at 1,550 *g* for 15 min at 4°C. Following centrifugation, plasma samples were stored at −80°C for batch analyses. Plasma triglycerides (TGs) (Pointe Scientific) and glucose (Pointe Scientific) were analyzed on a Chemwell 2910 automated analyzer (Awareness Technologies). Plasma insulin (Human Insulin ELISA, Crystal Chem) was analyzed on an iMark Microplate Absorbance Reader (Bio-Rad). Active proinsulin (ALPCO, STELLUX Chemi Human Total Proinsulin ELISA) was analyzed on a POLARstar Omega plate reader (BMG Labtech, Durham, NC). Plasma C-peptide was measured by Meso Scale Discovery C-peptide Singleplex (MD). All assays were run in duplicate. The coefficient of variation for duplicate samples was 2.9% (TGs), 3.0% (glucose), 5.0% (insulin), 4.5% (proinsulin), and 6.6% (C-peptide).

Inflammatory markers. Fasting plasma concentrations of tumor necrosis factor-α (TNF-α), monocyte chemoattractant protein-1 (MCP-1), interleukin-6 (IL-6), IL-10, and IL-18 were analyzed by multiplex immunoassay (U-PLEX Human Panel, Meso Scale Discovery) and read on a MESO Quickplex SQ 120. Plasma was centrifuged at 1,500 *g* for 15 min at 4°C to remove debris and analyzed in duplicate. The coefficient of variation was 7.6% (TNF-α), 4.6% (MCP-1), 5.7% (IL-6), 7.9% (IL-10), and 4.1% (IL-18).

Flow cytometry. Fc receptor-blocking reagent (130–059–901; Miltenyi Biotec, Bergisch Gladbach, Germany) was added to 90 μl of whole blood and incubated for 10 min at 4°C in the dark. Conjugated antibodies (all Miltenyi Biotec) for human CD14 (Vioblue, 130-094-364), TLR2 (phycoerythrin, 130-099-016), and TLR4 (allophycocyanin, 130-096-236) were added, followed by a 10-min incubation at 4°C in the dark. Next, 1 ml of red blood cell lysis buffer (120-001-339, Miltenyi Biotec) was added, followed by a 15-min incubation at room temperature (RT) in the dark. Two microliters of propidium iodide (130–093–233; Miltenyi Biotec) were added for dead cell exclusion, and samples were analyzed on a MACSQuant Analyzer 10 flow cytometer. Ten thousand monocytes were counted in each sample, and data were analyzed with MACSQuantify version 2.6 (Miltenyi Biotec). CD14+ monocytes were identified via a hierarchi-

Table 2. *Characteristics of participants*

Characteristic	Value
Number of participants (M/F)	11 (4/7)
Age, yr	64 (8)
Body mass index, kg/m^2	34.0 (8.0)
Waist circumference, cm	105 (13)
Systolic blood pressure, mmHg	124 (9)
Diastolic blood pressure, mmHg	77 (5)
Glycated hemoglobin, %	7.0 (1.0)
Time since diagnosis, yr	6.4 (4.3)
Medications	
MET, *n*	5
MET + SU, *n*	2
MET + GLP-1, *n*	1
MET + SU + DPP4, *n*	1
Statin, *n*	3
Antihypertensive, *n*	3

Data are presented as mean (SD), except for number of participants (*n*) (count; male/postmenopausal female) and medications (count). DPP4, dipeptidyl peptidase-4; F, female; GLP-1, glucagon-like-peptide-1; M, male; MET, metformin; SU, sulfonylurea.

cal gating strategy. Specifically, cells that stained positive for propidium iodide were excluded, and then cells were characterized as CD14+ and confirmed to be monocytes via a characteristic scatter profile. TLR2 and TLR4 median fluorescence intensity were then determined on CD14+ monocytes with fluorescence minus one control used to determine positive and negative populations. Total granulocyte, monocyte, and lymphocyte numbers were determined based on characteristic scatter profiles.

Monocyte and leukocyte-derived MPs. MMPs and leukocyte-derived MPs (LMPs) were characterized using flow cytometry, as previously described (4, 16). Plasma samples were centrifuged at 13,000 *g* for 2 min, and 200 μl of platelet-free plasma were transferred to TruCount tubes (BD Biosciences). MP size threshold was established using Megamix-Plus SSC calibrator beads (Biocytex, Marseille, France), and only events <1 μm in size were counted. MPs were defined as events falling within the established size ranges (0.16, 0.20, 0.24, and 0.5 μm). Monocyte- (CD14+) and leukocyte- (CD45+) specific antibodies (BioLegend, San Diego, CA) identified MMPs and LMPs for events falling within the respective MP size range. Samples were incubated with antibodies for 20 min in the dark at RT, fixed with 2% paraformaldehyde (ChemCruz Biochemicals, Santa Cruz, CA), diluted with PBS, and analyzed using BD Biosciences FACSAria I High Speed Cell sorter and flow cytometer (University of Colorado Anschutz Medical Campus, Allergy and Clinical Immunology/Infectious Disease Flow Core). The concentration of MMPs and LMPs was determined using the formula: [(number of events in region containing MPs/number of events in absolute count bead region) × (total number of beads per test/total volume of sample)]. MP analyses were performed on $n = 8$ subjects.

Western blotting analysis. PBMCs were extracted from ~8 ml of whole blood using Leucoep tubes (163288, Greiner Bio-One International, Kremsmunster, Austria), according to the manufacturer's instructions, and stored at −80°C for batch analyses. Samples were lysed by sonication in ice-cold radioimmunoprecipitation assay buffer (50 mM Tris·HCl, 1% Triton X-100, 0.5% sodium deoxycholate, 0.1% SDS, 150 mM NaCl) containing protease (cat. no. 04693159001; complete, Protease Inhibitor Cocktail, Roche) and phosphatase (100 mM $NaVO_3$, 20 mM NaF, 10 mM EDTA) inhibitors and centrifuged (14,000 *g* for 20 min). Protein was quantified in supernatants with the bicinchoninic acid assay (cat. no. 23225, Pierce BCA Protein Assay Kit), and 20-μg samples were prepared in Laemmli Buffer (cat. no. 7722, Blue Loading Buffer, 1.25 M DTT), heated at 95°C for 5 min, and separated using 12% SDS-polyacrylamide gel electrophoresis (cat. no. 170–3930, Mini Trans-Blot, Bio-Rad). Proteins were electrotransferred to polyvinylidene difluoride membranes using Towbin buffer (25 mM Tris·HCl, 192 mM glycine, 20% methanol, pH 8.3) for 3 h at 70 V. Membranes were stained with Ponceau S Solution (P7170, Sigma-Aldrich) to verify the efficiency of protein transfer. Membranes were blocked in Tris-buffered saline-Tween 20 (TBS-T) + iBlock solution (20 mM Tris·HCl, 140 mM NaCl, 0.1% Tween-20, 0.2% iBlock) for 1 h at RT and then incubated in primary phospho-stress-activated protein kinase (SAPK)/Jun NH_2-terminal kinase (JNK) (Thr183/Tyr185; p-JNK) antibody (1:1,000, cat. no. 4668, Cell Signaling Technology) in TBS-T + iBlock solution overnight at 4°C, followed by secondary anti-rabit IgG horseradish peroxidase-linked antibody (1:5,000, cat. no. 7074, Cell Signaling Technology) in TBS-T + iBlock solution for 2 h at RT. Proteins were detected using Luminata Forte HRP Substrate (cat. no. WBLUF0100, Millipore Sigma), and the images were acquired using Amersham Hyperfilm ECL (cat. no. 28906835, GE Life Sciences). Membranes were stripped (cat. no. 21059, Thermo Fisher Scientific) for 20 min at RT and blocked again in TBS-T + iBlock. The same procedure was adopted to quantify total SAPK/JNK (1:1,000, cat. no. 9252, Cell Signaling Technology). Results were quantified using Adobe Photoshop CC 2017 and relativized to the protein content using the Ponceau staining as described by Gilda et al. (21). Samples from each participant were run on the same gel, and bands were expressed relative to an internal standard containing pooled human PMBC lysate included in each blot. PBMC analyses were performed on $n = 6$ participants because of sample availability.

Statistical analysis. Data were analyzed using SPSS v.21 (SPSS, Chicago, IL). Normality was assessed using Q-Q plots and Shapiro-Wilk tests within each experimental condition. Four-day CGM data were analyzed using a one-way repeated-measures ANOVA with Fisher's least-significant difference post hoc testing. A linear mixed-effects model (condition and time as fixed factors, subject as random factor) was used to determine the treatment effects for all inflammatory and metabolic markers. Significant interactions and time effects were followed up with preplanned contrasts comparing pre- versus postintervention within each condition. Cohen's *d* effect size was calculated for these preplanned comparisons within each condition.

Table 3. *Four-day continuous glucose monitoring*

	GL	LC	LC+Ex	ANOVA	GL vs. LC	GL vs. LC+Ex	LC vs. LC+Ex
Mean, mmol/l	7.4 (1.6)	6.5 (1.2)	6.2 (1.1)	**0.001**	**0.000**	**0.001**	**0.049**
SD, mmol/l	1.7 (0.9)	0.8 (0.3)	0.8 (0.4)	**0.001**	**0.002**	**0.003**	0.476
MAGE, mmol/l	4.3 (2.2)	2.0 (1.0)	1.7 (0.9)	**0.000**	**0.000**	**0.001**	0.109
CONGA, mmol/l	6.6 (1.4)	6.2 (1.1)	5.9 (1.0)	**0.004**	**0.005**	**0.005**	**0.037**
Time > 10 mmol/l, %	13.5 (18.1)	2.3 (5.5)	1.3 (3.9)	**0.020**	**0.018**	**0.022**	0.218
Time < 4 mmol/l, %	0.3 (0.7)	0.5 (1.1)	1.1 (2.4)	0.332			

Data are presented as mean (SD). *P* values are shown for the overall one-way repeated-measures ANOVA and for the Fisher least-significant difference post hoc tests. Please see text for effect sizes. CONGA, continuous overall net glycemic action; GL, low-fat low-glycemic index guidelines diet; LC, low-carbohydrate high-fat diet; LC+Ex, low-carbohydrate high-fat diet and exercise; MAGE, mean amplitude of glycemic excursion. Values in bold denote *P* values with statistical significance.

Table 4. *Fasting hormones and metabolites before (pre) and after (post) each four-day diet intervention*

	GL		LC		LC+Ex		Linear Mixed-Model		
	Pre	Post	Pre	Post	Pre	Post	Time	Condition	Time × Condition
Glucose, mmol/l	8.3 (2.1)	8.1 (2.0)	8.4 (1.9)	7.6 (1.7)*	7.8 (1.8)	7.0 (1.5)*	**0.000**	0.059	0.144
Triglycerides, mmol/l	2.0 (1.1)	1.9 (0.7)	1.9 (1.0)	2.1 (1.2)	1.9 (0.9)	1.9 (1.3)	0.723	0.506	0.540
Insulin, pmol/l	63.9 (33.5)	58.0 (29.2)	64.8 (29.7)	62.1 (46.4)	59.6 (38.7)	50.1 (38.9)	0.077	0.655	0.558
Proinsulin, pmol/l	33.5 (14.7)	30.7 (16.1)	35.5 (15.1)	26.0 (12.8)†	35.3 (21.1)	22.8 (14.0)*	0.173	**0.034**	**0.000**
C-peptide, nmol/l	1.11 (0.39)	1.18 (0.52)	1.18 (0.38)	1.07 (0.45)	1.20 (0.64)	1.05 (0.51)	0.558	0.341	0.268
Proinsulin-insulin ratio	0.7 (0.5)	0.6 (0.3)	0.7 (0.5)	0.6 (0.3)	0.6 (0.3)	0.6 (0.5)	0.141	0.711	0.516
Proinsulin-C-peptide ratio	0.031 (0.013)	0.027 (0.012)	0.030 (0.013)	0.025 (0.008)*	0.032 (0.016)	0.022 (0.011)‡	**0.000**	0.563	0.331

Data are presented as mean (SD). GL, low-fat low-glycemic index guidelines diet; LC, low-carbohydrate high-fat diet; LC+Ex, low-carbohydrate high-fat diet and exercise. *$P \leq 0.01$, †$P = 0.001$, ‡$P < 0.05$ for preplanned contrast vs. pre within condition. Values in bold denote P values with statistical significance.

Significance was set at $P < 0.05$. Data in Tables 2–5 and Figs. 2–4 are presented as mean (SD).

RESULTS

Baseline characteristics and body weight changes. Characteristics of the participants who completed the study are shown in Table 2. Leisure-time exercise per week was 0.1 ± 0.3 for strenuous exercise, 1.7 ± 0.9 for moderate exercise, and 2.3 ± 0.7 for light exercise. The average total score [arbitrary units calculation: (9 × strenuous) + (5 × moderate) + (3 × light)] at baseline was 16.3 ± 4.5. A significant overall time effect was observed for body weight, which decreased by −2.0 ± 1.0 kg, −2.0 ± 0.8 kg, and −1.9 ± 1.1 kg for LC, LC+Ex, and GL, respectively ($P < 0.001$), with no condition by time interaction ($P = 0.969$).

Accelerometer. One-way repeated measures ANOVA showed a significant effect for length of time at moderate intensity over the 4-day interventions (235 ± 136) in LC+Ex compared with LC (75 ± 92) and GL (117 ± 137) ($P < 0.001$). As expected, total moderate intensity minutes in LC+Ex were higher when compared with LC (161 ± 107 min, $P = 0.01$, $d = 4.5$) and GL (119 ± 88 minutes, $P = 0.02$, $d = 3.8$) with no significant difference between LC and GL. Time spent in sedentary, light, and vigorous intensity activity was similar between all conditions (data not shown).

CGM. Four-day CGM data are presented in Table 3. One-way repeated measures ANOVA showed a significant effect for 4-day mean, standard deviation, MAGE, CONGA, and time over 10 mmol/l (all $P < 0.02$). Four-day mean glucose was significantly lower in the LC+Ex as compared with LC (−5%, $P < 0.05$, $d = 0.8$), whereas both LC+Ex and LC conditions were lower than GL (−16%, $P < 0.001$, $d = 1.9$ and −12%, $P < 0.001$, $d = 2.3$, respectively) (Fig. 2*A*). CONGA was also significantly lower in LC+Ex as compared with LC (−5%, $P < 0.05$, $d = 0.9$), whereas both LC+Ex and LC conditions were lower than GL (−11%, $P < 0.01$, $d = 1.2$ and −6%, $P < 0.01$, $d = 1.2$, respectively). LC+Ex and LC were not significantly different but were both respectively lower than GL for standard deviation (both −53%, $P < 0.01$, respectively, $d = 1.6$ and $d = 2.4$), MAGE (−61%, $P \leq 0.001$, $d = 2.1$ and −54%, $P < 0.001$, $d = 2.8$, respectively) (Fig. 2*B*), and time over 10 mmol/l (−90%, $P < 0.05$, $d = 2.0$ and −83%, $P < 0.05$, $d = 2.5$). No significant difference between conditions was observed for time under 4 mmol/l.

Metabolites and hormones. Metabolites and hormone data are presented in Table 4, and the change from baseline for fasting glucose, C-peptide, insulin, and proinsulin is presented in Fig. 3. A significant condition by time interaction was found for proinsulin ($P < 0.001$), with a significant decrease in LC (−27%, $P = 0.001$, $d = 1.5$) and LC+Ex (−35%, $P = 0.005$,

Table 5. *Fasting inflammatory markers before (pre) and after (post) each four-day diet intervention*

	GL		LC		LC+Ex		Linear Mixed-Model		
	Pre	Post	Pre	Post	Pre	Post	Time	Condition	Time × condition
TNF-α, pg/ml	14.6 (4.6)	15.7 (4.6)	14.1 (3.6)	14.8 (4.6)	14.8 (4.1)	15.7 (4.8)	0.200	0.660	0.968
MCP1, pg/ml	725 (201)	710 (222)	727 (217)	686 (229)	769 (173)	680 (156)*	**0.045**	0.859	0.449
IL-6, pg/ml	8.1 (2.8)	9.1 (4.7)	8.2 (3.8)	8.8 (3.6)	9.9 (3.1)	9.2 (3.2)	0.608	0.272	0.559
IL-18, pg/ml	2,810 (1,217)	2,760 (1,152)	2,838 (1,199)	2,818 (1,527)	2,691 (997)	2,658 (1,081)	0.755	0.646	0.994
IL-10, pg/ml	3.6 (2.9)	3.3 (2.8)	3.5 (3.4)	3.3 (2.4)	3.8 (2.9)	3.8 (2.9)	0.461	0.830	0.887
p-JNK, A.U.	100 (30)	68 (20)†	105 (40)	58 (19)†	73 (39)	41 (16)†	**0.000**	**0.008**	0.616
TLR2, MFI	7.5 (1.3)	6.9 (0.9)	7.4 (1.9)	6.9 (0.7)	7.8 (1.4)	7.7 (2.2)	0.211	0.228	0.852
TLR4, MFI	4.7 (0.3)	4.8 (0.3)	5.0 (0.4)	5.0 (0.4)	5.0 (0.4)	5.2 (0.5)	0.149	**0.014**	0.617
MMPs, count/ml	404 (330)	97 (55) †	245 (203)	73 (58)	238 (183)	239 (192)	**0.002**	0.236	**0.040**
LMPs, count/ml	1,313 (999)	490 (311)	1,055 (1,317)	890 (1,380)	620 (281)	565 (260)	0.067	0.837	0.206
Granulocytes, count/ml $\times 10^6$	2.4 (0.6)	2.9 (0.6)	2.6 (0.5)	2.6 (0.4)	2.9 (1.1)	2.5 (0.5)	0.539	0.640	0.204
Lymphocytes, count/ml $\times 10^6$	1.2 (0.2)	1.4 (0.4)	1.3 (0.3)	1.3 (0.3)	1.3 (0.2)	1.2 (0.4)	0.591	0.586	0.215
Monocytes, count/ml $\times 10^5$	2.7 (0.6)	3.0 (0.7)	2.6 (0.8)	2.7 (0.6)	3.0 (1.1)	2.6 (0.4)	0.980	0.578	0.273

Data are presented as mean (SD). A.U., arbitrary units; GL, low-fat low-glycemic index guidelines diet; LC, low-carbohydrate high-fat diet; LC+Ex, low-carbohydrate high-fat diet and exercise; LMPs, leukocyte-derived microparticles; MFI, median fluorescence intensity; MMPs, monocyte-derived microparticles; p-JNK, phosphorylated c-Jun NH_2-terminal kinase; TLR, toll-like receptor; TNF-α, tumor necrosis factor-α. *$P < 0.01$, †$P < 0.05$ for preplanned contrast vs. pre within condition. Values in bold denote P values with statistical significance.

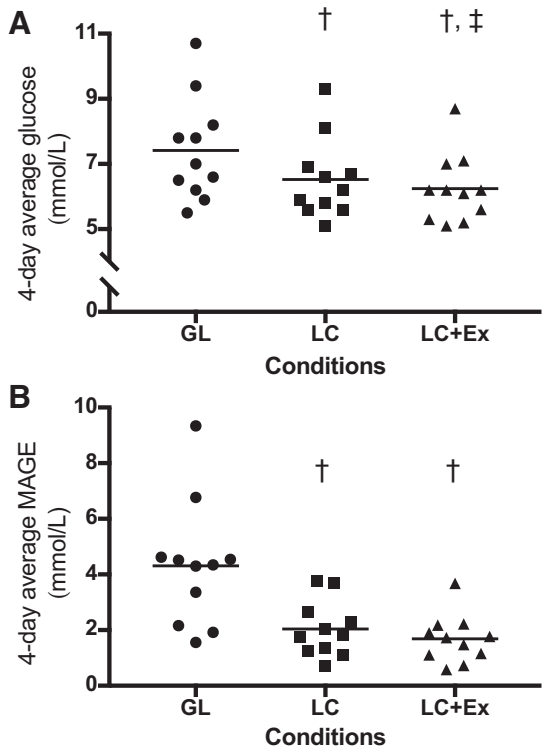

Fig. 2. Mean glucose and mean amplitude of glycemic excursions (MAGEs) from continuous glucose monitoring during each 4-day diet intervention. Mean glucose (*A*) and MAGE (*B*) calculated from continuous glucose monitoring throughout each intervention. Fisher least-significant difference post hoc tests following significant one-way repeated-measures ANOVA; GL, low-fat low-glycemic index guidelines diet; LC, low-carbohydrate high-fat diet; LC+Ex, low-carbohydrate high-fat diet and exercise. †$P \leq 0.001$ vs. CON. ‡$P < 0.05$ vs. LC.

$d = 1.5$) but not for GL (−8%, $P = 0.065$, $d = 0.7$). A significant main effect of time was observed for fasting glucose and proinsulin-C-peptide ratio (both $P < 0.001$). In preplanned contrasts, only LC+Ex and LC decreased fasting glucose (−10%, $P = 0.007$, $d = 1.3$ and −10%, $P = 0.011$, $d = 1.0$, respectively) and proinsulin-C-peptide ratio (−31%, $P = 0.045$, $d = 0.7$ and −17%, $P = 0.004$, $d = 1.2$, respectively).

Inflammatory markers. Inflammatory markers are presented in Table 5. Western blot images of p-JNK and total JNK in PBMCs, as well as change from baseline for p-JNK, are shown in Fig. 4, and MMPs and LMPs are presented in Fig. 5. A significant overall time effect was observed for MCP-1 ($P = 0.045$) and p-JNK ($P < 0.001$). In preplanned contrasts, only LC+Ex decreased MCP-1 (−12%, $P = 0.003$, $d = 1.3$), whereas p-JNK decreased in all three conditions (all $P < 0.05$, GL: −32%, $d = 1.6$; LC: −45%, $d = 1.7$; LC+Ex, −44%, $d = 1.4$). Despite the randomized crossover design, a main effect of condition was observed for p-JNK and TLR4 (both $P \leq 0.01$). Levels of p-JNK were overall lower in LC+Ex as compared with GL and LC, whereas overall TLR4 was lower in GL as compared with LC and LC+Ex. There were no differences in total granulocyte, monocyte, or lymphocyte cell counts with any of the interventions (Table 5). A significant condition by time interaction was observed for MMPs ($P = 0.040$) with a significant decrease in GL (−76%, $P = 0.035$, $d = 1.8$) and a tendency for a reduction in LC (−70%, $P = 0.064$, $d = 0.9$), whereas there was no significant change in LC+Ex (0.5%, $P = 0.990$, $d = 0.0$). For LMPs, there was a tendency for a significant main effect of time ($P = 0.067$), with exploratory pairwise comparisons within conditions showing tendencies for reductions in GL (−63%, $P = 0.055$, $d = 1.2$) and LC (−16%, $P = 0.057$, $d = 0.9$) but not LC+Ex (−9%, $P = 0.516$, $d = 0.3$).

DISCUSSION

The main objective of this study was to determine whether reducing hyperglycemia by following an LCHF diet alone, or in combination with postmeal walking, could lower markers of innate immune cell activation and systemic inflammation in people with T2D. Our study showed that while LC and LC+Ex led to superior improvements in glucose control and fasting proinsulin levels as compared with GL, all three diets appeared to lower PBMC p-JNK (a marker of cellular inflammation) over the short-term.

LCHF diets improve glucose control. Recently, LCHF diets have been recommended as a first-line treatment for improving glucose control in T2D (14). In studies lasting between 6 and 12 mo, LCHF diets have been shown to reduce HbA1c and fasting glucose to a greater extent than a traditional western diet or a low-glycemic diet (25, 33). In line with our results, the glucose-lowering effect of carbohydrate restriction can be observed quickly with no or very minimal weight-loss (18, 45). In addition to restricting carbohydrates, light walks performed around meal times have been shown to lower glucose excursions (7, 11). To the best of our knowledge, our study is the first to test the combined strategy of an LCHF diet with postmeal walks (LC+Ex) with findings showing that this approach can improve 24-h glucose control to a greater extent than both an LC diet alone or a GL diet. The LC diet alone was also clearly effective at lowering 24-h glucose when compared with GL. Change in fasting glucose was not different between conditions, but a significant decrease within LC and LC+Ex of a similar magnitude as a previous 7-day low-carbohydrate diet study in individuals who were overweight/obese was observed (49). The short duration of the current study might explain the absence of change in fasting glucose, as significant weight loss or longer-term metabolic adaptations may be needed to reduce hepatic glucose output and/or insulin resistance (50, 60). The dysglycemia of diabetes is characterized by sustained chronic hyperglycemia, postprandial glucose fluctuations, and increased glucose variability (40). As compared with GL, both LC and LC+Ex improved all of these glycemic outcomes, including MAGE, which has been shown to be strongly and positively correlated to oxidative stress and inflammation markers (41). The activation of oxidative stress and inflammatory pathways contributes to insulin resistance and diabetes complications (19, 28). Therefore, lowering overall hyperglycemia and glycemic variability in LC and LC+Ex may be advantageous in T2D.

Impact of short-term dietary interventions on inflammation. The phosphorylation of JNK is regarded as a key signaling node controlling inflammation by activating transcription factors that result in the production of proinflammatory cytokines and chemokines (6, 61). Murine studies support a causal role of myeloid JNK activation in the progression of insulin resistance, adipose tissue macrophage accumulation, and systemic

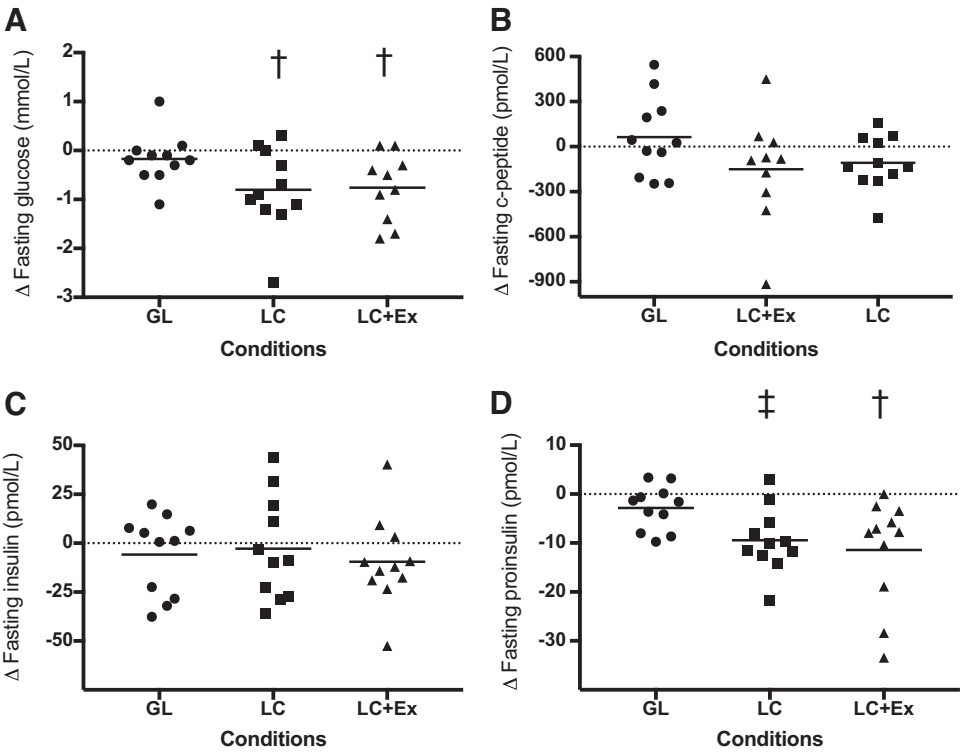

Fig. 3. Changes in (Δ) fasting glucose, C-peptide, insulin, and proinsulin following each 4-day diet intervention. Fasting glucose (*A*), fasting C-peptide (*B*), fasting insulin (*C*), and fasting proinsulin (*D*) presented as change scores (post − pre within each condition). Linear mixed models revealed a significant main effect of time for fasting glucose ($P < 0.001$) and a significant condition × time interaction for proinsulin ($P < 0.001$). GL, low-fat low-glycemic index guidelines diet; LC, low-carbohydrate high-fat diet; LC+Ex, low-carbohydrate high-fat diet and exercise. †$P \leq 0.01$ and ‡$P = 0.001$ for preplanned contrast of post vs. pre within condition.

inflammation (26). JNK activation is increased in T2D and can be triggered by several metabolic stressors, such as elevated glucose, circulating free fatty acids, insulin, cytokines, and oxidative stress (54, 56, 57). Thus, activation of JNK in PBMCs may represent both a systemic marker of inflammation and an underlying signaling pathway involved in T2D pathophysiology. In the present study, all three short-term dietary interventions led to significant reductions in PBMCs p-JNK. It is possible that the small (~2 kg) weight loss experienced in each intervention could contribute to the reduction in JNK activation. Since body weight went back to preintervention during washout periods, it is likely that participants were consuming slightly fewer calories during the three 4-day periods as compared with baseline. Alternatively, all three dietary conditions may have been less proinflammatory than the typical dietary pattern of participants, given that we provided minimally processed foods with low sugar and refined carbohydrate content.

Cell-derived MPs are small (~100–1,000 μm) plasma membrane vesicles that are released by most eukaryotic cells in response to activation and/or apoptosis. Depending on the cell of origin, circulating MPs exert distinct biological effects (38, 53). MPs derived from monocytes and leukocytes are known to play a role in inflammation (2, 24). For example, MMPs have been shown to induce an upregulation in endothelial cell expression of intracellular cell adhesion molecule-1 and promote T cell infiltration into the vessel wall, enhancing plaque formation (32). In addition, LMPs impact the endothelial monolayer, stimulating inflammatory processes, such as the JNK signaling pathway (39), and the recruitment of chemotactic cytokines (2). In the present study, circulating concentrations of MMPs were markedly lower in response to the GL (~75%) and LC (~70%) diets. While the main effect of each diet on LMPs was not as great as MMPs, the GL and LC diets tended to reduce circulating LMP concentrations. Reductions in circulating MMPs and LMPs support anti-inflammatory effects of each diet, potentially via reduced monocyte and leukocyte activation. Interestingly, there were no significant reductions in either MMPs or LMPs in response to a combined LC diet with postmeal light-to-moderate exercise. It is plausible that exercise induced a moderate, transient increase in monocyte and leukocyte activation and, in turn, MP vesiculation, which counteracted the diet effects. Indeed, acute exercise has been reported to increase circulating MPs, whereas chronic aerobic exercise is associated with reduced MP formation (30). It is likely that a longer intervention period, resulting in a more chronic exercise stimulus, may yield different results.

Among the five cytokines assessed in our study, only MCP-1 showed a significant decrease over the 4-day interventions. In a 7-day study in individuals who were overweight, an LCHF diet with and without antioxidants (vitamin C/E) led to a similar reduction in MCP-1, whereas, in line with our results, C-reactive protein and IL-6 levels were not affected (49). Thus, despite the reduction in PBMC JNK activation, plasma cytokines were largely unchanged by short-term diet interventions. Longer term low-carbohydrate diets typically result in substantial reductions in body and fat mass (43), which can confound interpretation of obesity-related inflammatory parameters. In our short-term study, the lack of change in proinflammatory cytokines could be viewed as support for the utilization of LCHF diets in the treatment of T2D. These findings suggest that previous findings of inflammatory activation after hypercaloric high-fat meals (~950 kcal) may not translate to heightened inflammation when eucaloric/hypocaloric LCHF foods are consumed over several days (13, 22). However, the use of plasma-borne inflammatory markers, such as cytokines, has

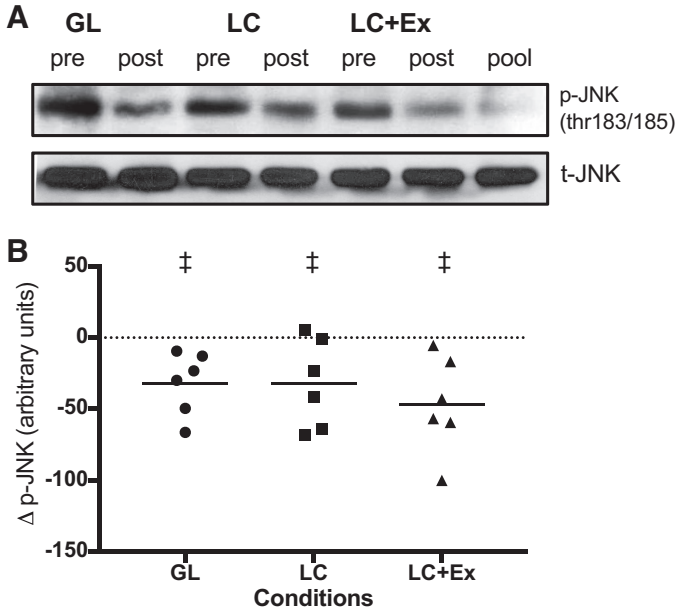

Fig. 4. Changes in (Δ) peripheral blood mononuclear cells (PBMCs) phosphorylated c-Jun NH_2-terminal kinase (p-JNK) following each 4-day diet intervention. *A*: representative Western blot image showing total and p-JNK before (pre) and after (post) each intervention. Band intensities were expressed relative to a pooled sample of human PBMCs included in every blot (pool). *B*: data for p-JNK/total JNK presented as change scores (post − pre within each condition). Linear mixed models revealed a significant main effect of time for p-JNK ($P < 0.05$). GL, low-fat low-glycemic index guidelines diet; LC, low-carbohydrate high-fat diet; LC+Ex, low-carbohydrate high-fat diet and exercise. ‡$P < 0.05$ for preplanned contrast of post vs. pre within condition.

recently been questioned, as they may not reflect inflammatory processes occurring in cells (29). Our findings are in agreement with this notion, as changes were seen in PBMCs p-JNK and MPs with minimal effects of the dietary interventions on key cytokines, such as IL-6 and TNF-α.

LCHF diets lower proinsulin. Elevated proinsulin, as well as proinsulin-insulin or the proinsulin-C-peptide ratio, is a strong predictor of insulin resistance and is associated with beta cell dysfunction (37, 51). In our study, LC and LC+Ex decreased fasting proinsulin, whereas no changes were seen following the GL diet. Although the change in proinsulin-C-peptide was not significantly different between conditions (i.e., nonsignificant condition × time interaction), within-condition comparisons showed that only LC and LC+Ex decreased this ratio. Here, we speculate that by reducing carbohydrate intake, and thus the demand for insulin, LCHF diets can potentially provide the beta cells with some transient rest, decrease endoplasmic reticulum stress, and improve proinsulin processing within beta cells (36). The long-term effects of such diets on proinsulin levels and beta cell function are unknown, but since both proinsulin and glucose levels are independent cardiovascular risk factors (23, 42), an LCHF diet with and without postmeal walks could contribute to improving the metabolic profile and cardiovascular risk of individuals with T2D.

Limitations and perspectives. Despite our efforts to maintain energy balance, a small (~1–2 kg) but significant weight loss occurred in all conditions. The short duration of each intervention did not permit adjusting the caloric intake in attempts to prevent weight loss, but energy intake was matched between conditions. Therefore, the small weight loss may be related to an overall increase in diet quality and/or strictly controlling food intake over the 4 days. The clinical relevance of this small reduction is unclear, but we acknowledge that weight loss could have played a role in the modulation of inflammation.

All food consumed by participants was provided and consisted of healthy whole foods. Thus, it is likely that most participants improved the quality of their diet as compared with what they usually eat by removing processed foods. This might have contributed to the absence of significant differences between conditions for some outcomes since each diet seems to have provided a certain degree of improvement over habitual patterns of participants. Furthermore, despite providing fresh whole foods, the strict protocol may have reduced generalizability as three participants were unable to complete all conditions, citing inability to comply with study procedures.

Like most people with T2D, the participants in the current investigation were on medications. We made sure to instruct each participant to continue to take their regular medications throughout the entire study and used a daily log to confirm this, but the interaction between medications, diets, and inflammatory outcomes are unknown. The relatively small sample size did not allow for an examination of interactions with medications or by sex, but the randomized crossover design was a strength in this regard.

In conclusion, an LCHF diet with or without daily postmeal walks improved four-day glycemic control and fasting proin-

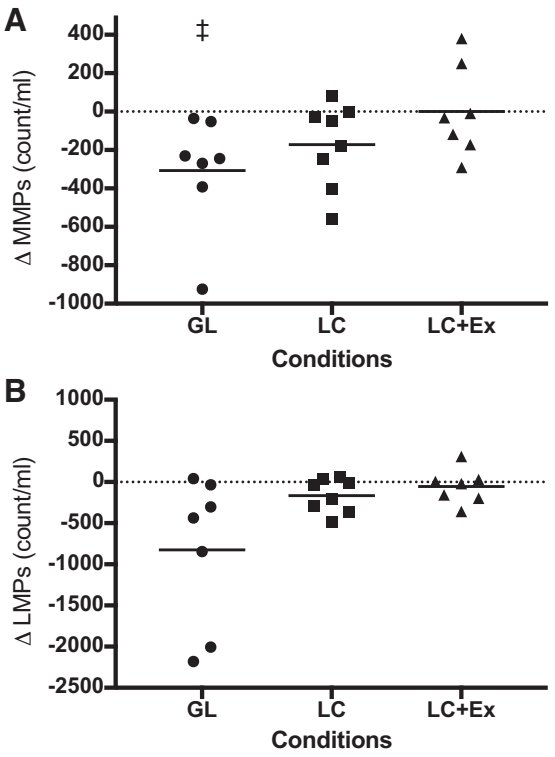

Fig. 5. Changes in (Δ) monocyte-derived microparticles (MMPs) and leukocyte-derived microparticles (LMPs) following each 4-day diet intervention. MMPs (*A*) and LMPs (*B*) presented as change scores (post − pre within each condition). Linear mixed models revealed a significant condition × time interaction for MMPs ($P < 0.05$). GL, low-fat low-glycemic index guidelines diet; LC, low-carbohydrate high-fat diet; LC+Ex, low-carbohydrate high-fat diet and exercise. ‡$P < 0.05$ for preplanned contrast of post vs. pre within condition.

sulin levels compared with a GL diet. The addition of postmeal walks to an LCHF diet further improved glycemic control. There were no consistent effects of the individual interventions on inflammatory markers, although inflammatory activation of circulating PBMCs appeared to be reduced following each intervention, with no appreciable changes in characteristic proinflammatory plasma cytokines. Longer-term studies using LCHF diets and exercise looking at cardiometabolic and inflammatory markers are necessary to confirm the beneficial effects of such diets in individuals with T2D.

Perspectives and Significance

LCHF diets have recently regained popularity for the management of T2D. The macronutrient distribution characteristic of this kind of diet is in contradiction with the current dietary guidelines for diabetes. As demonstrated in this study, an LCHF diet with and without postmeal walks significantly and rapidly normalized blood glucose levels. The elevated amount of fat consumed on this diet did not negatively affect inflammation or circulating TGs, which combined with the glucose-lowering effect and suggests that LCHF can be a beneficial treatment strategy for T2D. Metabolic adaptations to LCHF can take several weeks to months, and deepening our knowledge on their chronic effect on inflammatory and metabolic status will likely help us formulate better therapeutic dietary approaches for individuals with impaired glycemic regulation.

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DISCLOSURES

J. P. Little and J. D. Johnson are co-Chief Scientific Officers for the Institute for Personalized Therapeutic Nutrition, a not-for-profit organization that supports a food-first approach to treating and preventing chronic disease. J. P. Little holds shares in Metabolic Insights Inc., a for-profit company that is developing techniques for noninvasive metabolic monitoring.

AUTHOR CONTRIBUTIONS

É.M.-C. and J.P.L., conceived and designed research; É.M.-C., C.D., H.N., T.D.B., J.D.B., J.D.J., and C.A.D. performed experiments; É.M.-C., C.D., H.N., T.D.B., J.D.B., J.D.J., C.A.D., and J.P.L. analyzed data; É.M.-C., C.D., H.N., T.D.B., J.D.B., J.D.J., C.A.D., and J.P.L. interpreted results of experiments; É.M.-C., C.D., and J.P.L. prepared figures; É.M.-C., C.D., T.D.B., J.D.B., and J.P.L. drafted manuscript; É.M.-C., C.D., H.N., T.D.B., J.D.B., J.D.J., C.A.D., and J.P.L. edited and revised manuscript; É.M.-C., C.D., H.N., T.D.B., J.D.B., J.D.J., C.A.D., and J.P.L. approved final version of manuscript.

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