2018

Carbohydrate restriction with postmeal walking effectively mitigates postprandial hyperglycemia and improves endothelial function in type 2 diabetes

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Publication Details Citation

Francois, M. E., Myette-Cote, E., Bammert, T., Durrer, C., Neudorf, H., DeSouza, C., & Little, J. P. (2018). Carbohydrate restriction with postmeal walking effectively mitigates postprandial hyperglycemia and improves endothelial function in type 2 diabetes. Faculty of Science, Medicine and Health - Papers: Part B. Retrieved from https://ro.uow.edu.au/smhpapers1/63

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Abstract

Postprandial hyperglycemia has deleterious effects on endothelial function. Restricting carbohydrate intake and postmeal walking have each been shown to reduce postprandial hyperglycemia, but their combination and subsequent effects on endothelial function have not been investigated. Here, we sought to examine the effect of blunting postprandial hyperglycemia by following a low-carbohydrate diet, with or without postmeal walking exercise, on markers of vascular health in type 2 diabetes (T2D). In a randomized crossover design, individuals with T2D (n < 11) completed three 4-day controlled diet interventions consisting of 1) low-carbohydrate diet alone (LC), 2) low-carbohydrate diet with 15-min postmeal walks (LC > Ex), and 3) low-fat control diet (CON). Fasting blood samples and brachial artery flow-mediated dilation (%FMD) were measured before and after each intervention. Total circulating microparticles (MPs), endothelial MPs, platelet MPs, monocyte-platelet aggregates, and adhesion molecules were assessed as biomarkers of vascular health. There was a significant condition = time interaction for %FMD ($P < 0.01$), with post hoc tests revealing improved %FMD after LC > Ex (>0.8 ± 1.0%, $P < 0.02$), with no change after LC or CON. Endothelial MPs were significantly reduced with the LC diet by ~45% (from 99 ± 60 to 44 ± 31 MPs/μl, $P < 0.02$), with no change after LC > Ex or CON (interaction: $P < 0.04$). Total MPs were lower (main effect time: $P < 0.02$), whereas monocyte-platelet aggregates were higher (main effect time: $P = 0.01$) after all interventions. Plasma adhesion molecules and C-reactive protein were unaltered. Attenuating postprandial hyperglycemic excursions using a low-carbohydrate diet combined with postmeal walking appears to be an effective strategy to improve endothelial function in individuals with T2D. NEW & NOTEWORTHY Carbohydrate restriction and postmeal walking lower postprandial hyperglycemia in individuals with type 2 diabetes. Here, we show that the combination significantly improved endothelial function and that carbohydrate restriction alone reduced circulating endothelial microparticles in individuals with type 2 diabetes. Listen to this article's corresponding podcast at http://ajpheart.pod-bean.com/e/low-carb-diet-and-exercise-improve-endothelial-health/.

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Francois, M. E., Myette-Cote, E., Bammert, T. D., Durrer, C., Neudorf, H., DeSouza, C. A. & Little, J. P. (2018). Carbohydrate restriction with postmeal walking effectively mitigates postprandial hyperglycemia and improves endothelial function in type 2 diabetes. American Journal of Physiology - Heart and Circulatory Physiology, 314 (1), H105-H113.

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Carbohydrate-restriction with Postmeal Walking Effectively Mitigates Postprandial Hyperglycemia and Improves Endothelial Function in Type 2 Diabetes

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Running Title: low-carbohydrate and exercise improves endothelial health

Word Counts: Abstract 246 and Manuscript 3,752 words

4 Tables and 3 Figures.

Authors have no conflicts of interest to declare.

Key Words: LCHF, walking, glycemic control, high-fat, microparticles, flow-mediated dilation, glucose.

Abstract

Postprandial hyperglycemia has deleterious effects on endothelial function. Restricting carbohydrate intake and postmeal walking have each been shown to reduce postprandial hyperglycemia but their combination and subsequent effects on endothelial function have not been investigated. Here, we sought to examine the effect of blunting postprandial hyperglycemia by following a low-carbohydrate diet, with or without postmeal walking exercise, on markers of vascular health in type 2 diabetes (T2D). In a randomized crossover design, individuals with T2D (N=11) completed three four-day controlled diet interventions consisting of i) low-carbohydrate diet alone (LC), ii) low-carbohydrate diet with 15-minute postmeal walks (LC+Ex), and iii) Low-fat control diet (CON). Fasting blood samples and brachial artery flow-mediated dilation (%FMD) were measured before and after each intervention. Total circulating microparticles (MPs), endothelial MPs (EMPs), platelet MPs (PMPs), monocyte-platelet aggregates (MPAs), and adhesion molecules were assessed as biomarkers of vascular health. There was a significant conditionXtime interaction for %FMD ($p=0.01$), with post-hoc tests revealing improved %FMD after LC+Ex (+0.8±1.0%, $p=0.02$), with no change after LC or CON. EMPs were significantly reduced with the LC diet by ~45% (from 99±60 to 44±31 MP/μL, $p=0.02$), with no change after LC+Ex or CON (interaction: $p=0.04$). Total MPs were lower (main effect time: $p=0.02$), whereas, MPAs were higher (main effect time: $p<0.01$) after all interventions. Plasma adhesion molecules and c-reactive protein were unaltered. Attenuating postprandial hyperglycemic excursions using a low-carbohydrate diet combined with postmeal walking appears to be an effective strategy to improve endothelial function in individuals with T2D.

New and Noteworthy

Carbohydrate-restriction and post-meal walking lowers postprandial hyperglycemia in individuals with type 2 diabetes. Here, we show that the combination significantly improves endothelial function, and carbohydrate restriction alone reduces circulating endothelial microparticles, in individuals with type 2 diabetes.

Abbreviations

AUC = Area Under the Curve
CON = Control low-fat diet
CGM = Continuous glucose monitoring
EMPs = Endothelial Microparticles
FMD = Flow-Mediated Dilation
HbA_{1c} = Glycated Hemoglobin
ICAM-1 = Intracellular Adhesion Molecule 1
LC = Low-carbohydrate
LC+Ex = Low-carbohydrate plus exercise
MPs = Microparticles
MPAs = Monocyte-platelet aggregates
PMPs = Platelet Microparticles
T2D = Type 2 diabetes
VCAM-1 = Vascular Cell Adhesion Molecule 1

Introduction

Prevention of cardiovascular disease in individuals with type 2 diabetes (T2D) is a major treatment goal (29, 42). Within this, diet and exercise remain the cornerstone lifestyle therapies (42). Separately, and in combination, diet and exercise interventions significantly improve cardiovascular risk factors (24, 54, 71). Increased risk for cardiovascular disease (CVD) in individuals with T2D is attributed to a multitude of factors including hyperglycemia, inflammation, oxidative stress and dyslipidemia (59). In addition, impaired flow-mediated dilation (FMD), a measure of endothelial function, is an early manifestation of CVD that disproportionately affects individuals with T2D (36). Markers of endothelial activation are also elevated in individuals with T2D (45); for example endothelial and platelet derived microparticles (extracellular vesicles which are released from apoptotic or activated cells) and monocyte-platelet aggregates (which reflect platelet activation and inflammation) are markedly elevated under hyperglycemic conditions, and are important novel pathogenic markers of vascular disease (2, 7, 43).

Postprandial hyperglycemia has emerged as an independent risk factor for the development of diabetes complications, including vascular disease (8, 10). Postprandial hyperglycemia is particularly detrimental to endothelial function, as various studies have shown that postprandial glucose excursions can directly promote oxidative stress, activate inflammatory pathways, reduce nitric oxide bioavailability, and impair FMD (11, 13, 50). Combined epidemiological and

100 experimental studies suggest that hyperglycemia-induced endothelial dysfunction
101 could be a mechanistic link between postprandial glucose spikes and CVD risk
102 (10).

103 Dietary carbohydrate restriction is reemerging as an effective approach for
104 glycemic control (1, 26). Given that the rise in blood glucose concentration
105 following a meal is largely dependent on the carbohydrate composition (57),
106 reducing exogenous carbohydrate intake at each meal is a logical strategy to
107 lower postprandial glucose and insulin responses (31, 35, 53). However, despite
108 the immediate improvements in hyperglycemia observed with carbohydrate
109 restriction (52, 61, 65), there is strong apprehension surrounding the adoption of
110 a low-carbohydrate diet because it is typically high in fat (i.e., a low-carbohydrate
111 high-fat, LCHF diet) (46, 68). Following a high-fat meal, several studies have
112 observed a transient impairment in endothelial function and increase in
113 endothelial microparticles, which is typically attributed to postprandial
114 hypertriglyceridemia (25, 64, 66, 70). Thus, by targeting one risk factor for
115 endothelial dysfunction and CVD risk (i.e., postprandial hyperglycemia) a LCHF
116 diet may introduce another (postprandial hypertriglyceridemia). However, studies
117 showing detrimental effects of dietary fat on the endothelium have been acute
118 single meal studies, often involving combined high-fat and high-carbohydrate
119 loads using shakes and/or fast food meals (25, 64, 66, 70). The response to
120 several days of meals reflecting contemporary LCHF meals in T2D patients is
121 unknown.

Exercise has well-established benefits for vascular and overall health (34), and thus may be an attractive addition to a LCHF diet to maximize glucose lowering effects while mitigating any potential impairments caused by increasing dietary fat. Specifically, postmeal walking has been shown to markedly reduce postprandial hyperglycemia (22, 54) and lipemia (38, 56) in individuals with, and at risk for, T2D. However, to the best of our knowledge, no study has combined these two lifestyle approaches in an effort to optimize a lifestyle strategy for T2D.

The aim of the present study was to examine the effects of four days of a low-carbohydrate diet, with or without daily postmeal walking, on endothelial function and biomarkers of vascular health, in individuals with T2D. Given that carbohydrate restriction leads to significant weight loss and metabolic adaptations in the first few weeks to months, we chose a short-term (four days) intervention in this initial study in order to reduce the confounding effect of these factors on endothelial function outcomes (6, 16).

Methods

Overview

Individuals with physician-diagnosed T2D (HbA1c >6.5%, FPG >7.0 mmol/L, or 2-h glucose OGTT >11.1 mmol/L; CDA (32)) were recruited to complete three, short-term controlled intervention periods in a randomized crossover design. Interventions for this proof-of-concept study were conducted across four days, to reduce the confounding influence of body composition changes, and to improve compliance and standardization. All food was provided

to participants and diets were matched for energy and protein content. Accelerometers (Actigraph wGTX3+) were worn to monitor activity for all conditions and confirm the completion of post-meal walks. This trial was registered with clinicaltrials.gov (#NCT02683135) and approved by the UBC Clinical Research Ethics Board. Prior to study commencement participants provided written informed consent.

Participants

Sixteen (n=8 males/females) aged between 48-72 y, not on exogenous insulin and without diagnosed cardiovascular, kidney or any other diabetes complications were recruited from the local community. Individuals currently involved in a regular exercise routine (>3 days of structured exercise per week for last three months), following a low-carbohydrate diet or unwilling to consume the provided meat-containing diets were excluded. Five of the sixteen participants did not complete all three conditions, due to family reasons (n=1), inability or unwilling to follow study diets (n=3) and change of medications (n=1, addition of SGLT2 inhibitor after completing one condition). Therefore, eleven participants (7 females, who were all postmenopausal) were included in analyses and their baseline characteristics are shown in Table 1.

Experimental Protocol

Each participant attended a baseline screening session, which included the measurement of anthropometrics, a physical activity readiness questionnaire

(PARQ+) and a Godin leisure time exercise questionnaire, followed by the three four-day interventions in a random order. The day before each intervention diet and activity were standardized, and a washout of 9-14 days occurred between each intervention where participants were asked to return to their normal diet and physical activity habits. Fasting blood for biomarkers of vascular health and brachial artery flow-mediated dilation (FMD) were measured before and after each four-day intervention at the same time of day following a consumption of a standardized mixed meal on the evening before the start of each intervention. During each intervention, a continuous glucose monitor (CGM) was worn to measure postprandial glucose responses. Incremental AUC was calculated using the trapezoid method (44). All intervention diets were isoenergetic, with calories estimated using the Harris benedict equation (39) and habitual intake (i.e., matched from first trial). An example meal plan for one-day of each diet is provided in Table 2. For the low-carbohydrate plus exercise condition (LC+Ex), the estimated individualized energy utilization for the postmeal walking (48) (~70 kcal) was added to each meal to assure energy equilibrium between the three diets.

Low-fat control diet (CON)

Participants were provided a diet with meals based on the current dietary guidelines for adults with T2D comprising low-fat, low glycemic index, whole foods (23). Each meal comprised ~55% of total energy from carbohydrate (predominately from low glycemic index and high fibre carbohydrate sources),

20% energy from fat (aiming for <7% saturated fatty acids); and 25% protein (primarily from lean meats).

Low-carbohydrate high-fat diet (LC)

Participants consumed a diet that provided the same energy content as the CON diet but with carbohydrates reduced 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).

Low-carbohydrate diet and exercise (LC+Ex)

Participants performed 15 minutes of walking beginning ~30 minutes after breakfast, lunch and dinner. A similar strategy has been previously shown to reduce postprandial hyperglycemia in individuals with impaired glucose tolerance (22). The exercise intensity of the postmeal walking was light-to-moderate, which was confirmed on day-one 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 (RPE; CR-10 scale) of 3 ‘moderate’ (equating to ~60% of maximal heart rate). Participants were instructed to replicate this pace at home for each of the 15-minute postmeal walks for the remainder of the intervention. Accelerometers were worn to confirm compliance and intensity. Participants consumed the same diet as the LC intervention, but with the addition of ~70 kcal to each meal to account for the estimated energy expenditure of 15 minutes of walking.

Physiological Measures

Brachial artery flow-mediated dilation (FMD)

Endothelial function was assessed with brachial artery FMD using high-resolution ultrasound (Terason 3200) according to current guidelines (17, 62). First, a longitudinal section, 2-3 cm from the antecubital fossa of the brachial artery, was imaged for 1 minute using B mode ultrasound (insonation angle maintained at 60°). Then, a rapid inflation cuff positioned 1-2 cm distal from the olecranon process of the forearm was inflated to >60 mmHg above systolic blood pressure for 5 minutes. Simultaneous diameter and velocity measurements continued throughout and were recorded 30 seconds before and for 3 minutes after the cuff was rapidly deflated. Brachial blood pressure was measured using a manual sphygmomanometer and stethoscope. Mean arterial blood pressure (MAP) was calculated as 1/3*systolic blood pressure (SBP) + 2/3*diastolic blood pressure (DBP).

Analyses for the synchronized diameter and velocity measures were performed using edge detection software (33, 72). FMD is expressed as the percent change in artery diameter from baseline (%FMD = 100*(Post – $\text{Preocclusion}_{\text{mean diameter}}$/$\text{preocclusion}_{\text{mean diameter}}$).

Biomarkers of vascular health

Collection of blood samples

For adhesion molecules, venous blood was collected into EDTA containing tubes (BD Vacutainer) and the plasma obtained after centrifugation at 1550Xg for 15 minutes at 4 °C. For monocyte-platelet aggregate and microparticles analyses, venous blood was collected from the antecubital vein by venipuncture into sodium citrate tubes (BD Vacutainer). Monocyte-platelet aggregates were analyzed from whole blood 10 min after blood collection (details below). Plasma was generated by centrifugation for 15 min at 1550 g and stored at -80 °before batch analyses were performed (described below).

Monocyte platelet aggregates (MPA)

Exactly 10 minutes following blood collection, 90 µL of whole blood was transferred from the sodium citrate vacutainer into a TruCount tube (BD Biosciences, New Jersey, USA). 10 µL of FcR blocking reagent (Miltenyi Biotec, Germany) was added and the sample was then incubated in the dark at room temperature for 10 minutes. Following this, 2 µL of CD14-Vioblue (Miltenyi Biotec) and 10 µL of CD42b-APC (BD Biosciences) were added and gently mixed before incubation under the same conditions. Finally, 1 mL of red blood cell lysis buffer was added before incubation on a rocking platform for 15 minutes. The sample was then analyzed on a flow-cytometer (Miltenyi Biotec MACSQuant Analyzer) with monocyte platelet aggregates defined as events positive for both CD14 and CD42b (51). Fluorescence-minus-one controls were used to determine positive staining for both CD14 and CD42b events.

Circulating Microparticles

Microparticles were characterized using flow cytometry, as previously described (4, 30). For the quantification of total microparticles (MPs), endothelial microparticles (EMP), and platelet microparticle (PMP) subspecies, plasma samples were centrifuged at 13,000g for 2 minutes and 200μL of platelet free plasma was then transferred to a TruCount tube (BD Biosciences, New Jersey, USA). MP size threshold was established using Megamix-Plus SSC calibrator beads (Megamix-Plus SSC beads, Biocytex, Marseille, France), and only events <1 μm in size were counted (Nielsen et al, 2014). Total MPs were defined as events falling within the Megamix-Plus SSC established size range (0.16, 0.20, 0.24 and 0.5 μm). Cellular specific MP lineage was determined by flourochrome staining for endothelial (CD62e) and platelet (CD62p) specific antibodies and falling within the respective MP size range (BioLegend, San Diego, California). Samples were incubated with antibodies for 20 minutes in the dark at room temperature. Following incubation, samples were fixed with 2% paraformaldehyde (ChemCruz Biochemicals, Santa Cruz, California), 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 total MPs, EMPs and PMPs were determined using the formula: ([number of events in region containing MPs /number of events in absolute count bead region] x [total number of beads per test / total volume of sample]).

Adhesion molecules, c-reactive protein (CRP) and Serum amyloid A (SAA)

Plasma was thawed, mixed and diluted 1000-fold before analyses of Intracellular Adhesion Molecule 1 (ICAM-1), Vascular Cell Adhesion Molecule 1 (VCAM-1), CRP and SAA were made using the V-PLEX Vascular Injury Panel 2 Human Kit (Meso Scale Discovery, Maryland, USA), according to the manufacturer's instructions. Measures were made in duplicate and analyzed on a MESO QuickPlex SQ 120 (Meso Scale Discovery), with an intra-assay coefficient of variation 3.7%.

Statistics

All data were first tested for normality using Q-Q plots and are reported as mean and standard deviation (SD) or 95% confidence intervals. Data were analyzed using linear mixed model, with repeated measures of condition (CON, LC and LC+Ex) and time (pre, post) as fixed factors with SPSS 22.0 (SPSS, Chicago, Illinois). Post-hoc analyses using Tukey's procedure were used to evaluate within condition changes (i.e., pre versus post) following significant interactions. Statistical significance was set at $p < 0.05$. Magnitude-based inference analyses were performed according to contemporary views on statistical reporting, allowing for clinically meaningful inference (5) using the spreadsheet available from http://www.sportsci.org. The smallest clinically beneficial threshold for %FMD was +1%, based on a recent meta-analyses which showed a 13% reduced risk of future cardiovascular events for every 1%

improvement in %FMD (95% CI: 9% to 17%) (41). For measurements with an unknown smallest clinical change threshold, the default Cohen's *d* of 0.2 was used.

Results

Figure 1A shows the 24 hour CGM curves for CON, LC, and LC+Ex conditions (n=11). LC and LC+Ex for four-days reduced the incremental blood glucose area under the curve (iAUC) by ~86 ± 21% and ~94 ± 22%, respectively compared to CON (p=0.01, Figure 1B). The change in body mass was not different between interventions (-1.9 ± 0.8 kg, main effect of time, p<0.01). However, the change in body mass was not different between conditions (Interaction: p=0.82) supporting successful matching of energy intake across diets.

Flow-mediated dilation (%FMD)

There was a significant condition X time interaction (p=0.01) for the change in %FMD. %FMD was significantly increased after LC+Ex (by +0.81 ± 0.95%, p=0.02, Figure 2), with no change following CON and LC (both p>0.12, Figure 2). The probability that the change in %FMD with LC+Ex is beneficial/negligible/harmful based on the clinically meaningful change (+1%) was 25/75/0% (95% CI 1.6, 0.04%), respectively. For the CON and LC conditions the probability was 0/61/40% and 11/88/1%, beneficial/negligible/harmful,

329 respectively. Baseline diameter and time to peak diameter did not change across
330 time or between conditions (Table 3).
331
332 *Microparticles*
333 There was a significant condition X time interaction (p=0.04) for the
334 change in EMPs. EMPs were significantly reduced after the LC condition (by
335 65%, p=0.04, Figure 3), with no change following CON and LC+Ex (both p>0.12,
336 Figure 3). The probability that the change in EMPs with LC is
337 beneficial/negligible/harmful was 95/4/1%, respectively (Cohen's *d* 0.81). For the
338 CON and LC+Ex conditions the probability was 6/26/68% and 17/42/41%,
339 beneficial/negligible/harmful, respectively. A significant main effect of time
340 (p=0.02) revealed a collective 45% reduction in total microparticles (from 8790 ±
341 6036 to 5865 ± 4327 MP/μL). The probability that the change in circulating
342 microparticles is beneficial/negligible/harmful was 94/6/0%, respectively (Cohen's
343 *d* 0.52, Table 4). Platelet microparticles (PMPs) did not differ significantly
344 between conditions (Interaction: p=0.07, Table 3).
345
346 *Monocyte platelet aggregates (MPA), c-reactive protein and adhesion molecules*
347 A significant main effect of time (p<0.01) revealed a collective 14%
348 increase in total (count/mL) monocyte-platelet aggregates (interaction: p=0.15,
349 Table 4). The probability that the change in monocyte-platelet aggregates is
350 beneficial/negligible/harmful was 1/5/94%, respectively (Cohen's *d* 0.71, Table
351 4). %MPA (interaction: p=0.78, time: p=0.08, Table 3), plasma adhesion

molecules (ICAM-1, VCAM-1), SAA and c-reactive protein (all $p>0.12$) were not significantly changed following all conditions (Table 3). Pairwise comparisons for the magnitude based inference for each condition is provided in Table 4.

Discussion

The present study examined the short-term effects of a low-carbohydrate diet, with and without postmeal walking exercise, on endothelial function and markers of vascular health in individuals with T2D. We tested whether the attenuation of postprandial hyperglycemia with a low-carbohydrate diet and postmeal walking (LC+Ex) might represent an optimal strategy for improving endothelial function and markers of vascular health. The main findings of the present study were that i) LC+Ex significantly improved endothelial function assessed by FMD, and ii) LC alone lowered circulating endothelial microparticles. No changes were observed in the selected measures of vascular health following a low-fat CON diet based on current diabetes guidelines (3, 14). Additionally, total circulating microparticles were reduced, however monocyte-platelet aggregates were slightly increased, following all short-term conditions. The present study shows that attenuating postprandial hyperglycemia by restricting carbohydrates and postmeal walking can improve vascular health in individuals with T2D. The addition of postmeal walking to a low-carbohydrate high-fat diet may mitigate the purported deleterious effects of high-fat meals on endothelial function seen in some (19, 25, 66, 70) but not all (67) investigations and further research is warranted to determine this in larger, longer interventions.

Postprandial hyperglycemia, exacerbated by carbohydrate consumption at meals, contributes to the excess CVD risk in individuals with T2D (8, 11, 18). Elevated blood glucose levels following an oral glucose load (13) or oscillating glucose infusion (11) impairs endothelial function. However, antioxidant, statin and/or insulin therapies that reduce glycemia and oxidative stress, can restore endothelial function, at least in an acute setting (12). In the present study, the aim was to reduce postprandial hyperglycemic excursions with lifestyle interventions, namely carbohydrate-restriction and postmeal walking. Continuous glucose monitoring confirmed the reduction in postprandial hyperglycemia with LC alone and in combination with postmeal walking, compared to the currently recommended low-fat diet (CON). Indeed, postprandial hyperglycemia assessed by iAUC was reduced by 86% and 94%, respectively, with short-term LC and LC+Ex. Furthermore, endothelial function was increased after LC+Ex. The observed reduction in postprandial hyperglycemia with LC+Ex was larger than that typically seen with Acarbose treatment (a drug to delay carbohydrate digestion and thus postprandial hyperglycemia) (20). Acute Acarbose administration before a sucrose load has been shown to mitigate postprandial endothelial dysfunction (69). As well, long-term trials show that Acarbose treatment reduces the incidence of diabetes and prevents CVD (15, 37). However, there is a high prevalence of adverse gastrointestinal symptoms with Acarbose use (15, 37). Here, we show for the first time that lowering postprandial hyperglycemia with the non-pharmacological combination of a low-carbohydrate

high-fat diet and postmeal walking improves endothelial function in individuals with T2D. Therefore, this lifestyle combination may be effective for reducing vascular dysfunction in T2D.

The postprandial period is associated with a cascade of proatherogenic events, including endothelial and immune cell activation (9). Circulating microparticles are biologically active submicron particles that are shed from the membrane of cells under conditions of stress/injury (21). Studies have shown that circulating microparticles are indicative of endothelial dysfunction in individuals with T2D (27). Indeed, endothelial microparticles carry and express endothelial proteins such as adhesion molecules and integrins, and thus disturb vascular homeostasis (21). Acutely, previous studies have shown an increase in endothelial microparticles (EMPs) following a high-fat meal, indicating endothelial activation (28, 60). However, in the present study EMPs were reduced following four-days of a low-carbohydrate high-fat diet. This is likely attributed to the reduction in postprandial hyperglycemic excursions with carbohydrate-restriction compared to the low-fat diet in individuals with T2D. Indeed, oscillating blood glucose is more deleterious for oxidative stress than constant high glucose in those with T2D (11). However, it is unclear why the same decrease in EMPs was not seen following the LC+Ex condition. There is some evidence to suggest that this may be due to exercise induced shear-stress, which mediates microparticle shedding from the vascular wall (49). Following a single session of exercise, studies have reported a transient increase in microparticles, and have attributed

this to increased shear stress and/or oxidative stress (47, 49, 58). In this regard, acute exercise prior to a high-fat meal may not acutely lower endothelial microparticles (40), however repetitive exposure to exercise over several weeks may improve defense systems. Indeed, regular exercise improves endothelial function and vascular health, which appears to be primarily mediated by shear stress and increased nitric oxide bioavailability (55, 63). Thus, it is possible that the addition of three postmeal walks in the LC+Ex condition was promoting vascular remodeling in the previously inactive T2D participants, which may have led to a different EMP response when compared to the LC diet alone.

The present study did not include an exercise only, low-fat CON condition. Although it is hypothesized that the reduced postprandial hyperglycemia with the LC diet combined with exercise has additive and may have unique effects on the vasculature, further research is needed to compare exercise alone to low-carbohydrate diet approaches. Previous research has shown that postmeal walking while following a low-fat diet reduces 24-h blood glucose (22) and postprandial iAUC (54) by ~11%. The present study shows that the combination of low-carbohydrate diet and postmeal exercise lowers four-day iAUC by 94%, compared to low-fat CON diet alone. A low-carbohydrate diet alone lowered the four-day iAUC by ~86%. Therefore it appears that a low-carbohydrate diet approach is much more potent than postmeal walking for lowering glucose levels but our study cannot ascertain the independent and combined effects of each approach.

444 Interestingly, the total microparticle count was reduced after all conditions
445 suggesting that all diets altered circulating microparticle concentration. This is
446 most likely driven by the decrease in EMPs following the LC and PMPs following
447 the LC+Ex conditions. However the CON diet, which comprised of low-fat, low
448 glycemic index, whole foods (23), is likely a less processed and 'healthier' diet
449 than typically consumed by participants. Thus, this improvement in overall food
450 quality may underlie the resulting decrease in microparticles after all conditions
451 (independent of changes in glycemic control). The reduction in body mass
452 experienced after all three short-term diet conditions generally supports that the
453 provided diets were healthier or lower in calories and this could have played a
454 role. It is unknown whether the observed change in microparticle count might be
455 the result of altered microparticle production and/or clearance, which are areas
456 that require further research. Furthermore, the microparticle species, cargo and
457 subsequent physiological signaling could be different regardless of total
458 microparticle concentration (73).
459
460 *Conclusion*
461 In individuals with T2D, postprandial hyperglycemia is particularly
462 concerning as it contributes to CVD risk through impairing endothelial function,
463 increasing oxidative stress, and promoting inflammation (11, 13, 50). The results
464 of the present study show that controlling postprandial hyperglycemia with a low-
465 carbohydrate high-fat diet combined with postmeal walking exercise improves
466 endothelial function in individuals with T2D. Microparticles, as markers of

endothelial activation, were reduced with short-term carbohydrate restriction. Carbohydrate restriction and postmeal exercise may therefore represent an effective strategy to mitigate the negative effects of postprandial hyperglycemia and reduce CVD risk in individuals with T2D. Further research is needed to elucidate the long-term impact of carbohydrate restriction and postmeal exercise on CVD risk factors in individuals with, and at risk for, type 2 diabetes.

Author Contributions

MEF, EMC and JPL designed the study. MEF and EMC collected the data. MEF, EMC, CD, HN and TDB analyzed the data. MEF, EMC, CAD and JPL interpreted the data. MEF drafted the manuscript, and MEF, EMC, CD, HN, TDB, CAD and JPL edited and approved the final manuscript.

Disclosures

No conflicts of interest, financial or otherwise, are declared by the author(s).

Tables and Figures

Table 1. Baseline characteristics of participants (n=11).

Age (y)	Body mass (kg)	BMI (kg/m^2)	Waist circumference (cm)	HbA_{1c} (%)	Years of diagnosis	Blood Pressure (mmHg)
64 ± 8	91 ± 18	34 ± 8	105 ± 13	7 ± 1	6 ± 4	93 ± 4
Medications: Metformin = 9, GLP-1 = 1, DPP4 = 1, Sulfonylurea = 1, Statin = 3						

HbA_{1c} = glycated hemoglobin, BMI = Body mass index, Duration T2D = years diagnosed with T2D.

Table 2. Example meal plan showing the three meals provided for one day of each intervention (500 kcal/meal version).

Diet	CON (low-fat)	LC (low-carb)	LC+Ex
Goal macronutrient ratio (CHO:PRO:fat)	55%:25%:20%	10%:25%:65%	10%:25%:65%
Breakfast	95g-Oats 25g-Whey 30g-Blueberries 30g-Raspberries	150g-Whole Egg 110g-Egg Whites 55g-Avocado 30g-Peppers 40g-Onions 40g-Carrots 10g-Almonds	150g-Whole Egg 110g-Egg Whites 55g-Avocado 30g-Peppers 40g-Onions 40g-Carrots 10g-Almonds
Lunch	105g-Chicken Breast 230g-Yams 40g-Green Beans 17g-Cashew	105g-Ground Turkey 38g-Cashew nuts 15g-Olive oil 35g-Spinach 30g-Carrots 30g-Cucumber	105g-Ground Turkey 38g-Cashew nuts 15g-Olive oil 35g-Spinach 30g-Carrots 30g-Cucumber
Dinner	100g-Turkey 85g-Brown Rice 14g-Cashew 40g-Brocolli	100g-Steak (Rib eye) 30g-Cahsew nuts 13g-Olive Oil 30g-Apple 30g-Spinach 50g-Cucumber	100g-Steak (Rib eye) 30g-Cahsew nuts 13g-Olive Oil 30g-Apple 30g-Spinach 50g-Cucumber

CON = Control low-fat moderate-carbohydrate diet, LC = low-carbohydrate diet, LC+Ex = low-carbohydrate plus postmeal walking, CHO= carbohydrate, PRO = protein.

Table 3. Flow-mediated dilation (n=11), microparticles (n=9), monocyte-platelet aggregates (n=11), adhesion molecules, serum amyloid and c-reactive protein (n=11) data before and after each four-day condition.

	CON		LC		LC+Ex		Interaction	Main effect
	Pre	Post	Pre	Post	Pre	Post	P value	P value
Baseline diameter (mm)	0.39 ± 0.07	0.41 ± 0.09	0.39 ± 0.06	0.40 ± 0.06	0.40 ± 0.06	0.40 ± 0.07	0.15	0.11
Peak diameter (mm)	0.42 ± 0.07	0.44 ± 0.08	0.42 ± 0.06	0.44 ± 0.07	0.43 ± 0.06	0.44 ± 0.06	0.33	0.11
Time to peak (s)	51.3 ± 35.3	50.0 ± 26.7	51.1 ± 27.1	47.2 ± 30.4	44.6 ± 36.9	60.1 ± 47.1	0.48	0.67
PMPs (MP/µL)	42.1 ± 26.5	46.1 ± 31.7	33.2 ± 30.8	33.9 ± 21.5	50.8 ± 20.0	28.8 ± 25.6	0.07	0.34
Total MPA (cells/µL)	77.5 ± 21.7	95.5 ± 22.7	80.2 ± 20.5	92.7 ± 27.9	77.3 ± 20.0	87.1 ± 19.0	0.15	<0.01
%MPA	29.8 ± 5.5	31.3 ± 5.5	31.0 ± 6.4	32.7 ± 5.4	31.0 ± 6.4	31.3 ± 7.8	0.78	0.08
CRP (mg/L)	8.0 ± 8.3	7.9 ± 8.5	5.3 ± 4.9	8.2 ± 8.4	7.3 ± 7.2	9.3 ± 11.4	0.12	0.29
SAA (mg/L)	5.7 ± 4.2	6.4 ± 4.3	3.9 ± 2.5	5.3 ± 5.6	5.3 ± 3.4	5.1 ± 4.2	0.72	0.30
ICAM-1 (ng/mL)	461 ± 89	478 ± 83	368 ± 111	402 ± 77	389 ± 102	422 ± 89	0.44	0.19
VCAM-1 (ng/mL)	392 ± 249	441 ± 255	320 ± 208	395 ± 217	389 ± 252	400 ± 248	0.48	0.06

Data are mean ± SD. PMP = Platelet microparticle, MPA = Monocyte-platelet aggregate, CRP = c-reactive protein, SAA = Serum amyloid A, ICAM-1 = Intracellular adhesion molecule, VCAM-1 = Vascular cell adhesion molecule.

Table 4. Pairwise comparisons using magnitude based inferences for the change in selected markers of vascular health measured before and after each four-day condition

		CON	LC	LC+Ex
Total MP	Cohens *d*	0.4	0.9	0.6
	Beneficial/trivial/harmful	65/22/13%	96/3/1%	88/10/2%
	Qualitative Inference	*Possibly beneficial*	*Very likely beneficial*	*Likely beneficial*
PMPs	Cohens *d*	0.1	0.8	0.1
	Beneficial/trivial/harmful	23/40/37%	97/3/0%	23/37/40%
	Qualitative Inference	*Unclear*	*Very likely beneficial*	*Unclear*
MPA count	Cohens *d*	0.9	0.6	0.5
	Beneficial/trivial/harmful	0/3/97%	2/11/88%	2/15/83%
	Qualitative Inference	*Very likely harmful*	*Likely harmful*	*Likely harmful*
MPA %	Cohens *d*	0.6	*0.3*	*0.2*
	Beneficial/trivial/harmful	2/11/88%	8/33/59%	9/41/50%
	Qualitative Inference	*Likely harmful*	*Unclear*	*Unclear*
CRP	Cohens *d*	0.0	0.5	0.2
	Beneficial/trivial/harmful	31/46/23%	3/17/80%	10/35/55%
	Qualitative Inference	*Unclear*	*Likely harmful*	*Unclear*
SAA	Cohens *d*	0.3	0.3	0.1
	Beneficial/trivial/harmful	11/29/60%	8/31/61%	40/34/25%
	Qualitative Inference	*Unclear*	*Unclear*	*Unclear*
ICAM	Cohens *d*	0.3	0.3	0.3
	Beneficial/trivial/harmful	11/29/60%	6/29/65%	8/32/60
	Qualitative Inference	*Unclear*	*Unclear*	*Unclear*
VCAM	Cohens *d*	0.6	0.9	0.1
	Beneficial/trivial/harmful	2/10/88%	1/3/96%	21/41/39%
	Qualitative Inference	*Likely harmful*	*Very likely harmful*	*Unclear*

MP = Microparticle, PMP = Platelet microparticle, MPA = Monocyte-platelet aggregate, CRP = c-reactive protein, SAA = Serum amyloid A, ICAM-1 = Intracellular adhesion molecule, VCAM-1 = Vascular cell adhesion molecule.

Figure 1. Continuous blood glucose data (n=11) showing **1A)** the blood glucose excursions across four-days of a control low-fat diet (CON), low-carbohydrate diet (LC) and low-carbohydrate plus exercise (LC+Ex), and **1B)** the incremental AUC for each four-day condition. # = $p < 0.05$ interaction, * = $p < 0.05$ post-hoc.

Figure 2. Changes in flow-mediated dilation (FMD) before and after short-term control low-fat diet (CON), low-carbohydrate diet (LC) and low-carbohydrate plus exercise (LC+Ex) conditions. Group mean (Bar: n=11) and individual data (lines). # = $p < 0.05$ interaction, * = $p < 0.05$ post-hoc.

Figure 3. Changes in endothelial microparticles (EMP) before and after short-term control low-fat diet (CON), low-carbohydrate diet (LC) and low-carbohydrate plus exercise (LC+Ex) conditions. Group mean (Bar: n=9) and individual data (lines). # = $p < 0.05$ interaction, * = $p < 0.05$ post-hoc.

References

1. **American Diabetes Association**. Nutrition recommendations and interventions for diabetes. *Diabetes care* 31: S61-S78, 2008.
2. **Arteaga RB, Chirinos JA, Soriano AO, Jy W, Horstman L, Jimenez JJ, Mendez A, Ferreira A, de Marchena E, and Ahn YS**. Endothelial microparticles and platelet and leukocyte activation in patients with the metabolic syndrome. *The American journal of cardiology* 98: 70-74, 2006.
3. **Association AD**. *American Diabetes Association complete guide to diabetes*. Bantam, 2003.
4. **Bain AR, Ainslie PN, Bammert TD, Hijmans JG, Sekhom M, Hoiland RL, Flück D, Donnelly J, and DeSouza CA**. Passive heat stress reduces circulating endothelial and platelet microparticles. *Experimental Physiology* 2017.
5. **Batterham AM, and Hopkins WG**. Making meaningful inferences about magnitudes. *International journal of sports physiology and performance* 2006.
6. **Berk K, Oudshoorn T, Verhoeven A, Mulder M, Roks A, Dik W, Timman R, and Sijbrands E**. Diet-induced weight loss and markers of endothelial dysfunction and inflammation in treated patients with type 2 diabetes. *Clin Nutr ESPEN* 15: 101-106, 2016.
7. **Boden G, and Rao AK**. Effects of hyperglycemia and hyperinsulinemia on the tissue factor pathway of blood coagulation. *Current diabetes reports* 7: 223-227, 2007.
8. **Brownlee M**. The pathobiology of diabetic complications. *Diabetes* 54: 1615-1625, 2005.
9. **Ceriello A**. The post - prandial state and cardiovascular disease: relevance to diabetes mellitus. *Diabetes Metab Res Rev* 16: 125-132, 2000.
10. **Ceriello A**. Postprandial hyperglycemia and diabetes complications. *Diabetes* 54: 1-7, 2005.
11. **Ceriello A, Esposito K, Piconi L, Ihnat MA, Thorpe JE, Testa R, Boemi M, and Giugliano D**. Oscillating glucose is more deleterious to endothelial function and oxidative stress than mean glucose in normal and type 2 diabetic patients. *Diabetes* 57: 1349-1354, 2008.
12. **Ceriello A, Kumar S, Piconi L, Esposito K, and Giugliano D**. Simultaneous control of hyperglycemia and oxidative stress normalizes endothelial function in type 1 diabetes. *Diabetes care* 30: 649-654, 2007.
13. **Ceriello A, Taboga C, Tonutti L, Quagliaro L, Piconi L, Bais B, Da Ros R, and Motz E**. Evidence for an independent and cumulative effect of postprandial hypertriglyceridemia and hyperglycemia on endothelial dysfunction and oxidative stress generation. *Circulation* 106: 1211-1218, 2002.
14. **Cheng A**. Canadian Diabetes Association Clinical Practice Guidelines Expert Committee: Canadian diabetes association 2013 clinical practice guidelines for the prevention and management of diabetes in Canada. *Can J Diabetes* 37: S1-S212, 2013.
15. **Chiasson J-L, Josse RG, Gomis R, Hanefeld M, Karasik A, Laakso M, and Group S-NTR**. Acarbose treatment and the risk of cardiovascular disease

and hypertension in patients with impaired glucose tolerance: the STOP-NIDDM trial. *Jama* 290: 486-494, 2003.

16. **Clifton PM, Keogh JB, Foster P, and Noakes M**. Effect of weight loss on inflammatory and endothelial markers and FMD using two low-fat diets. *Int J Obes* 29: 1445-1451, 2005.

17. **Corretti MC, Anderson TJ, Benjamin EJ, Celermajer D, Charbonneau F, Creager MA, Deanfield J, Drexler H, Gerhard-Herman M, and Herrington D**. Guidelines for the ultrasound assessment of endothelial-dependent flow-mediated vasodilation of the brachial artery: a report of the International Brachial Artery Reactivity Task Force. *Journal of the American College of Cardiology* 39: 257-265, 2002.

18. **Creager MA, Lüscher TF, Cosentino F, and Beckman JA**. Diabetes and vascular disease. *Circulation* 108: 1527-1532, 2003.

19. **Cuevas AM, Guasch V, Castillo O, Irribarra V, Mizon C, San Martin A, Strobel P, Perez D, Germain AM, and Leighton F**. A high-fat diet induces and red wine counteracts endothelial dysfunction in human volunteers. *Lipids* 35: 143-148, 2000.

20. **Derosa G, Salvadeo SA, D'Angelo A, Ferrari I, Mereu R, Palumbo I, Maffioli P, Randazzo S, and Cicero AF**. Metabolic effect of repaglinide or acarbose when added to a double oral antidiabetic treatment with sulphonylureas and metformin: a double-blind, cross-over, clinical trial. *Curr Med Res Opin* 25: 607-615, 2009.

21. **Dignat-George F, and Boulanger CM**. The many faces of endothelial microparticles. *Arterioscler Thromb Vasc Biol* 31: 27-33, 2011.

22. **DiPietro L, Gribok A, Stevens MS, Hamm LF, and Rumpler W**. Three 15-min bouts of moderate postmeal walking significantly improves 24-h glycemic control in older people at risk for impaired glucose tolerance. *Diabetes Care* 36: 3262-3268, 2013.

23. **Dworatzek PD, Arcudi K, Gougeon R, Husein N, Sievenpiper JL, and SL. W**. Canadian Diabetes Association Clinical Practice Guidelines Expert Committee. Canadian Diabetes Association 2013 Clinical Practice Guidelines for the Prevention and Management of Diabetes in Canada: nutrition therapy. *Can J Diabetes* 37: 45-55, 2013.

24. **Elhayany A, Lustman A, Abel R, Attal - Singer J, and Vinker S**. A low carbohydrate Mediterranean diet improves cardiovascular risk factors and diabetes control among overweight patients with type 2 diabetes mellitus: a 1 - year prospective randomized intervention study. *Diabetes Obes Metab* 12: 204-209, 2010.

25. **Esposito K, Ciotola M, Sasso FC, Cozzolino D, Saccomanno F, Assaloni R, Ceriello A, and Giugliano D**. Effect of a single high-fat meal on endothelial function in patients with the metabolic syndrome: role of tumor necrosis factor-α. *Nutrition, metabolism and cardiovascular diseases* 17: 274-279, 2007.

26. **Feinman RD, Pogozelski WK, Astrup A, Bernstein RK, Fine EJ, Westman EC, Accurso A, Frassetto L, Gower BA, and McFarlane SI**. Dietary

carbohydrate restriction as the first approach in diabetes management: critical review and evidence base. *Nutrition* 31: 1-13, 2015.

27. **Feng B, Chen Y, Luo Y, Chen M, Li X, and Ni Y**. Circulating level of microparticles and their correlation with arterial elasticity and endothelium-dependent dilation in patients with type 2 diabetes mellitus. *Atherosclerosis* 208: 264-269, 2010.

28. **Ferreira AC, Peter AA, Mendez AJ, Jimenez JJ, Mauro LM, Chirinos JA, Ghany R, Virani S, Garcia S, and Horstman LL**. Postprandial hypertriglyceridemia increases circulating levels of endothelial cell microparticles. *Circulation* 110: 3599-3603, 2004.

29. **Fox CS, Coady S, Sorlie PD, D'Agostino RB, Pencina MJ, Vasan RS, Meigs JB, Levy D, and Savage PJ**. Increasing cardiovascular disease burden due to diabetes mellitus the Framingham Heart Study. *Circulation* 115: 1544-1550, 2007.

30. **Francois ME, and Little JP**. The impact of acute high-intensity interval exercise on biomarkers of cardiovascular health in type 2 diabetes. *Eur J Appl Physiol* 1-10, 2017.

31. **Gannon MC, and Nuttall FQ**. Effect of a high-protein, low-carbohydrate diet on blood glucose control in people with type 2 diabetes. *Diabetes* 53: 2375-2382, 2004.

32. **Goldenberg R, and Punthakee Z**. Definition, classification and diagnosis of diabetes, prediabetes and metabolic syndrome. *Can J Diabetes* 37: S8-S11, 2013.

33. **Green D, Cheetham C, Reed C, Dembo L, and O'Driscoll G**. Assessment of brachial artery blood flow across the cardiac cycle: retrograde flows during cycle ergometry. *Journal of Applied Physiology* 93: 361-368, 2002.

34. **Green DJ, Hopman MT, Padilla J, Laughlin MH, and Thijssen DH**. Vascular Adaptation to Exercise in Humans: Role of Hemodynamic Stimuli. *Physiological Reviews* 97: 495-528, 2017.

35. **Gutniak M, Grill V, and Efendlć S**. Effect of composition of mixed meals—low-versus high-carbohydrate content—on insulin, glucagon, and somatostatin release in healthy humans and in patients with NIDDM. *Diabetes care* 9: 244-249, 1986.

36. **Hadi HA, and Al Suwaidi J**. Endothelial dysfunction in diabetes mellitus. *Vascular health and risk management* 3: 853, 2007.

37. **Hanefeld M, Schaper F, and Koehler C**. Effect of acarbose on vascular disease in patients with abnormal glucose tolerance. *Cardiovascular Drugs and Therapy* 22: 225-231, 2008.

38. **Hardman AE, and Aldred HE**. Walking during the postprandial period decreases alimentary lipaemia. *Journal of cardiovascular risk* 2: 71-78, 1995.

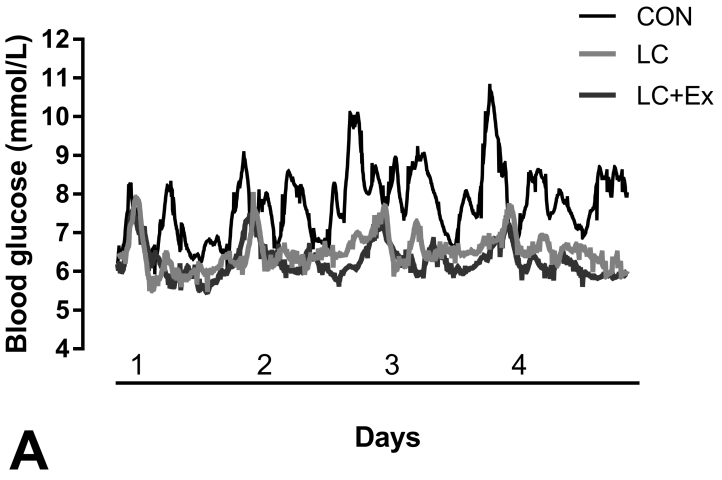
39. **Harris JA, and Benedict FG**. A biometric study of human basal metabolism. *Proceedings of the National Academy of Sciences* 4: 370-373, 1918.

40. **Harrison M, Murphy RP, O'Connor PL, O'Gorman DJ, McCaffrey N, Cummins PM, and Moyna NM**. The endothelial microparticle response to a high fat meal is not attenuated by prior exercise. *Eur J Appl Physiol* 106: 555, 2009.

41. **Inaba Y, Chen JA, and Bergmann SR**. Prediction of future cardiovascular outcomes by flow-mediated vasodilatation of brachial artery: a meta-analysis. *The international journal of cardiovascular imaging* 26: 631-640, 2010.
42. **Inzucchi SE, Bergenstal R, Buse JB, Diamant M, Ferrannini E, Nauck M, Peters A, Tsapas A, Wender R, and Matthews D**. Management of hyperglycaemia in type 2 diabetes: a patient-centered approach. Position statement of the American Diabetes Association (ADA) and the European Association for the Study of Diabetes (EASD). *Diabetologia* 55: 1577-1596, 2012.
43. **Jimenez JJ, Jy W, Mauro LM, Horstman LL, Bidot CJ, and Ahn YS**. Endothelial microparticles (EMP) as vascular disease markers. *Advances in clinical chemistry* 39: 131-157, 2005.
44. **Le Floch J-P, Escuyer P, Baudin E, Baudon D, and Perlemuter L**. Blood glucose area under the curve: methodological aspects. *Diabetes care* 13: 172-175, 1990.
45. **Leroyer A, Tedgui A, and Boulanger C**. Microparticles and type 2 diabetes. *Diabetes & metabolism* 34: 27-32, 2008.
46. **Lustig RH, Schmidt LA, and Brindis CD**. Public health: The toxic truth about sugar. *Nature* 482: 27-29, 2012.
47. **Marsh SA, and Coombes JS**. Exercise and the endothelial cell. *Int J Cardiol* 99: 165-169, 2005.
48. **Mikus C, Oberlin D, Libla J, Boyle L, and Thyfault J**. Glycaemic control is improved by 7 days of aerobic exercise training in patients with type 2 diabetes. *Diabetologia* 55: 1417-1423, 2012.
49. **Miyazaki Y, Nomura S, Miyake T, Kagawa H, Kitada C, Taniguchi H, Komiyama Y, Fujimura Y, Ikeda Y, and Fukuhara S**. High shear stress can initiate both platelet aggregation and shedding of procoagulant containing microparticles. *Blood* 88: 3456-3464, 1996.
50. **Monnier L, Mas E, Ginet C, Michel F, Villon L, Cristol J-P, and Colette C**. Activation of oxidative stress by acute glucose fluctuations compared with sustained chronic hyperglycemia in patients with type 2 diabetes. *Jama* 295: 1681-1687, 2006.
51. **Newby DE**. Flow cytometric analysis of circulating platelet-monocyte aggregates in whole blood: methodological considerations. *Thromb Haemost* 98: 451-456, 2007.
52. **Nielsen JV, and Joensson E**. Low-carbohydrate diet in type 2 diabetes. Stable improvement of bodyweight and glycemic control during 22 months follow-up. *Nutrition & metabolism* 3: 22, 2006.
53. **Papakonstantinou E, Triantafillidou D, Panagiotakos D, Iraklianou S, Berdanier C, and Zampelas A**. A high protein low fat meal does not influence glucose and insulin responses in obese individuals with or without type 2 diabetes. *Journal of human nutrition and dietetics* 23: 183-189, 2010.
54. **Reynolds AN, Mann JI, Williams S, and Venn BJ**. Advice to walk after meals is more effective for lowering postprandial glycaemia in type 2 diabetes mellitus than advice that does not specify timing: a randomised crossover study. *Diabetologia* 59: 2572-2578, 2016.

55. **Rush JW, Denniss SG, and Graham DA**. Vascular nitric oxide and oxidative stress: determinants of endothelial adaptations to cardiovascular disease and to physical activity. *Can J Appl Physiol* 30: 442-474, 2005.
56. **Schlierf G, Dinsenbacher A, Kather H, Kohlmeier M, and Haberbosch W**. Mitigation of alimentary lipemia by postprandial exercise—phenomena and mechanisms. *Metabolism* 36: 726-730, 1987.
57. **Sheard NF, Clark NG, Brand-Miller JC, Franz MJ, Pi-Sunyer FX, Mayer-Davis E, Kulkarni K, and Geil P**. Dietary carbohydrate (amount and type) in the prevention and management of diabetes. *Diabetes care* 27: 2266-2271, 2004.
58. **Sossdorf M, Otto GP, Claus RA, Gabriel HH, and Lösche W**. Cell-derived microparticles promote coagulation after moderate exercise. *Med Sci Sports Exerc* 43: 1169-1176, 2011.
59. **Stamler J, Vaccaro O, Neaton JD, Wentworth D, and Group MRFITR**. Diabetes, other risk factors, and 12-yr cardiovascular mortality for men screened in the Multiple Risk Factor Intervention Trial. *Diabetes care* 16: 434-444, 1993.
60. **Strohacker K, Breslin WL, Carpenter KC, Davidson TR, Agha NH, and McFarlin BK**. Moderate-intensity, premeal cycling blunts postprandial increases in monocyte cell surface CD18 and CD11a and endothelial microparticles following a high-fat meal in young adults. *Appl Physiol Nutr Metab* 37: 530-539, 2012.
61. **Tay J, Luscombe-Marsh ND, Thompson CH, Noakes M, Buckley JD, Wittert GA, Yancy WS, and Brinkworth GD**. A very low-carbohydrate, low–saturated fat diet for type 2 diabetes management: a randomized trial. *Diabetes Care* 37: 2909-2918, 2014.
62. **Thijssen DH, Black MA, Pyke KE, Padilla J, Atkinson G, Harris RA, Parker B, Widlansky ME, Tschakovsky ME, and Green DJ**. Assessment of flow-mediated dilation in humans: a methodological and physiological guideline. *American Journal of Physiology-Heart and Circulatory Physiology* 300: H2-H12, 2011.
63. **Tinken TM, Thijssen DH, Hopkins N, Dawson EA, Cable NT, and Green DJ**. Shear stress mediates endothelial adaptations to exercise training in humans. *Hypertension* 55: 312-318, 2010.
64. **Tushuizen ME, Nieuwland R, Scheffer PG, Sturk A, Heine RJ, and Diamant M**. Two consecutive high - fat meals affect endothelial - dependent vasodilation, oxidative stress and cellular microparticles in healthy men. *Journal of thrombosis and haemostasis* 4: 1003-1010, 2006.
65. **Unwin D, and Unwin J**. Low carbohydrate diet to achieve weight loss and improve HbA1c in type 2 diabetes and pre - diabetes: experience from one general practice. *Practical Diabetes* 31: 76-79, 2014.
66. **Vogel RA, Corretti MC, and Plotnick GD**. Effect of a single high-fat meal on endothelial function in healthy subjects. *The American journal of cardiology* 79: 350-354, 1997.
67. **Volek JS, Ballard KD, Silvestre R, Judelson DA, Quann EE, Forsythe CE, Fernandez ML, and Kraemer WJ**. Effects of dietary carbohydrate restriction versus low-fat diet on flow-mediated dilation. *Metabolism* 58: 1769-1777, 2009.

68. **Wang H, Steffen LM, Zhou X, Harnack L, and Luepker RV**. Consistency between increasing trends in added-sugar intake and body mass index among adults: the Minnesota Heart Survey, 1980–1982 to 2007–2009. *American journal of public health* 103: 501-507, 2013.
69. **Wascher T, Schmoelzer I, Wiegratz A, Stuehlinger M, Mueller - Wieland D, Kotzka J, and Enderle M**. Reduction of postchallenge hyperglycaemia prevents acute endothelial dysfunction in subjects with impaired glucose tolerance. *Eur J Clin Invest* 35: 551-557, 2005.
70. **Wei-Chuan T, Yi-Heng L, Chih-Chan L, Ting-Hsing C, and Jyh-Hong C**. Effects of oxidative stress on endothelial function after a high-fat meal. *Clinical Science* 106: 315-319, 2004.
71. **Wing R, Bolin P, Brancati F, Bray G, Clark J, Coday M, Crow R, Curtis J, Egan C, and Espeland M**. Cardiovascular effects of intensive lifestyle intervention in type 2 diabetes. *The New England journal of medicine* 369: 145-154, 2013.
72. **Woodman R, Playford D, Watts G, Cheetham C, Reed C, Taylor R, Puddey I, Beilin L, Burke V, and Mori T**. Improved analysis of brachial artery ultrasound using a novel edge-detection software system. *Journal of Applied Physiology* 91: 929-937, 2001.
73. **Yáñez-Mó M, Siljander PR-M, Andreu Z, Bedina Zavec A, Borràs FE, Buzas EI, Buzas K, Casal E, Cappello F, and Carvalho J**. Biological properties of extracellular vesicles and their physiological functions. *J Extracell Vesicles* 4: 27066, 2015.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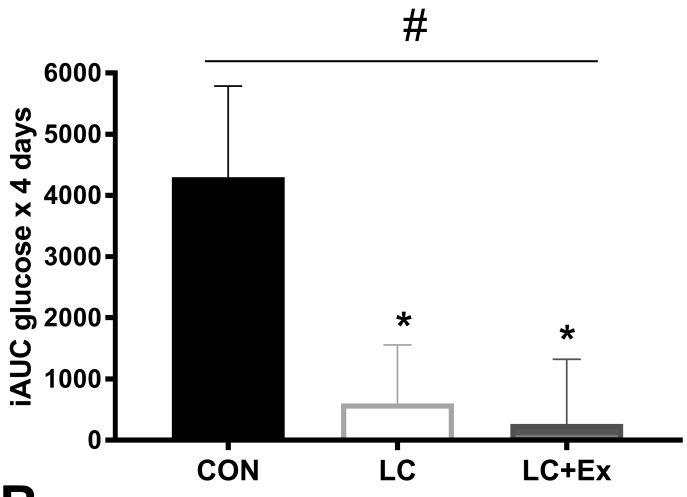
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CON
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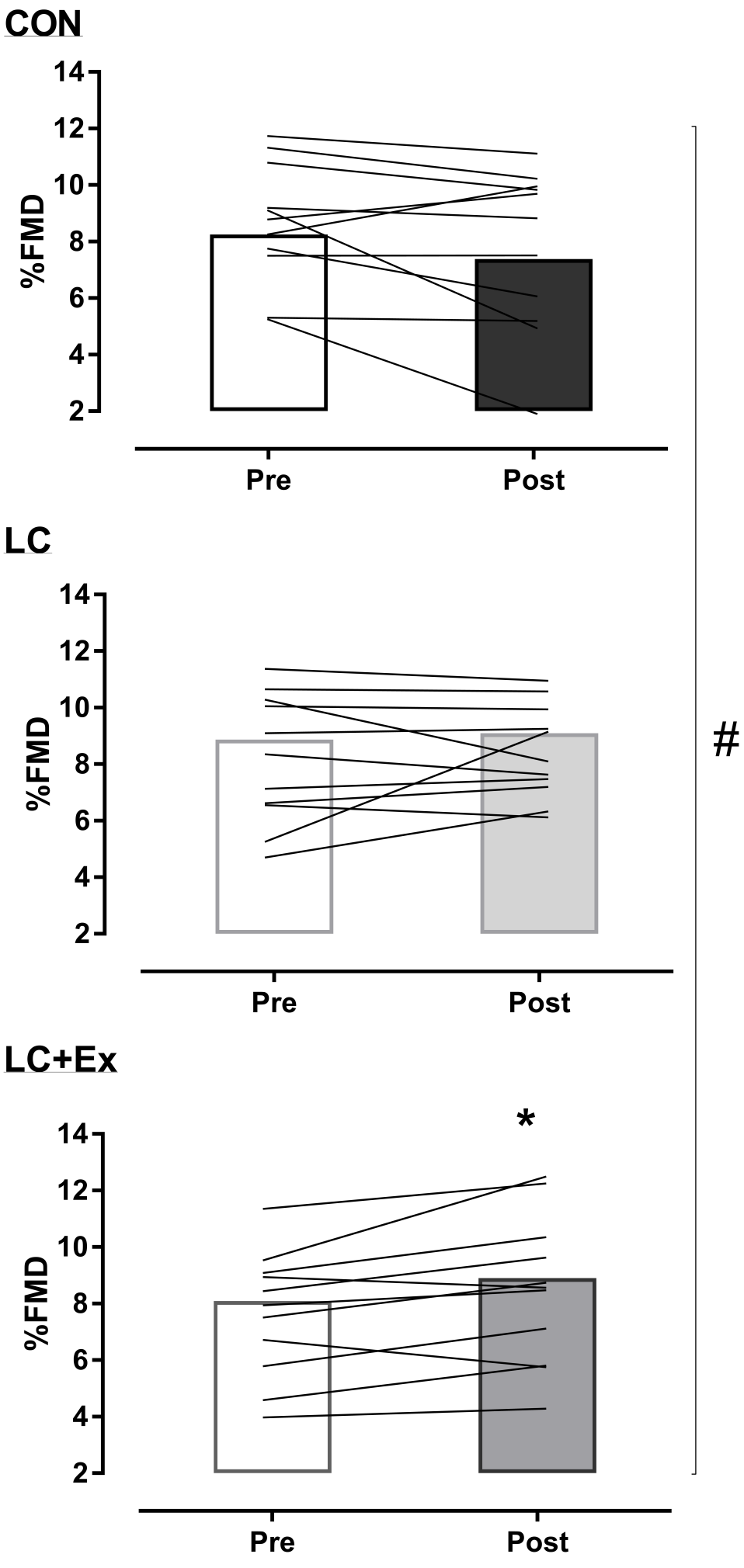
LC+Ex

B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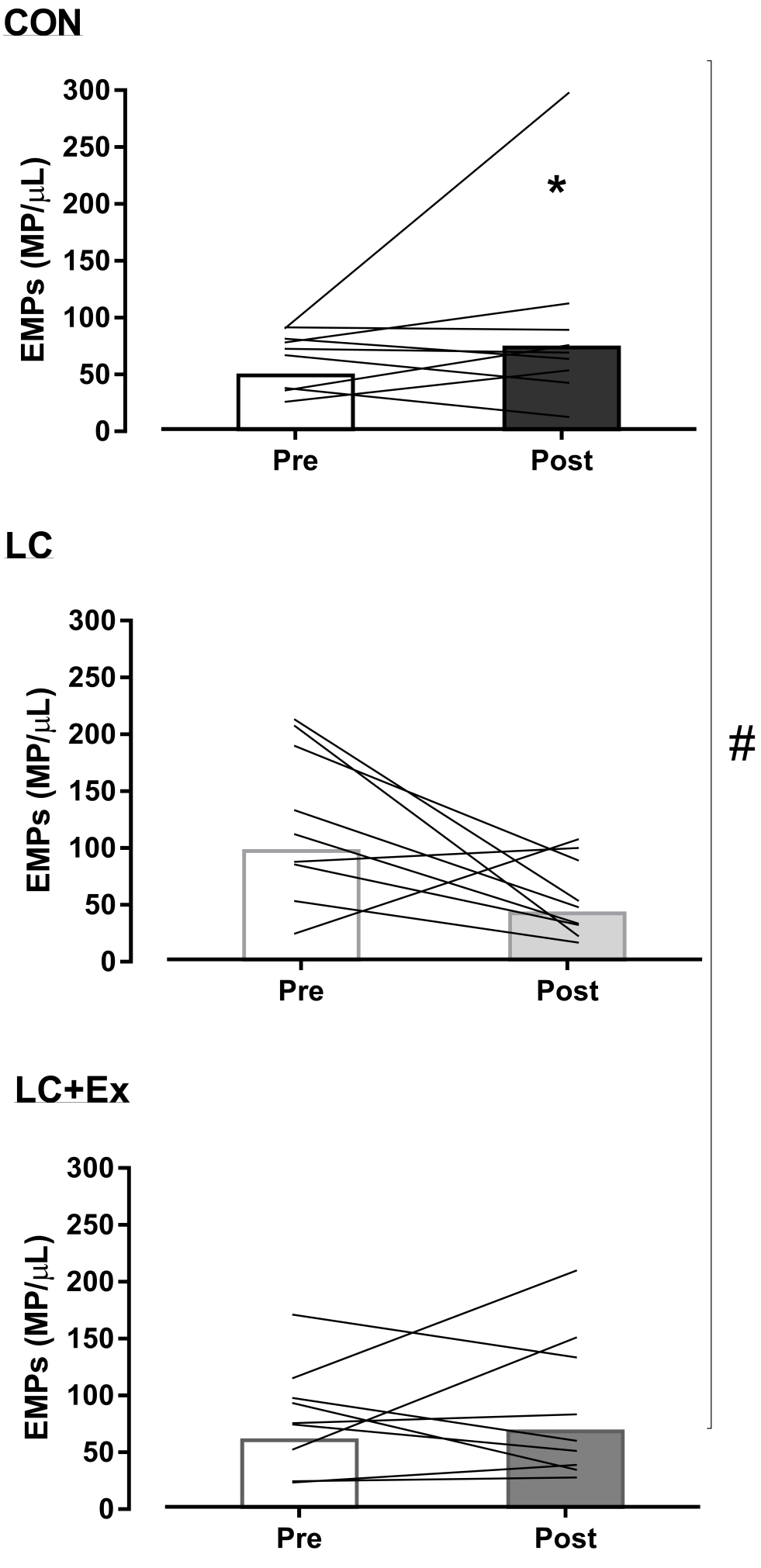
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