

A single Mediterranean meal does not impair postprandial flow-mediated dilatation in healthy men with subclinical metabolic dysregulations

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Abstract: Cardiovascular risk factors are known to exacerbate high-saturated fatty acid meal (HSFAM)-induced endothelial dysfunction, but the influence of subclinical metabolic dysregulations and the acute impact of a single mixed Mediterranean-type meal (MMM) remains unknown. Thus, this study has the objective to evaluate the metabolic and vascular effect of such meals in healthy subjects with or without subclinical fasting metabolic dysregulations. Twenty-eight healthy males without overt cardiovascular risk factors randomly ingested 1 of 2 isocaloric meals on separate days. Plasma metabolic markers, fatty acid (FA) profile, and endothelial function (flow-mediated dilatation; FMD) were assessed at baseline and 2 and 4 h after meal ingestion. Unsupervised hierarchical clustering identified 2 subgroups of participants ($n = 11$ and 17) differing by their baseline metabolic profiles. The MMM did not significantly alter postprandial endothelial function in all subjects, irrespective of baseline metabolic parameters. In contrast, the HSFAM induced postprandial endothelial dysfunction ($\Delta\%FMD_{\text{absolute}} = -5.28 \pm 2.54$, $p < 0.01$ vs. MMM) in a subgroup of individuals with significantly greater body mass index, fasting insulinemia, and lipid parameters ($n = 11$). Finally, the postprandial plasma FA profiles were differentially enriched by the HSFAM and MMM, notably with saturated FAs and omega-3 polyunsaturated FAs, respectively. Collectively, our results highlight the detrimental impact of a single HSFAM on endothelial function in healthy individuals displaying subclinical fasting metabolic dysregulations. Such individuals could benefit from MMM, demonstrated herein to be without any acute detriment to endothelial function.

Key words: endothelial function, plasma fatty acid composition, inflammation, oxidative stress, cardiovascular disease risk factors.

Résumé : Les facteurs de risque cardiovasculaire sont reconnus pour exacerber la dysfonction endothéliale suivant la consommation de repas riche en acides gras saturés (« HSFAM »). Cependant, l'impact de dérèglements métaboliques sous-cliniques demeure méconnu comme l'est l'effet d'un repas de type méditerranéen (« MMM »). Cette étude a donc pour objectif d'évaluer les réponses métabolique et endothéliale à de tels repas chez des sujets sains avec ou sans dérèglement métabolique sous-clinique. Vingt-huit hommes sains, sans facteurs de risque cardiovasculaires ont reçu de façon randomisée un des repas à l'étude. Des marqueurs métaboliques et le profil d'acides gras (AG) plasmatiques, ainsi que la fonction endothéliale (*flow-mediated dilatation*; « FMD ») ont été évalués à jeun et 2 et 4 heures postprandiales. Un algorithme de regroupement hiérarchique non-supervisé a identifié deux sous-groupes de participants ($n = 11$ and 17) sur la base de certains paramètres métaboliques à jeun. Aucune altération endothéliale postprandiale significative n'a été observée suivant le MMM. Le HSFAM a induit une dysfonction endothéliale postprandiale ($\Delta\%FMD_{\text{absolute}} = -5,28 \pm 2,54$, $p < 0,01$ vs. MMM) seulement chez les individus à l'indice de masse corporelle, l'insulinémie et aux paramètres lipidiques à jeun plus élevés ($n = 11$). Finalement, le profil d'AGs postprandial a été enrichi différemment par le HSFAM et le MMM, notamment en AGs saturés et polyinsaturés oméga-3, respectivement. Nos résultats démontrent l'impact négatif d'un HSFAM sur la fonction endothéliale postprandiale de sujets sains avec dérèglements métaboliques sous-cliniques. Ces individus pourraient bénéficier de la consommation de MMM, démontré ici pour ne pas altérer la fonction endothéliale.

Mots-clés : fonction endothéliale, composition d'acides gras plasmatiques, inflammation, stress oxydatif, facteurs de risque de maladie cardiovasculaire.

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Introduction

Cardiovascular and metabolic diseases are a growing global burden (Chen et al. 2012; Mozaffarian et al. 2015) as is the industrialization of the food supply and the westernization of daily habits. In fact, in the past few decades, consumption of sodium, refined sugars, saturated fatty acids (SFA), as well as the proportion of omega-6 versus omega-3 polyunsaturated fatty acids (PUFA) has increased (Carrera Bastos et al. 2011) and is at least partly to blame for the deterioration of global cardiometabolic health. In contrast, increased adherence to a Mediterranean-type diet was shown to improve cardiometabolic health and prevent cardiovascular events (Estruch et al. 2013; Sofi et al. 2014).

Another hallmark of recent westernization of habits is increasing periods of time spent in the postprandial state, which was shown to be associated with detrimental vascular consequences (Cordain et al. 2005; Lopez-Miranda et al. 2007). One notable manifestation of repeated postprandial insults is the impairment of endothelial function, a well-established step in atherogenesis initiation (Zilvermit 1979; Bonetti et al. 2003). Indeed, endothelial dysfunction alone is predictive of future cardiovascular events in healthy individuals and in patients with established cardiovascular disease (Witte et al. 2005; Inaba et al. 2010; Matsuzawa et al. 2015). In this regard, the investigation of the body's ability to restore homeostasis in the few hours following physiological stressors such as meal ingestion can help identify early metabolic defects that would otherwise not be captured during the fasting state (van Ommen et al. 2009). The first demonstration by Vogel et al. (1997) of postprandial impairments in metabolic and endothelial functions following a single high-SFA meal (HSFAM) has since been corroborated on multiple occasions and is more frequently observed in individuals with cardiovascular risk factors (Lacroix et al. 2012). Meal fatty acid (FA) composition and/or antioxidant content would appear to influence the whole-meal effect on endothelial function. The addition of single elements from the cardioprotective Mediterranean diet, such as olive oil, fish, or fish oil, was previously shown to attenuate the deleterious effects of a HSFAM on vascular reactivity (Plotnick et al. 1997; Vogel et al. 2000; Fahs et al. 2010; Lacroix et al. 2012). Most of these studies were, however, conducted with high and unrealistic quantities of isolated Mediterranean ingredients, thereby disregarding the potential synergy between multiple constituents of the Mediterranean diet.

Hence, the objective of the present study was to assess the acute impact of a complete mixed Mediterranean-type meal (MMM) versus HSFAM on endothelial and metabolic functions in healthy males with or without subclinical fasting metabolic dysregulations.

Materials and methods

Study population

Twenty-eight male subjects, aged 18 to 50 years and free of cardiovascular disease or risk factors were recruited on a self-referral basis from the cardiovascular prevention and research centres of the Montreal Heart Institute. Potential participants were screened on the basis of their medical and familial history, medication use, and blood work. Exclusion criteria were known/documentary vascular disease (coronary, cerebral, or peripheral), heart failure, left ventricular ejection fraction <40%, renal insufficiency (creatinine clearance < 90 mL/min), hypertension (blood pressure > 140/90 mm Hg or treated), dyslipidemia (low-density lipoprotein (LDL) cholesterol > 3.5 mmol/L), diabetes mellitus (Hb1Ac > 6.5% or treated), abdominal obesity (waist circumference > 102 cm), acute infection, any medication use, nitroglycerin intolerance, history of alcohol abuse, smoking (active or within the past year), and supplementation with omega-3 PUFA (past 3 months) or vitamin C (past 2 weeks). This study was conducted according to the guidelines laid down in the Declaration of Helsinki and the Montreal

Table 1. Nutritional composition of both test meals.

	HSFAM	MMM
Total calories (kcal)	858	885
Total lipids (g)	56.91	51.30
Cholesterol (mg)	252	76
Total calories from fat (%)	58	51
SFA (g)	14.78	7.87
MUFA (g)	22.62	25.94
Total PUFA (g)	11.15	8.59
Omega-3 PUFA (g)	—	2.29
DHA:EPA	—	2:1
Carbohydrates (g)	68.74	63.81
Fibers (g)	6.56	13.00
Proteins (g)	24.16	37.82
Sodium (mg)	1806	1284
Vitamin C (mg)	4.77	87.93

Note: DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids.

Heart Institute ethics committee approved all procedures. Written informed consent was obtained from all subjects.

Intervention

Study participants were randomly assigned to 2 different meal sequences using a crossover design (MMM – HSFAM or HSFAM – MMM). Both isocaloric test meals were separated by a minimum of 7 and a maximum of 30 days. The MMM consisted of fresh salmon with onion and almond pesto served with green beans, cherry tomatoes, and potatoes all baked in extra-virgin olive oil (EVOO). Although salmon does not occur naturally in the Mediterranean sea, we refer to our MMM as a Mediterranean-type meal because of its nutritional composition (rich in omega-3 PUFAs, oleic acid, polyphenols, and other antioxidant content) consistent with a Mediterranean-type diet, and the use of other ingredients that are typical of such a diet (EVOO, almonds, beans, and tomatoes). The HSFAM consisted of a sausage, egg, and cheese breakfast sandwich with 3 servings of hash brown potatoes obtained from a multinational fast-food chain on the day of each evaluation. The nutritional composition of both test meals is listed in Table 1. Nutritional compositions of both meals were obtained from the Food Processor Nutrition Analysis software (ESHA Research, USA). Content of most macro- and micronutrients was similar between meals with the exception of SFA, omega-3 PUFA, cholesterol, fibres, and vitamin C, which are inherent differences between the Mediterranean and western-type diets and their representative meals. Meals were consumed in the early morning (0900–0930 h) after an overnight fast. Subjects were asked to refrain from strenuous exercise and alcohol consumption for 24 h prior to both study visits. Medical personnel performing evaluations were blinded to intervention sequence.

Anthropometric measures and blood samples

Subjects underwent a medical history, physical examination with measurement of height, weight, and body composition with tetrapolar bio-electrical impedance (Tanita BC418, Tanita Corp., Japan). Blood was drawn in the fasted state (baseline) and at 2 (T2) and 4 (T4) hours into the postprandial period (i.e., following meal intake). These time points were chosen since meals rich in FAs elicit most of their effect between 2 and 4 h into the postprandial period (Vogel et al. 1997). Quantifications of blood glucose, insulin, lipid profile, and high-sensitivity C-reactive protein (hsCRP) were performed by a Siemens Dimension RxL (Siemens AG, Germany). LDL cholesterol levels were calculated with the Friedewald formula, which is highly dependent on triglyceride levels. For that reason, postprandial LDL levels will be provided but not further interpreted. High sensitivity interleukin-6 (hsIL6) and myeloperoxidase (MPO) were evaluated by immunoassays (R&D Systems

HS600B and Alpc Diagnostics 30-6631A, respectively). Of note, hsIL6 and MPO were quantified only in a subset of 6 individuals and served as an exploratory investigation.

Background dietary habits, and more specifically those related to FA intake, were evaluated using a validated 14-item food frequency questionnaire (FFQ) for cardiovascular prevention that results in a score reflecting global dietary pattern and subscores reflecting SFA, monounsaturated FAs (MUFA), omega-3 PUFA, omega-6 to omega-3 PUFA ratio, and fruit and vegetable intakes (Laviolle et al. 2005). This questionnaire was recently shown to appropriately estimate the erythrocyte levels of vegetable and marine sources of omega-3 PUFAs and omega-6 PUFAs (Turcot et al. 2015). Furthermore, past and present physical activity habits were assessed using a validated 10-item questionnaire that estimates maximal oxygen uptake ($\dot{V}O_{2max}$) levels with very high reliability (Trivel et al. 2004).

Endothelium-dependent dilatation

Endothelial function was evaluated in the fasted state and 2 and 4 h following ingestion of the test meal (T2 and T4, respectively). Brachial ultrasonography was performed according to published guidelines (Corretti et al. 2002) following a 5-min brachial artery occlusion using a pressure cuff connected to an automated cuff inflator (D.E. Hokanson Inc., model E20, USA) positioned above the right elbow and inflated at 80 mm Hg over the systolic blood pressure. Images were analyzed offline using the Brachial analyzer for Research software (Medical Imaging Applications LLC, USA) by a single technician blinded to the meal sequence to minimize variability and potential bias. Flow-mediated dilatation (FMD) was defined as the maximal percent of arterial dilatation from baseline during postocclusive reactive hyperemia. Using this method, the intra- and inter-observer variability for brachial artery diameter determinations are 0.056 ± 0.024 and 0.073 ± 0.031 mm, respectively (Nigam et al. 2003). The intra-subject coefficient of variation between fasting FMD measurements was 9%, which is lower than previously published values (Herrington et al. 2001). Resting and hyperemic blood flow was also measured at rest and following cuff deflation.

Endothelium-independent dilatation

Fasting nitroglycerin-mediated dilatation (NMD), a marker of endothelium-independent dilatation, was assessed 5 min after completion of the evaluation of FMD according to published guidelines (Corretti et al. 2002). NMD was defined as the maximal percent of arterial dilatation during a 3-min period following administration of a sublingual vaporization of 0.4 mg of nitroglycerin. Endothelium-independent dilatation was evaluated to ensure normal vascular smooth muscle vasomotor function.

Total plasma FA profile

Total fasting and postprandial (T4) plasma FAs, free and bound to triglycerides and phospholipids, were characterized by gas chromatography coupled to mass spectroscopy following a previously described methodology (Gelinas et al. 2011). Individual FAs were expressed as a percentage of total plasma FA concentration and thus represent a proportion of total plasma FAs.

Statistical analysis

The study population size of 28 subjects provided 90% power to detect a 2% absolute variation in FMD, which was previously shown to be clinically significant (Corretti et al. 2002; Nigam et al. 2003) and to be associated to adjusted risk of future cardiovascular events (Matsuzawa et al. 2015). A post hoc unsupervised agglomerative hierarchical clustering (hclust R function) analysis identified 2 subgroups of participants based on the similarity of their baseline characteristics (age, body mass index (BMI), glucose, insulin, homeostasis model assessment insulin resistance (HOMA-IR), total, LDL and high-density lipoprotein (HDL) cholesterol, triglycerides,

Table 2. Averaged baseline characteristics of study participants for both study visits.

	All subjects	Group 1	Group 2
n	28	11	17
Age (y)	33.8±8.3	35.5±7.0	32.7±9.0
BMI (kg/m ²)	25.2±3.1	26.9±3.1*	24.1±2.5
Total body fat (%)	18.3±6.3	21.3±4.0	16.6±6.8
Trunk fat mass (%)	20.5±7.3	23.5±4.7	18.4±8.1
Glucose (mmol/L)	4.8±0.4	4.9±0.3	4.7±0.3
Insulin (pmol/L)	38 [28, 55]	62 [53, 86] [†]	29 [28, 35]
HOMA-IR	1.0 [0.8, 1.8]	2.0 [1.7, 2.8] [†]	0.8 [0.7, 1.0]
Total cholesterol (mmol/L)	4.5±1.0	5.1±1.0	4.2±0.7
LDL (calculated) (mmol/L)	2.8±0.9	3.3±0.9 [†]	2.5±0.8
HDL (mmol/L)	1.3±0.4	1.1±0.2 [†]	1.5±0.4
Non-HDL (mmol/L)	3.2±1.1	4.0±1.0 [†]	2.8±0.8
Triglycerides (mmol/L)	0.8 [0.7, 1.3]	1.4 [1.1, 1.8] [†]	0.7 [0.6, 0.8]
Cholesterol/HDL	1.4±0.9	5.0±1.2 [†]	3.1±1.0
TG/HDL	0.6 [0.5, 1.4]	1.6 [0.9, 1.9] [†]	0.5 [0.4, 0.6]
hsCRP (mg/L)	0.8 [0.3, 0.9]	0.8 [0.6, 1.0]	0.8 [0.2, 0.8]
FMD (%)	10.7±2.8	11.0±3.6	10.5±2.3
Resting diameter (mm)	3.8±0.4	3.9±0.3	3.7±0.4
NMD (%)	11.8±3.1	12.1±3.3	11.5±3.0

Note: Brackets [] are used to detail [Q1, Q3] in cases of non-normally distributed values. BMI, body mass index; FA, fatty acids; FMD, flow-mediated dilatation; HDL, high-density lipoprotein; HOMA-IR, homeostatic model assessment of insulin resistance; hsCRP, high-sensitivity C-reactive protein; LDL, low-density lipoprotein; NMD, nitroglycerin-mediated dilatation; Q, quartile; TG, triglycerides.

* $p < 0.05$.

[†] $p < 0.01$ versus group 2.

and cholesterol/HDL). Shapiro–Wilk test was used to evaluate normality of distribution and data not following normality were log-transformed before analysis or analyzed with nonparametric tests such as the case of plasma FA. No evidence of meal sequence carry-over effect (2-way ANOVA using meal sequence as interaction term) and no significant differences in fasting (baseline) parameters between study visits were observed. Baseline parameters were thus averaged to simplify data presentation. Postprandial area under the curve was calculated according to the trapezoid method (pracma R package). All tests were 2-sided and conducted at the 0.05 significance level. Normally distributed values are presented as means \pm SD, while those not following normality are presented as median [quartile (Q)1, Q3]. Analyses were performed with the R software version 3.0.1 (R Development Core Team 2011).

Results

Averaged baseline characteristics for both study visits are shown in Table 2 and are reflective of a healthy cohort without evidence of cardiovascular risk factors and with normal fasting endothelial function (i.e., FMD of $10.7\% \pm 2.8\%$). Moreover, the average total plasma FA content was 1329 [1079, 1570] nmol/L.

Identification of metabolic subgroups and baseline characteristics

An unsupervised hierarchical clustering algorithm defined 2 subgroups of participants based on the similarity between the baseline characteristics detailed in Table 2. The subgroups generated comprised 11 (group 1) and 17 (group 2) individuals, respectively. Post hoc power calculations revealed that the sample sizes of the aforementioned subgroups provided 80% power to detect a 2.5% and a 2% absolute variation in FMD, respectively. Group 1 (also referred to as the *dysmetabolic group*) comprised subjects with significantly higher BMI, fasting insulin and HOMA-IR, fasting total and non-HDL cholesterol, and fasting triglycerides and triglyceride to HDL ratio in comparison with subjects from group 2 (healthy group) ($p < 0.05$ vs. group 2) (Table 2). Moreover, total body fat and trunk fat percentages tended to be higher in group 1 ($p < 0.1$ vs. group 2). Subjects within the dysmetabolic group

Table 3. Scores of food frequency questionnaire evaluating dietary habits and habitual physical activity questionnaire evaluating fitness level.

	All subjects (n = 28)	Group 1 (n = 11)	Group 2 (n = 17)
Global dietary score	8.7±4.3	8.3±4.1	9.1±4.6
SFA score	3.0±1.5	3.1±1.6	2.9±1.5
MUFA score	2.0 [1.0, 4.0]	2.0 [1.5, 3.0]	2.5 [1.0, 4.0]
Omega-3 PUFA score	4.0 [2.0, 8.0]	4.0 [3.5, 8.0]	3.5 [2.0, 8.0]
Fruit and vegetables score	3.0±1.5	3.9±1.4*	5.1±1.7
Omega-6:omega-3 PUFA	3.6 [2.7, 4.3]	3.4 [2.9, 4.0]	3.7 [2.4, 4.5]
Estimated $\dot{V}O_{2max}$ (mL/(kg·min))	49.7 [45.2, 55.8]	49.0 [42.8, 51.2]	51.5 [46.3, 55.8]

Note: Brackets [] are used to detail [Q1, Q3] in cases of non-normally distributed values. MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; Q, quartile; SFA, saturated fatty acids.

* $p < 0.05$ versus group 2.

(group 1) also had significantly lower HDL cholesterol ($p < 0.01$ vs. group 2, Mann-Whitney (MannW)). Importantly, fasting endothelial parameters (%FMD, resting arterial diameter, and %NMD) were not significantly different between subgroups. Finally, baseline total FA levels were significantly greater in dysmetabolic subjects (group 1) than those within group 2 (1585 [1437, 1885] vs. 1188 [1053, 1355] nmol/L respectively, $p < 0.05$, MannW).

As for the dietary habits and fitness levels, most of the FFQ items were not significantly different between groups 1 and 2 with the exception of daily fruit and vegetable consumption, which was significantly lower for dysmetabolic individuals (group 1) ($p < 0.05$, MannW) (Table 3). Fitness levels (estimated $\dot{V}O_{2max}$) were not significantly different and within normal age-adjusted ranges for all individuals (Table 3).

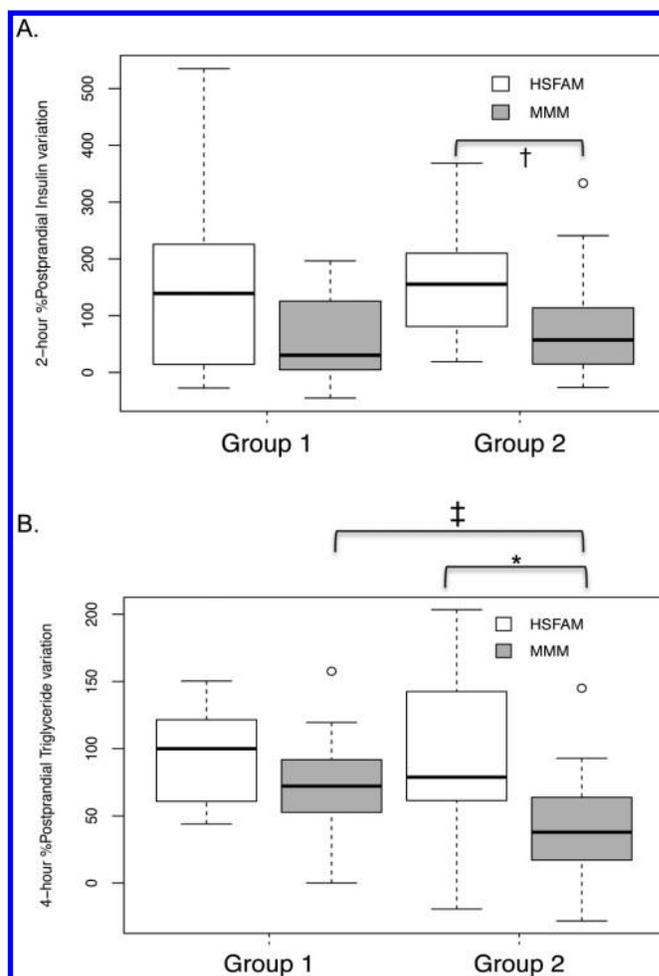
Postprandial metabolic and endothelial responses to test meals

The ingestion of both test meals resulted in significant elevations in insulin and triglycerides in both groups (Figs. 1A and 1B and Supplementary Table S1¹). Meal composition influenced the amplitude of the hypertriglyceridemic response solely in group 2 ($p < 0.005$, Wilcoxon between meals). Group 2 also experienced a less significant increase in triglycerides following the MMM in comparison with group 1 ($p < 0.05$, MannW between groups). Area under the triglyceride curve (AUC_{TC}) was significantly higher in dysmetabolic subjects (group 1) ($p < 0.05$ vs. group 2, MannW) and was higher following the HSFAM within group 2 ($p < 0.05$ vs. MMM, Wilcoxon). Finally, no significant postprandial variations in hsCRP, hsIL6, or MPO were observed (Supplementary Table S2¹).

The HSFAM induced significant postprandial endothelial dysfunction 4 h following meal ingestion only in dysmetabolic individuals (group 1) ($\Delta\%FMD_{absolute} = -5.28 \pm 2.54$, $p < 0.01$ vs. MMM, and $p < 0.01$ vs. group 2), while the MMM had no significant effect on postprandial endothelial function in either group (Fig. 2 and Supplementary Table S1¹). Additionally, the 95% confidence interval (CI) of the observed 4-h postprandial FMD following the HSFAM in group 1 individuals (i.e. 95%CI from 4.34% to 7.34%FMD) does not exceed their baseline FMD level. This adds robustness to the postprandial FMD alterations observed in these dysmetabolic individuals.

Plasma FA profiles following test meals

No significant group differences in postprandial variations in FA profile to meals were observed. Postprandial FAs results are therefore presented for the entire cohort ($n = 28$). Both meals induced postprandial total FA elevation that was significantly greater following the HSFAM ($\Delta Total FA_{HSFAM} = 295 [130, 519]$ nmol/L) than the

Fig. 1. Metabolic response to meals. Postprandial insulin (A) and triglyceride (B) variations (expressed in percentage of variation relative to baseline) to test meal ingestions. White circles represent data outside of 1.5x the interquartile range (Q3-Q1). *, Wilcoxon signed-rank, $p < 0.05$; †, Wilcoxon signed-rank, $p < 0.01$; ‡, Mann-Whitney $p < 0.05$. HSFAM, high-saturated fatty acid meal; MMM, mixed Mediterranean-type meal.

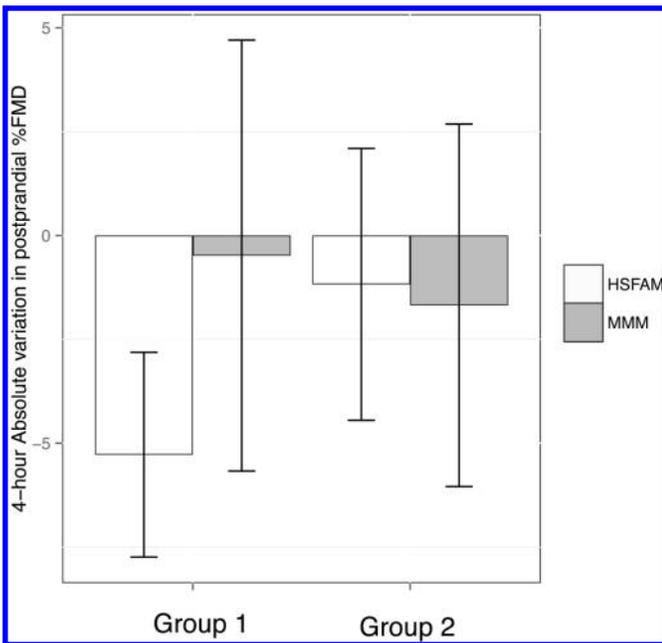
MMM ($\Delta Total FA_{MMM} = 139 [26, 333]$ nmol/L, $p = 0.01$ between meals). Both test meals also induced variation in postprandial FA composition (Fig. 3 and Supplementary Table S3¹). For instance, the proportions of SFA (C16:0), MUFA (C18:1n9, C20:4), omega-6 PUFA (C18:2n6), and each omega-3 PUFA were significantly modified in the postprandial state by both meals ($p < 0.05$ vs. baseline). Furthermore, the postprandial proportions of several FAs, namely C18:0, C18:1n9, 18:2n6, and each omega-3 PUFA were significantly influenced by meal composition ($p < 0.05$ between meals). Notably, postprandial plasma omega-3 to omega-6 PUFA ratios were significantly increased only following the MMM ($\Delta\omega\text{-3}:\omega\text{-6} = 0.006 [0.001, 0.02]$ vs. $-0.002 [-0.007, 0.003]$ following the HSFAM, $p < 0.001$).

Discussion

This study is, to our knowledge, the first to have (i) investigated the influence of a single and complete meal reflective of a Mediterranean-type diet on postprandial endothelial function, (ii) concomitantly evaluated postprandial circulating FA composition, and (iii) investigated the impact of subclinical metabolic dys-

¹Supplementary data are available with the article through the journal Web site at <http://nrcresearchpress.com/doi/suppl/10.1139/apnm-2015-0490>.

Fig. 2. Endothelial function. Absolute endothelial function variations 4 h following meal ingestion for both subgroups (expressed in %FMD units variation from baseline). *, Student's *t* test, $p < 0.01$; †, Student's *t* test, $p < 0.005$. FMD, flow-mediated dilatation; HSFAM, high-saturated fatty acid meal; MMM, mixed Mediterranean-type meal.



regulations on these parameters. Our results demonstrate for the first time that an MMM does not alter postprandial endothelial function in healthy males, regardless of the fasting metabolic profile. The fact that the MMM is less challenging for postprandial endothelial homeostasis adds to the many characteristics of the Mediterranean-type diet and presumably contributes to its cardiometabolic benefits. Interestingly, we also observed that an isocaloric HSFAM impairs postprandial endothelial function, but only in a subgroup of healthy individuals with subclinical metabolic dysregulations.

The neutral effect on endothelial function exhibited by the MMM in our cohort could be attributable to, among other factors, its EVOO content, which is known for its favorable FA content and anti-inflammatory and antioxidant polyphenol composition (Ghanim et al. 2011). The addition of EVOO to fat-rich meals was shown to improve postprandial glucose, LDL, and oxidized LDL, and to prevent increases in inflammation, endothelial adhesion molecules, and oxidative stress associated with consumption in healthy and hypertriglyceridemic subjects (Bogani et al. 2007; Pacheco et al. 2008; Violi et al. 2015). In the present study, MMM ingestion in fact resulted in plasma oleic acid (18:1n9) elevation that could have acted in synergy with polyphenols to explain the neutrality of the MMM on endothelial function. Furthermore, ingestion of the MMM, which was also an important source of long-chain omega-3 PUFAs, resulted in significant increases in their circulating levels and of the omega-3 to omega-6 PUFA ratio. The acute elevation of this class of PUFA is known to result in the production of eicosanoids and oxylipins with lesser inflammatory potential than those synthesized from omega-6 PUFA and SFA (Armah et al. 2008; Margioris 2009; Strassburg et al. 2014) and to attenuate HSFAM-induced endothelial impairments (Fahs et al. 2010). Altogether, these features contribute to decreasing the stress induced by the MMM on metabolic and vascular homeostatic mechanisms.

The impairments in endothelial function that occurred following the HSFAM in dysmetabolic subjects (group 1) are also of interest since they corroborate what was previously observed in a cohort of subjects with high cardiometabolic risk factors. In fact,

most trials reporting a significant impairment of postprandial endothelial function following a HSFAM did so in participants with overt cardiovascular risk factors, such as hypertriglyceridemia (Giannattasio et al. 2005; Norata et al. 2006), abdominal obesity (Wang et al. 2011), and/or type 2 diabetes mellitus (West et al. 2005). Recently, healthy subjects with elevated anthropometric and fasting metabolic parameters were found to exhibit metabolic, inflammatory, and endocrine responses to a high-SFA beverage that were intermediary between those of healthier subjects and subjects with metabolic syndrome (Kardinaal et al. 2015). In the present trial, those healthy individuals that displayed postprandial endothelial impairments following the HSFAM (group 1) had adiposity, fasting insulinemia, and lipemia (notably triglyceridemia), which were significantly higher than their counterparts (group 2), although all values remained within ranges regarded as "normal".

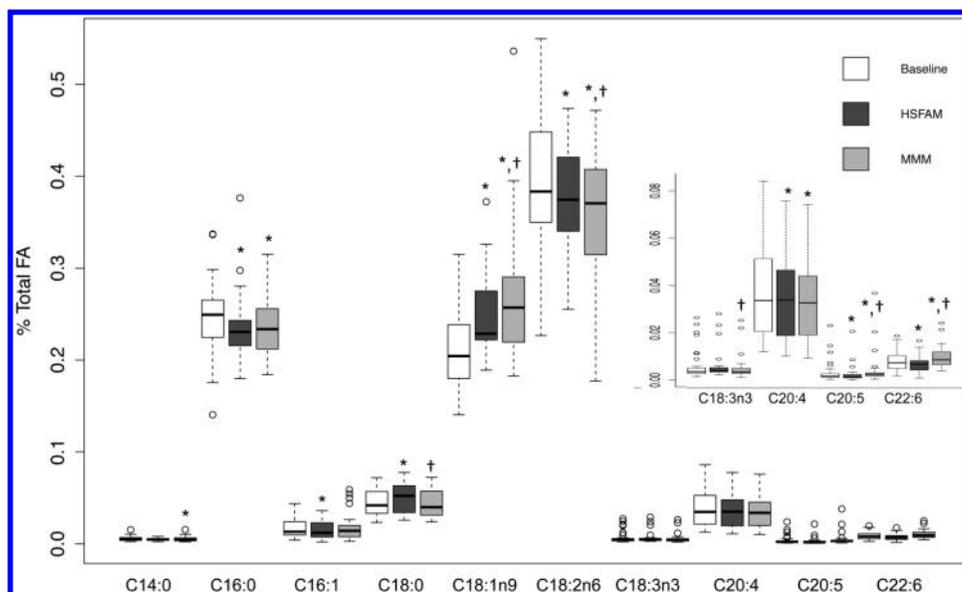
The aforementioned marginally altered anthropometric and fasting metabolic parameters in otherwise healthy individuals could thus exacerbate the postprandial endothelial – and metabolic – sensitivity to meal composition and be early indicators of increased risk of postprandial impairments. In fact, the subclinical metabolic alterations exhibited by group 1 seem to have increased their susceptibility to the unfavourable composition of the HSFAM (i.e., high SFA and cholesterol content, low omega-3 to omega-6 PUFA ratio, and low antioxidant content) in comparison with the MMM. For one, these phenotypes could impair the – or be the result of impaired – postprandial handling of the lipid load that inevitably arises from the ingestion of fat-rich meals. Indeed, dysmetabolic individuals (group 1) experienced a prolonged postprandial period spent under a greater triglyceridemic load (i.e., greater AUC_{TC} than group 2), which in addition, was enriched in SFA like stearic acid (C18:0) following the HSFAM. Second, the marginal metabolic profile exhibited by group 1 might also have blunted their ability to handle postprandial inflammatory and/or oxidative insults. Incidentally, dysmetabolic individuals (group 1) reported a lower fruit and vegetable consumption, which could also have contributed in lowering their basal anti-inflammatory and antioxidant status. This item of the FFQ was in fact shown to correlate positively with plasma vitamin C and folate concentrations, both of which exert antioxidant effects (Laviolle et al. 2005).

Some elements of the present trial might be regarded as limitations to the interpretation and generalization of results. First, the inclusion of participants was limited to healthy men below 50 years of age in an effort to minimize potential confounders, such as the effect of aging and of the female hormonal cycle on endothelial function (Harris et al. 2012; Higashi et al. 2012) and its evaluation by FMD (Witte et al. 2005). Second, the quantification of inflammatory and oxidative stress biomarkers in a statistically insufficient subset of participants might have led to misinterpretation of the involvement of these processes in the observed postprandial endothelial events. This, however, does not eliminate the potential involvement of low-grade oxidative stress and/or inflammation processes at the systemic and/or vascular endothelial level (Wopereis et al. 2013). Elevation of postprandial plasma inflammation and/or oxidative stress are rarely and still inconsistently reported in healthy subjects (Lacroix et al. 2012) and should be interpreted with caution since they often only reflect local inflammatory response to venous cannulation, a procedure not performed here (Herieka and Erridge 2013; Wopereis et al. 2013). Finally, the choice of a short semiquantitative FFQ aimed specifically at evaluating the quality of FA intake may not have adequately taken into account the impact of habitual intake of nutrients other than FAs on endothelial responses to test meals.

Conclusions

Altogether, our observations suggest that subclinical metabolic dysregulations are early signs of increased endothelial and meta-

Fig. 3. Plasma total fatty acid profile. Fasted and postprandial plasma fatty acid composition. Values represent the proportion of each fatty acid with respect to total plasma fatty acid content, free and bound to triglycerides and phospholipids. Omega-6 and omega-3 polyunsaturated fatty acids values have been reproduced with a larger scale (in %Total FA) for ease of visualization. White circles represent data outside of 1.5× the interquartile range (Q3-Q1). *, Wilcoxon, $p < 0.05$ vs. baseline; †, Wilcoxon, $p < 0.05$ vs. HSFAM. FA, fatty acid; HSFAM, high-saturated fatty acid meal; MMM, mixed Mediterranean-type meal.



bolic sensitivity to meal composition. Individuals with such a phenotype could thus be at increased risk for postprandial endothelial dysfunction following meals, inducing a greater stress on homeostatic mechanisms such as HSFAM. These individuals would thus benefit even more from consuming meals representative of a Mediterranean-type diet demonstrated herein for their nondeleterious endothelial properties. This study also further highlights the importance of challenging homeostasis to uncover otherwise unrecognized postprandial abnormalities, thus improving the evaluation of the cardiovascular risk and personalization of dietary recommendations.

Upon confirmation in a larger cohort of healthy individuals with marginally dysregulated fasting parameters, these findings ultimately will deepen the understanding of homeostatic mechanisms that maintain and determine vascular and overall health and will highlight the importance of the nutritional composition of each and every meal, even in seemingly healthy individuals.

Conflict of interest statement

The authors of this study have no relevant conflict of interest to disclose.

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