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ORIGINAL DATA

Acute Effects of Dietary Fat on Inflammatory Markers and Gene Expression in First-Degree Relatives of Type 2 Diabetes Patients

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■ Abstract

BACKGROUND: Subjects with type 2 diabetes (T2D) and their relatives (REL) carry an increased risk of cardiovascular disease (CVD). Low-grade inflammation, an independent risk factor for CVD, is modifiable by diet. Subjects with T2D show elevated postprandial inflammatory responses to fat-rich meals, while information on postprandial inflammation in REL is sparse. **AIM**: To clarify whether medium-chain saturated fatty acids (SFA) and monounsaturated fatty acids (MUFA) have differential acute effects on low-grade inflammation in REL compared to controls (CON). **METHODS**: In randomized order, 17 REL and 17 CON ingested two fat-rich meals, with 72 energy percent from MUFA and 79 energy percent from mainly medium-chain SFA, respectively. Plasma high sensitivity C-reactive protein (hs-CRP), interleukin-6 (IL-6), adiponectin, and leptin were measured at baseline, 15 min, 60 min, and 240 min postprandially. Muscle and adipose tissue biopsies were taken at baseline and 210 min after the test meal, and expression of selected genes was analyzed. **RESULTS**: Plasma IL-6 increased ($p < 0.001$) without difference between REL and CON and between the meals, whereas plasma adiponectin and plasma hs-CRP were unchanged during the 240 min observation period. Plasma leptin decreased slightly in response to medium-chain SFA in both groups, and to MUFA in REL. Several genes were differentially regulated in muscle and adipose tissue of REL and CON. **CONCLUSIONS**: MUFA and medium-chain SFA elicit similar postprandial circulating inflammatory responses in REL and CON. Medium-chain SFA seems more proinflammatory than MUFA, judged by the gene expression in muscle and adipose tissue of REL and CON.

Keywords: type 2 diabetes · dietary fat · fat-rich meal · gene expression · genetic predisposition · interleukin 6 · postprandial inflammation · postprandial period

Introduction

Type 2 diabetes (T2D) has reached epidemic proportions worldwide, and is associated with a two- to three-fold increase in cardiovascular disease (CVD) [1]. Healthy first-degree relatives of patients with T2D (REL) carry an increased risk of developing both T2D and CVD [2].

In addition to conventional risk factors such as dyslipidemia, hypertension, smoking, obesity, male gender, and family history of CVD, various other factors can play important roles in the pathogenesis of atherosclerosis and CVD [3]. Adipose tissue is a source of inflammatory cytokines, e.g. interleukin 6 (IL-6) that stimulates production of the acute-phase reactant C-reactive protein (CRP). Elevated concentrations of circulating high-sensitivity CRP (hs-CRP) are associated with an increased risk of cardiovascular morbidity and mortality [4]. Adipose tissue also produces the adipokines adiponectin and leptin. While increased circulating adiponectin levels are negatively corre-

lated [5], increased leptin levels are strongly positively correlated to insulin resistance, T2D, levels of inflammatory markers, and CVD [6].

Intervention studies point to the habitual diet having moderate influence on the concentrations of inflammatory markers and adipokines, although these biomarkers do not vary in unison [7]. Dietary influences have been demonstrated for carbohydrates, fibres, and fat. A number of studies indicate that consumption of monounsaturated fatty acids (MUFA) is associated with decreased levels of inflammatory markers, while a diet high in saturated fatty acids (SFA) increases levels of inflammation [7]. These changes are not restricted to long-term effects, but also occur acutely. Thus, an SFA load increases hs-CRP levels in healthy individuals and in subjects with T2D [8], while MUFA decreases hs-CRP in healthy persons [9]. In both healthy and type 2 diabetic subjects, circulating adiponectin levels decline slightly after a fat-rich meal [10, 11]. Leptin decreases 0-240 min postprandially [12], but increases subsequently after a fat-rich meal [13]. Also, IL-6 has been found to increase in response to a fat-rich meal [8, 14, 15].

Little information is available on the impact of fat quality on inflammatory markers and adipokines in REL, a high-risk population for CVD. Recently, we have demonstrated a differential effect of fat quality on the independent CVD risk factor postprandial triglyceridemia in REL. Medium-chain SFA caused a higher increase in 240-min postprandial circulating triglyceride levels in REL than in healthy controls with no family history of T2D (CON). In contrast, MUFA had similar effects on triglycerides in both groups (unpublished results). The aim of the present study is to clarify whether medium-chain SFA and MUFA have differential acute effects on circulating hs-CRP, IL-6, leptin, and adiponectin in REL compared to CON. In addition, we investigated the acute impact of medium-chain SFA and MUFA on gene expression in muscle and adipose tissue in REL and CON.

Abbreviations:
ADA - American Diabetes Association
ADIPOQ - adiponectin gene
ADIPOR1/2 - adiponectin receptor 1/2
ANOVA - analysis of variance
BMI - body mass index
CCL2 - chemokine (C-C motif) ligand 2
CD16 - cluster of differentiation 16a
CI - confidence interval
CON - controls
CRP - C-reactive protein
CV - coefficient of variation
CVD - cardiovascular disease
DanORC - Danish Obesity Research Centre
ELISA - enzyme-linked immunosorbent assay
FCGR3A - Fc fragment of IgG low affinity IIIa receptor (formerly known as CD16a)
FDR - false discovery rate
FP - fasting plasma
FPG - fasting plasma glucose
HbA1c - glycated hemoglobin
HDL - high-density lipoprotein
HOMA-IR - homeostasis model of assessement - insulin resistance
hs-CRP - high-sensitivity CRP
IL-1B - interleukin-1β
IL-6 - interleukin-6
IL-6R - interleukin-6 receptor
LDL - low-density lipoprotein
LEP - leptin
LEPR - leptin receptor
MCP1 - monocyte chemoattractant protein 1
MUFA - monounsaturated fatty acids
NCoE - Nordic Centre of Excellence
PCR - polymerase chain reaction
RBP4 - retinol binding protein 4
REL - first-degree relatives of type 2 diabetes patients
RNA - ribonucleic acid
SEM - standard error of mean
SFA - saturated fatty acids
SYSDIET - Systems Biology in Controlled Dietary Interventions and Cohort Study
T2D - type 2 diabetes
TLR4 - toll-like receptor 4
TNF-α - tumor necrosis factor α
TNFRSF1A - tumor necrosis factor receptor superfamily member 1A
WHO - World Health Organization

Methods and subjects

Subjects

17 REL and 17 CON participated in the study. REL had either minimum two first-degree relatives with T2D, or minimum one relative with T2D, if the participant had a history of gestational diabetes. The subject characteristics are shown in Table 1. Total body fat percentage was measured by dual-energy x-ray absorptiometry. Fasting plasma glucose of the participants was < 7.0 mmol/l at the screening visit; 13 REL and 10 CON had fasting plasma glucose in the range of 5.6-6.9 mmol/l. None of the participants had any medical conditions or received any medications that could interfere with the outcomes of the study. Three REL and two CON were treated with the oral lipid-lowering drug simvastatin. All participants continued taking their medication without dose changes throughout the duration of the study. None of the subjects were smokers or had any abuse problems. The study was performed in ac-

Table 1. Clinical and biochemical characteristics of healthy first-degree relatives of patients with type 2 diabetes (REL) and controls (CON) at screening

Characteristic	REL (n = 17)	CON (n = 17)	p
Gender (F/M)	(12/5)	(11/6)	0.734
Age (yr)[a]	55.0 (48.0 - 62.0)	46.0 (37.5 - 57.5)	0.143
BMI (kg/m²)	25.1 (23.2 - 27.0)	24.5 (22.7 - 26.3)	0.646
FPG (mmol/l)	5.8 (5.5 - 6.1)	5.7 (5.4 - 5.9)	0.431
Fasting serum insulin (pmol/l)	28.4 (20.6 - 36.1)	35.5 (24.0 - 47.0)	0.267
HOMA-IR (mIU x mmol/l²)	1.2 (1.0 - 1.6)	1.3 (0.7 - 2.3)	0.487
HbA1c (%)	5.6 (5.4 - 5.7)	5.5 (5.3 - 5.6)	0.287
FP total cholesterol (mmol/l)	5.1 (4.6 - 5.5)	5.1 (4.6 - 5.6)	0.970
FP LDL cholesterol (mmol/l)	3.0 (2.6 - 3.3)	2.8 (2.3 - 3.3)	0.543
FP HDL cholesterol (mmol/l)[b]	1.5 (1.3 - 1.7)	1.7 (1.5 - 1.9)	0.237
FP triglyceride (mmol/l)[b]	1.0 (0.8 - 1.3)	1.1 (0.9 - 1.4)	0.602
Total body fat percentage (%)[b]	31.6 (25.2 - 35.0)	28.6 (16.8 - 35.9)	0.228

Legend: Data are mean and 95 % CI unless otherwise stated. [a] Reported as median and interquartile range. [b] Reported as median and 95 % CI. BMI: body mass index. FP: fasting plasma. FPG: fasting plasma glucose. HbA1c: glycated haemoglobin. HOMA-IR: homeostasis model of assessement - insulin resistance.

cordance with the Declaration of Helsinki, 6th revision (2008), and approved by the local Ethical Committee (Region Midtjylland, Denmark). All subjects gave their written consent to participate in the study.

Study design

The present trial was conducted as an acute, controlled, randomized cross-over study on the effects of a meal high in medium-chain SFA and MUFA, respectively, on circulating biomarkers and gene expression. For 48 h before the test meal, the subjects refrained from strenuous physical activity. For 24 h before the test meal, the subjects refrained from alcohol and ingested a standard diet delivered from the research unit with 56% energy (E%) from carbohydrate, 24% from fat and 20% from protein. The women's portions had an energy content of 7,000 kJ, the men's portions of 9,000 kJ. On the test meal days, the subjects arrived at the clinic after an overnight fast (8 h). The test meals of MUFA or medium-chain SFA (details given below) were served and ingested within 15 min. Muscle and adipose tissue biopsies were taken at baseline and 210 min after the meal. Blood samples were drawn from an intravenous catheter at baseline, 15, 60, and 240 min after the test meal.

The test meals

The test meals comprised 40 g white bread, 400 ml tap water and a soup. The soup was made of 150 ml chicken broth, 10 ml lime juice, 10 g finely chopped leek, 2.5 g curry powder and 80 g oil. The MUFA-rich test meal included macadamia nut oil (SpiZe, Zelected Foods A/S), while coconut oil (DinSundhed, CB Power Food) was used in the test meal high in medium-chain SFA. The energy content of the isocaloric meals was 3,547 kJ (847 kcal) with 11 E% originating from carbohydrate, 2 E% from protein and 87 E% from fat. The MUFA-rich meal had a total fat content of 87 E% (3,102 kJ), of which 2,549 kJ (609 kcal) equal to 72 E% came from MUFA, predominantly oleic acid and palmitoleic acid. The meal high in coconut oil had a total fat content of 87 E% (3,102 kJ) and was rich in SFA (2,786 kJ (666 kcal) ~79 E%), with 49 E% from medium-chain SFA (predominantly lauric acid) and 30 E% from long-chain SFA (predominantly myristic acid).

Blood analyses

The majority of screening blood samples was analyzed the same day (at the Department of Clinical Biochemistry, Aarhus University Hospital, Denmark). At meal test days, plasma samples were immediately separated by centrifugation at 2000 x relative gravity for 15 min at 4°C. Both plasma and serum samples were placed in a -20°C freezer for 24 h, and the next day stored in a -80°C freezer until analyses were performed.

Plasma hs-CRP was measured with high sensitivity ELISA human CRP kit (DRG Diagnostics GmbH, Marburg, Germany), with a detection range of 0.1-10.0 mg/l, intra-assay coefficient of

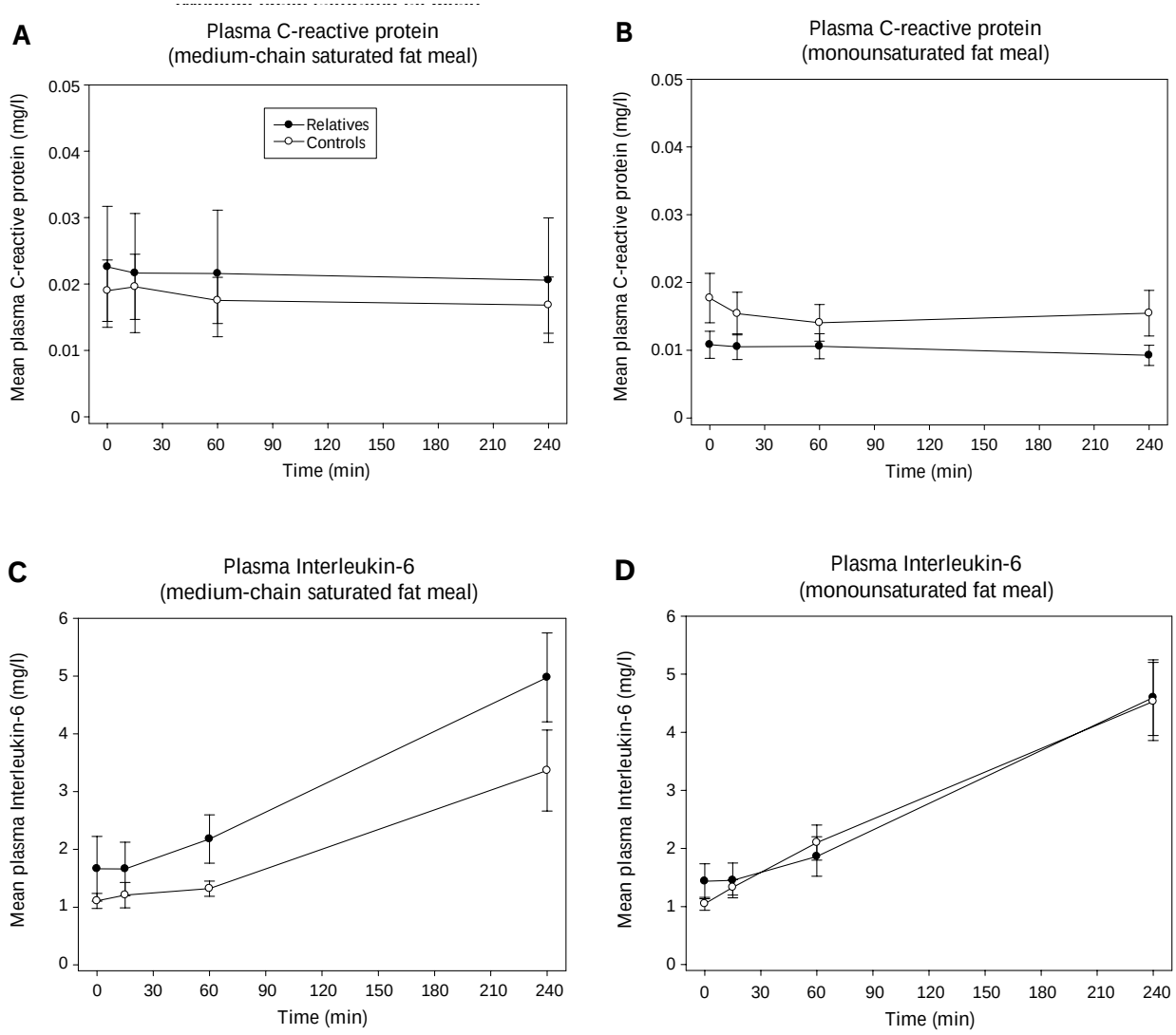

Figure 1. Mean (+/- SEM) plasma hs-C-reactive protein (**A**, **B**) and interleukin-6 (**C**, **D**) after meals high in medium-chain saturated fat and monounsaturated fat in healthy first-degree relatives of subjects with T2D (n = 17) and controls (n = 17). Comparison with repeated measurements analysis of variance (ANOVA) within meal types. No statistical differences were seen in hs-C-reactive protein and interleukin-6 between relatives and controls.

variation (CV) of 7.5%, and inter-assay CV of 4.1%. **Plasma IL-6** was measured with a high sensitivity ELISA human IL-6 kit (R&D Systems, Minneapolis, MN, USA), with a minimum detectable dose of 0.016-0.110 pg/ml, intra-assay CV of 6.9-7.8%, and inter-assay CV of 6.5-9.6%. **Total plasma adiponectin** was measured with an ELISA human adiponectin kit (B-Bridge International Inc., Cupertino, CA, USA), with a detection range of 0.4-12.0 ng/ml, intra-assay CV of 3.3-3.6%, and inter-assay CV of 3.2-7.3%. **Plasma leptin** was measured with an ELISA human leptin kit (Mediagnost GmbH, Reutlingen, Germany), with a minimum detectable dose of 0.2 ng/ml, intra-assay CV of 2.5%, and inter-assay CV of 7.7%.

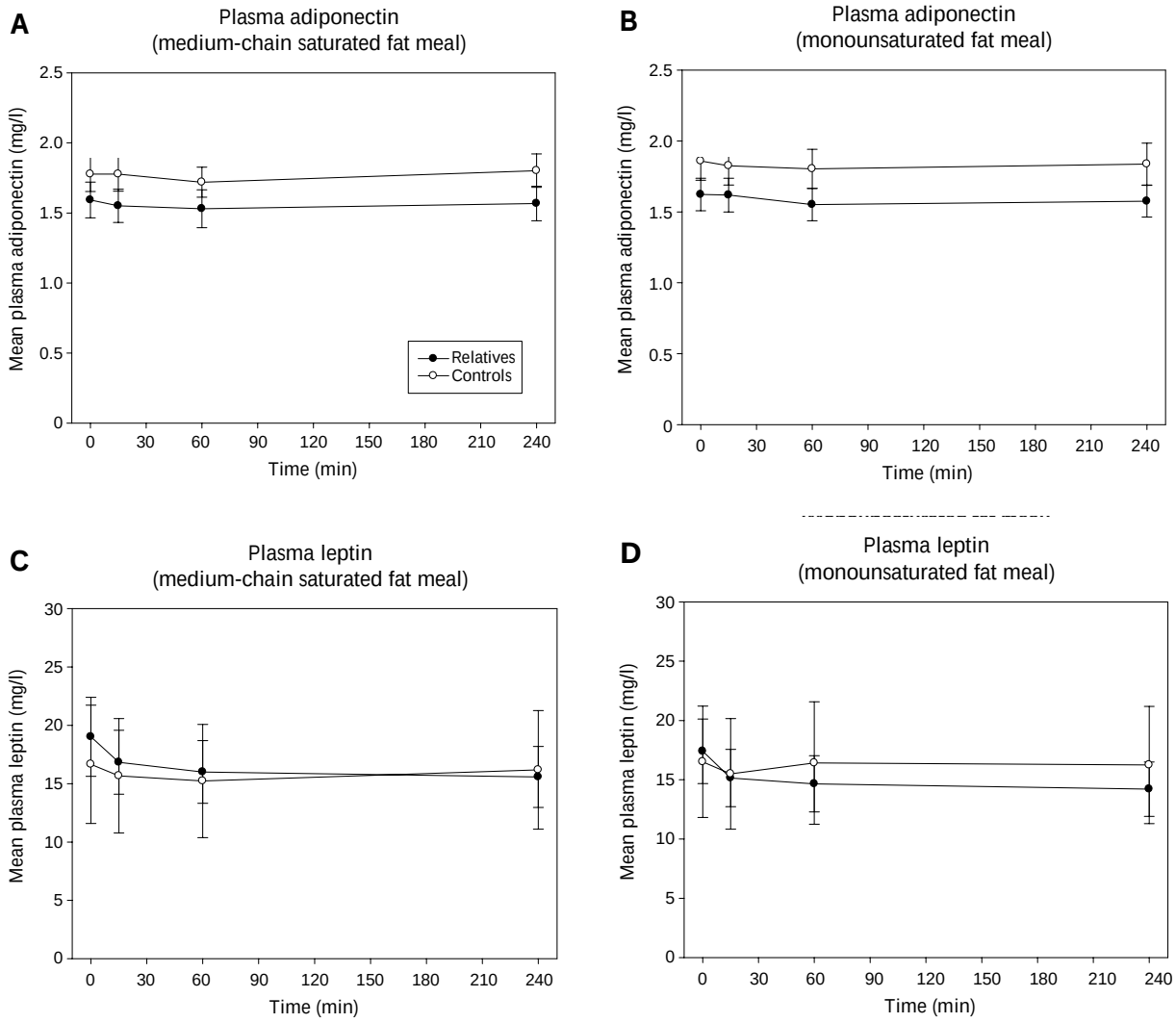

Figure 2. Mean (+/- SEM) plasma adiponectin (**A**, **B**) and leptin (**C**, **D**) after meals high in medium-chain saturated fat and monounsaturated fat in healthy first-degree relatives of patients with T2D (n = 17) and controls (n = 17). Comparison with repeated measurements analysis of variance (ANOVA) within meal types. No statistical differences were seen in plasma adiponectin and leptin between relatives and controls.

Biopsies

Biopsies were performed at baseline and 210 min after the test meal from the lateral vastus muscle and from the abdominal subcutaneous adipose tissue. The biopsies were performed with Bergström's needle under local analgesia with lidocaine (20 mg/ml, 8-10 ml) through a 1 cm incision with minimum 5 cm distance between the pre- and post-meal biopsies. The biopsy tissue was cleaned, snap frozen in liquid nitrogen, and stored in a -80°C freezer until analyses were performed.

Gene expression analyses

RNA was isolated from 25 mg muscle tissue and 250 mg adipose tissue using an acid guanidinium thiocyanate-phenol-chloroform extraction method (Trizol Reagent 15596-018, Invitrogen, Life Technologies, Carlsbad, California, USA). Gene expression analyses were performed by AROS Applied Biotechnology AS (Aarhus, Denmark) using Fluidigm BioMark real-time reverse transcriptase-PCR system. Premade primers and TaqMan Gene Expression Assays probes were pur-

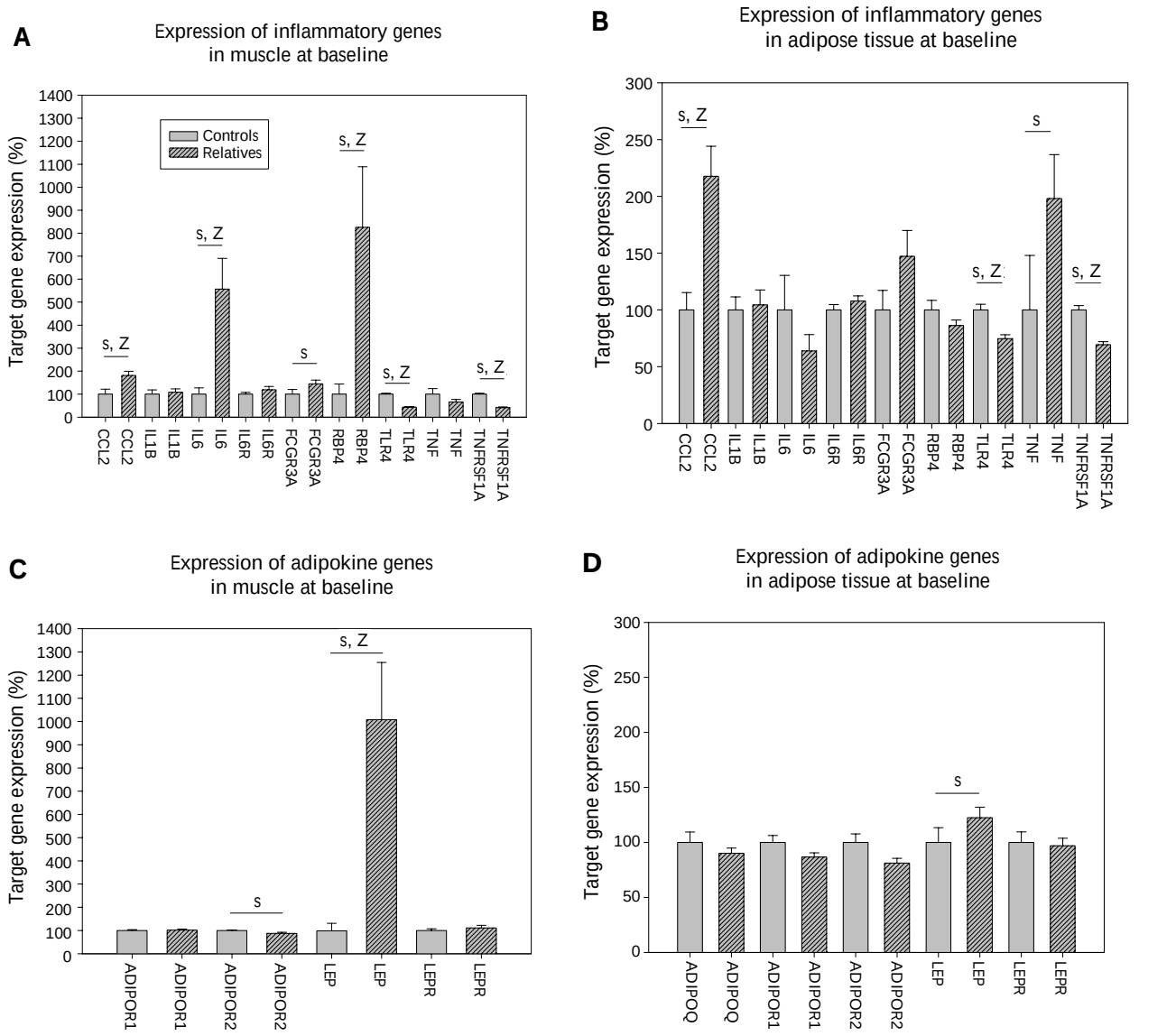

Figure 3. Expression of inflammatory genes (**A**, **B**) and adipokine genes (**C**, **D**) at baseline in skeletal muscle and adipose tissue of healthy first-degree relatives of patients with T2D (n = 15) and controls (n = 15). Gene expression was set at 100 percent (%) in controls. Gene expression in relatives is reported as percentage of that in controls. Correction for multiple testing was done with the false discovery rate (FDR) method. s: significant difference in gene expression between controls and relatives before FDR correction. Z: significant difference in gene expression between controls and relatives after FDR correction.

chased from Applied Biosystems (Life Technologies, Carlsbad, California, USA). The cycle threshold values were measured in duplicate for every sample and normalized to the geometric mean of the reference genes beta-actin and beta-2-microglobulin.

Expression of reference genes was not statistically different between groups at baseline and was not altered in response to the test meals. Correction for the remaining variation in expression of reference genes was carried out by the Pfaffl method, i.e. the relative expression ratio was calculated (target gene/reference gene), including both the variation in the target gene expression and in the reference gene expression [16]. We investigated the expression of several genes puta-

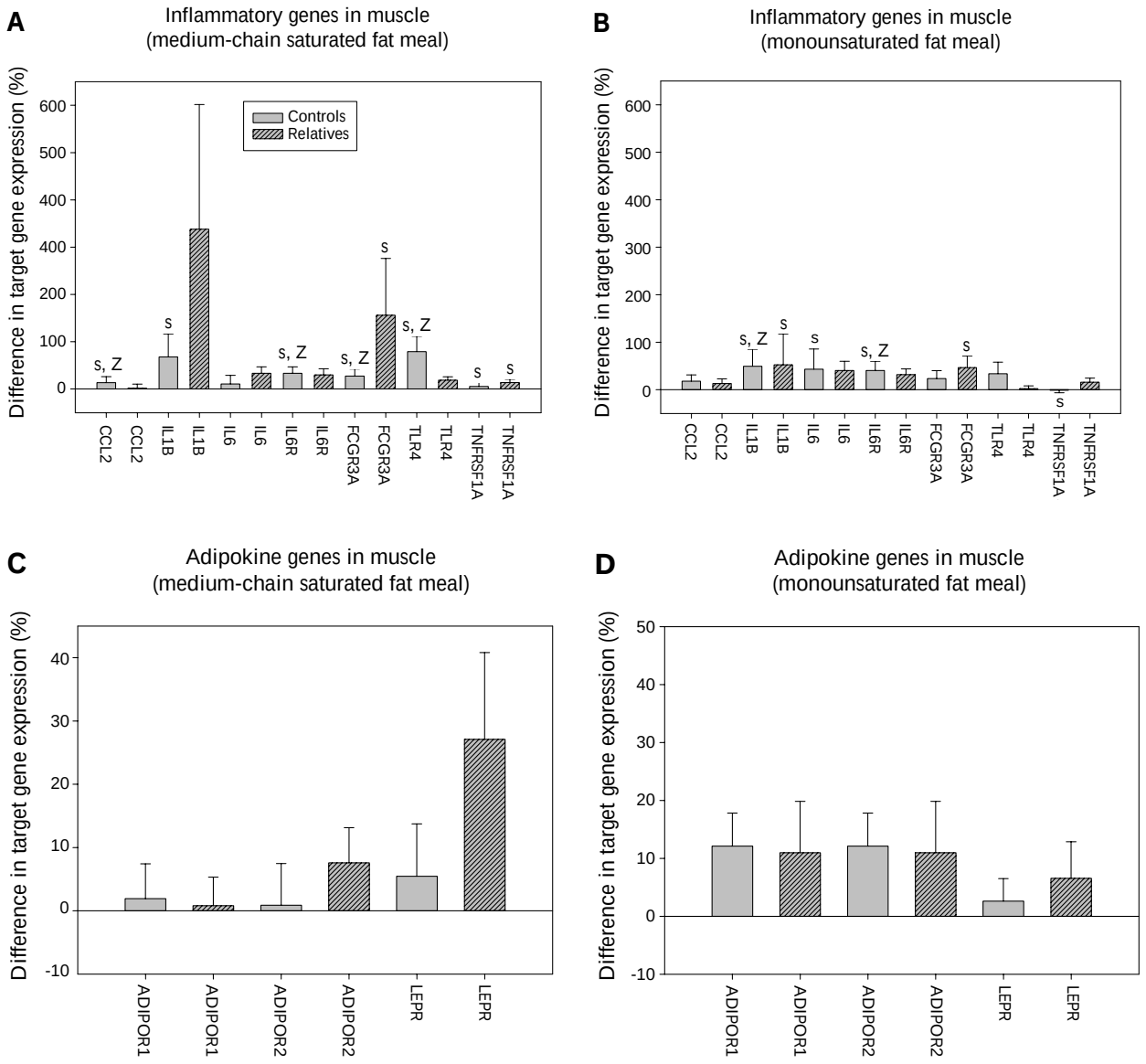

Figure 4. Changes in gene expression of inflammatory genes (**A**, **B**) and adipokine genes (**C**, **D**) in skeletal muscle from baseline till 210 min after the meal rich in medium-chain saturated and monounsaturated fat in healthy first-degree relatives of patients with T2D (n = 15) and controls (n = 15). Changes from baseline are reported in percent (%). Correction for multiple testing was done with the false discovery rate (FDR) method. TNF and LEP are for graphical reasons not depicted in the figure. LEP was significantly upregulated in relatives in response to monounsaturated fat before, but not after FDR correction, and to medium-chain saturated fat in controls after FDR correction (1722% ± 808%). TNF was significantly upregulated after FDR correction (250% ± 268%) in controls in response to monounsaturated fat (**B**). s: significant difference in gene expression from baseline till 210 min after the meal before FDR correction. Z: significant difference in gene expression from baseline till 210 min after the meal after FDR correction.

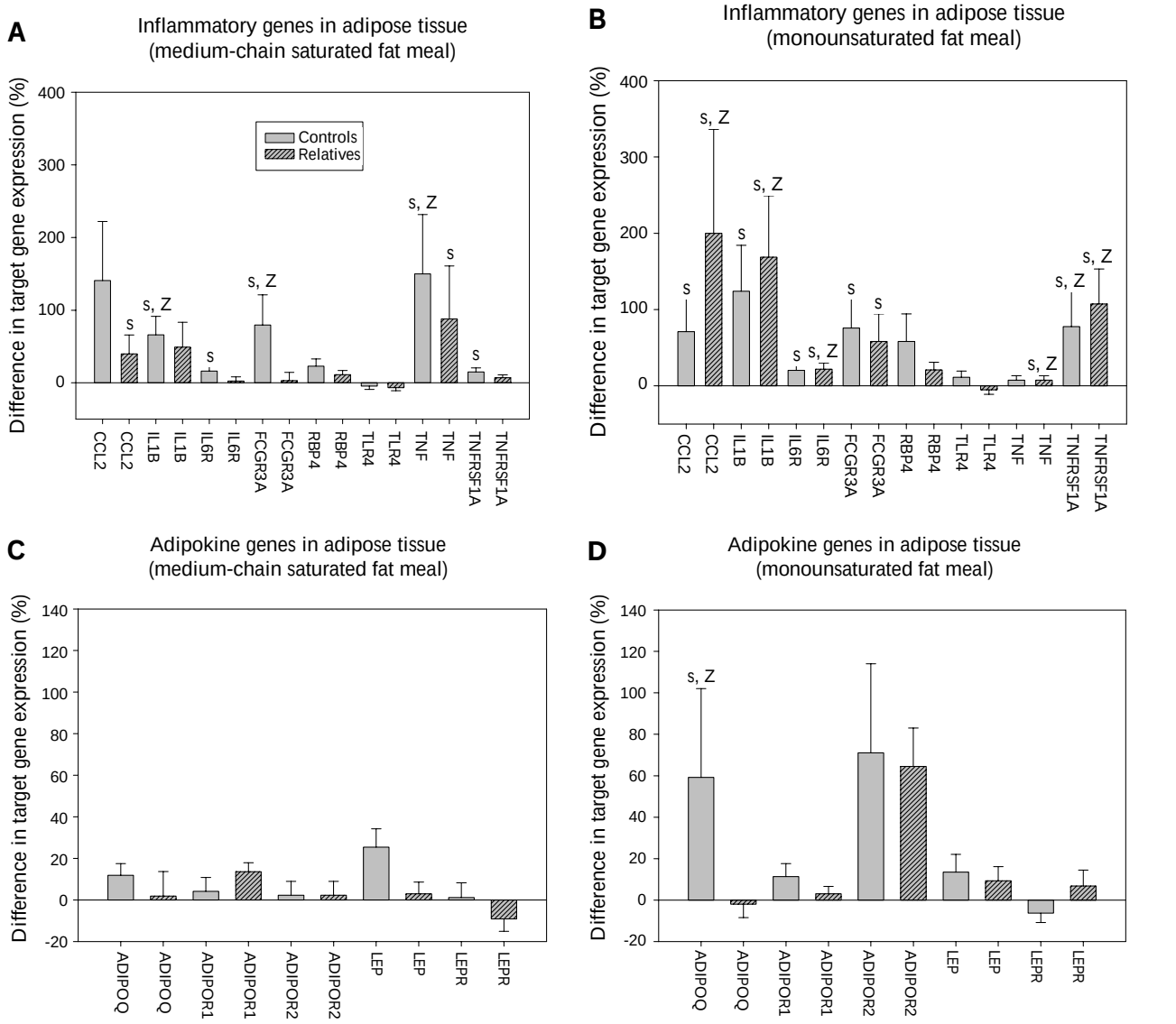

Figure 5. Changes in inflammatory gene expression (**A**, **B**) and adipokine gene expression (**C**, **D**) in adipose tissue from baseline till 210 min after the meal rich in medium-chain saturated and monounsaturated fat in healthy first-degree relatives of patients with T2D (n = 15) and controls (n = 15). Changes from baseline are reported in percent (%). Correction for multiple testing was done with the false discovery method (FDR). IL-6 is for graphical reasons not depicted in graphs **A** and **B**. IL-6 was significantly upregulated after FDR correction (515% ± 259%) in controls after medium-chain saturated fat (**A**) and (540 % ± 407%) in relatives after monounsaturated fat (**B**). s: significant difference in gene expression from baseline till 210 min after the meal before FDR correction. Z: significant difference in gene expression from baseline till 210 min after the meal after FDR correction.

tively involved in the inflammatory postprandial response in muscle and adipose tissue: adiponectin receptor 1 (ADIPOR1) and 2 (ADIPOR2), chemokine (C-C motif) ligand 2 (CCL2), formerly known as monocyte chemoattractant protein 1 (MCP1), interleukin-1β (IL1B), interleukin-6 (IL6), interleukin-6 receptor (IL6R), Fc fragment of IgG low affinity IIIa receptor (FCGR3A), formerly known as cluster of differentiation 16a (CD16a), leptin (LEP), leptin receptor (LEPR), retinol binding protein 4 (RBP4), toll-like receptor 4 (TLR4), tumor necrosis factor α (TNF-α), and tumor necrosis factor receptor superfamily member 1A (TNFRSF1A). In adipose tissue, we also studied the expression of adiponectin (ADIPOQ).

Statistical analyses and calculations

The characteristics of the two groups at screening, the baseline gene expression and the changes in gene expression in response to the test meals, were compared between groups using two-tailed Student's *t*-test, or Mann-Whitney Rank Sum Test in the case of non-normal distribution or unequal variances. The baseline and post-meal gene expression within groups was compared using two-tailed paired *t*-test or Wilcoxon Signed-Rank Test. Concentrations of circulating substances were compared using repeated measurements analysis of variance (ANOVA) adjusting for baseline values, age, gender, BMI, and total body fat percentage. Baseline gene expression in REL is reported as relative to baseline gene expression in CON (defined as 100%). Changes in gene expression in response to the test meals are reported as percent change from the baseline expression. The problem of multiple statistical testing was addressed using false discovery rate (FDR) correction [17].

When data were normally distributed, results were reported as mean and 95% CI. When data were logarithm transformed, results were reported as median and 95% CI. When a non-parametrical test was used, results were reported as median and interquartile range. For visual purposes, all curves are depicted using mean ± SEM. A p-value < 0.05 was considered statistically significant. All statistical calculations were performed using the Data Analysis and Statistical Software STATA, statistical software package version 10.1 (StataCorp LP, College Station, TX, USA) and Scientific Data Analysis and Graphing Software SigmaPlot, version 11.0 (Systat Software Inc.(SSI), San Jose, CA, USA).

Results

All 34 participants ingested both test meals and had all blood samples performed. Three subjects refused the second adipose tissue biopsy, and three subjects refused the second muscle biopsy. For those subjects, tissue obtained from the first completed biopsies was withdrawn from the analyses. 13 REL and 10 CON had fasting plasma glucose of 5.6-6.9 mmol/l (impaired fasting glucose according to the criteria of the American Diabetes Association, ADA), and 5 REL and 2 CON had fasting plasma glucose of 6.0-6.9 mmo/l (impaired fasting glucose according to the criteria of the World Health Organization, WHO). Applying HbA1c criteria, 8 REL and 5 CON had prediabetes according to ADA, i.e. HbA1c of 5.7-6.4%, while none of the participants had prediabetes according to WHO, i.e. HbA1c of 6.0-6.4%. The screening characteristics and the baseline concentrations of circulating hs-CRP, IL-6, adiponectin, and leptin were not statistically different between REL and CON.

Hs-CRP and IL-6 responses

Mean plasma hs-CRP responses to the meal rich in medium-chain SFA (adjusted for baseline concentrations, age, gender, BMI, and total body fat percentage) did not differ between REL and CON ($p = 0.810$) (Figure 1A), nor did mean hs-CRP responses to the MUFA-rich meal ($p = 0.416$) (Figure 1B). We detected no change in hs-CRP in response to either meal.

Plasma IL-6 increased in response to the meal high in medium-chain SFA in REL ($p < 0.001$) and CON ($p < 0.001$). It also increased in response to MUFA in REL ($p < 0.001$) and CON ($p < 0.001$). However, adjusted mean IL-6 responses to the meal rich in medium-chain SFA did not differ between REL and CON ($p = 0.315$) (Figure 1C), nor did mean IL-6 responses to the MUFA-rich meal ($p = 0.298$) (Figure 1D).

Adiponectin and leptin responses

Mean plasma adiponectin responses to the meal rich in medium-chain SFA (adjusted for baseline concentrations, age, gender, BMI, and total body fat percentage) were largely identical for REL and CON ($p = 0.137$) (Figure 2A), nor were the mean plasma adiponectin responses to the MUFA-rich meal ($p = 0.557$) (Figure 2B). The meals caused no change in adiponectin levels in either group.

Plasma leptin decreased in response to the meal rich in medium-chain SFA in REL ($p < 0.001$)

and CON ($p = 0.004$) and to MUFA in REL ($p = 0.001$), but not CON ($p = 0.207$). Yet again, mean adjusted leptin responses to the meal high in medium-chain SFA were not different between REL and CON ($p = 0.137$) (Figure 2C), nor were the mean leptin responses to the MUFA-rich meal ($p = 0.557$) (Figure 2D).

Gene expression

Basal expression of IL6, RBP4, and LEP was higher in muscle of REL than CON (Figure 3A and 3C). In muscle and adipose tissue, basal expression of CCL2 was higher in REL than in CON, while basal expression of TLR4 and TNFRSF1A was lower in REL than in CON (Figure 3A and 3B).

In skeletal muscle, several genes were significantly upregulated in response to medium-chain SFA (Figure 4A and 4C) in CON, namely CCL2, FCGR3A, IL6R, LEP, TNF, and TLR4. In response to the MUFA-rich meal (Figure 4B and 4D), IL1B, IL6R, and TNF were upregulated in muscle of CON, and LEP was upregulated in muscle of REL.

In adipose tissue, several genes were significantly upregulated in response to the meal high in medium-chain SFA (Figure 5A-B) in CON, namely FCGR3, IL1B, IL6, IL6R, and TNF. The MUFA-rich meal (Figure 5C-D) caused upregulation of CCL2, IL1B, IL6, IL6R, TNF, and TNFRSF1A in adipose tissue of REL, while ADIPOQ and TNFRSF1A were upregulated in adipose tissue of CON.

Discussion

In the present study, we evaluated the acute effects of a meal rich in medium-chain SFA or MUFA on plasma adiponectin, leptin, hs-CRP, and IL-6 responses, and on expression of selected genes in muscle and adipose tissues of REL compared with CON. Medium-chain fatty acids are taken up directly to the portal system, while long-chain fatty acids are transported through the lymphatic system. Previously, it has been demonstrated that medium-chain SFA induce lower plasma triglyceride responses than long-chain MUFA [18]. Whether there is a difference between medium-chain and long-chain fatty acids in relation to their influence on inflammatory markers has not yet been investigated.

Hs-CRP, an inflammatory acute-phase reactant, is associated with T2D [19] and CVD [4, 20]. We did not find any effects on plasma hs-CRP from a meal rich in medium-chain SFA or MUFA, nor were any differences in hs-CRP responses between REL and CON. This result agrees with the majority of studies on hs-CRP responses to meals high in mixed fat in healthy individuals [14, 21, 22] and subjects with T2D [22]. While MUFA is found to decrease the hs-CRP response in healthy persons [9], SFA is shown to have an increasing effect on hs-CRP in healthy and type 2 diabetic subjects [8]. However, in these studies, predominantly long-chain SFA is used, and fat comprised 96% of the energy in test meals given to the healthy subjects [8]. No postprandial effects on hs-CRP responses in REL were reported. Fasting hs-CRP levels were found to be higher in REL than CON in the majority [23, 24], but not all studies [25]. This may be due to different levels of adjustment for covariance, as a study including 3,187 REL showed a correlation between hs-CRP and age, gender, ethnicity, smoking status, systolic blood pressure, total cholesterol concentration, and BMI [26]. Also, differences in insulin sensitivity of the study populations as well as in quality and quantity of the test meals may influence the outcome.

IL-6, a cytokine with both pro- and anti-inflammatory properties, plays a controversial role in metabolism [27]. Increased IL-6 levels were found in subjects with T2D [28]. Yet, others found comparable IL-6 levels in subjects with T2D, REL, and CON [29], and there was no correlation between basal and insulin-stimulated IL-6 concentrations and insulin resistance [27]. IL-6 infusion was found to increase or not to affect glucose disposal, and to increase lipolysis and fat oxidation [27]. Thus, it is not clear whether IL-6 plays a beneficial or detrimental role in the pathogenesis of T2D. In our study, IL-6 increased in response to both test meals, and did not differ between REL and CON. These findings are in agreement with the majority of postprandial studies, where meals rich in mixed-fat or SFA were found to increase plasma IL-6 [14, 21]. While Nappo *et al*. found postprandial IL-6 levels to increase more in subjects with T2D than in non-diabetic individuals [15], our findings corroborate two other studies where neither a mixed meal nor insulin stimulation elicited a differential response in plasma IL-6 in REL and CON [23, 30]. Although circulating IL-6 levels did not differ between REL and CON [23, 30], elevated baseline IL-6 expression was found in adipose tissue (which secretes up to 35% of IL-6 in resting state [27]) of REL [23] and of insulin resistant subjects [31]. However, we also found higher baseline IL-6 expression in muscle (a tissue pro-

ducing very little IL-6 at rest) of REL. Previously, SFA (but not MUFA) was found to upregulate IL-6 in blood mononuclear cells [32]. In our study, IL-6 was upregulated in adipose tissue of CON in response to medium-chain SFA, and in adipose tissue of REL in response to MUFA, indicating that both fat qualities can upregulate IL-6 in both REL and CON.

Adiponectin is produced exclusively by adipose tissue [6]. It suppresses inflammation, increases peripheral insulin sensitivity, decreases gluconeogenesis, and stimulates adipose tissue lipid storage. In REL, low fasting plasma adiponectin was found in the majority of studies [33, 34], but not in all studies [23]. Baseline expression of ADIPOQ (the gene encoding adiponectin) was unchanged between female REL and CON [35], but it was lower in male REL than CON [23]. In our study on predominantly female REL, we found no differences in baseline ADIPOQ expression or in circulating adiponectin. Moreover, we found no effect of either test meal on plasma adiponectin. This agrees with some previous studies on the impact of fat on plasma adiponectin in insulin-sensitive and insulin-resistant individuals [36]. Whereas, in the majority of studies, plasma adiponectin decreased in response to a high-fat meal [10, 11]. The latter studies are characterized by a higher number of subjects, and the decrease was no more detectable 5 h postprandially in one study [10]. Thus, our study may be underpowered and of a too short duration to detect a decrease in plasma adiponectin. Despite unchanged circulating adiponectin levels, we found upregulated ADIPOQ in response to the MUFA-rich meal in CON, but not in REL. This finding confirms a previous study where lower insulin-stimulated ADIPOQ expression was found in REL than in CON [23]. Thus, REL seem to have a less beneficial adiponectin response to a MUFA-rich fat challenge than CON.

Leptin is primarily secreted by adipose tissue, and plays a central role in metabolism and energy expenditure [6]. Leptin levels are elevated in obesity, yet, the elevation is accompanied by leptin resistance [6]. Unchanged [35] or increased fasting leptin levels were found in REL [23], and increased postprandial leptin responses were seen in subjects with T2D [37]. In the present study, plasma leptin decreased in response to the meal rich in medium-chain SFA in both groups, and to MUFA in REL. However, mean leptin responses did not differ between REL and CON or between the fat types. Several previous studies have shown a decrease in leptin in response to mixed and high-fat meals in healthy individuals [12], but not in subjects with T2D [37]. Our results contrast to a longer-term study where lower plasma leptin responses to a test meal were observed in 11 REL after 3 weeks on a MUFA-rich diet versus a SFA-rich diet [38]. While others found decreased baseline and insulin-stimulated LEP expression in the main site of leptin synthesis (adipose tissue) in male REL [23], we only found differences in muscle. Baseline muscle LEP expression was higher in REL than in CON, while medium-chain SFA upregulated LEP in muscle of CON, but not REL. Skeletal muscle tissue plays a minor role in leptin production, and these differences were not reflected in the circulating leptin concentrations, as mentioned above.

TNF is a potent proinflammatory cytokine that stimulates CRP and IL-6 amongst others [6]. It is implicated in insulin resistance by inhibiting downstream signaling from the insulin receptor [39]. We found similar baseline expressions of TNF in REL and CON which agrees with previous studies [23, 34]. A fat-rich meal did not affect the levels of circulating TNF in healthy subjects [14], nor did it increase them in subjects with T2D [15]. In contrast, increased monocyte TNF expression was found in response to mixed fat [40] and to a SFA (but not MUFA) in healthy individuals [32]. We found upregulated TNF in adipose tissue of CON in response to medium-chain SFA and in muscle of CON and adipose tissue of REL in response to MUFA. In conclusion, both fat types seem to upregulate TNF without any distinct differences between REL and CON.

The results of the present study indicate that a fat-rich meal upregulates expression of the proinflammatory cytokine IL-1B and the tissue macrophage infiltration marker CCL2 [41] with no overall differences between REL and CON or the fat qualities. In agreement with a previous study [23], we found that REL have a more detrimental baseline CCL2 expression than CON, while medium-chain SFA (but not MUFA) induced upregulation of another marker of macrophage infiltration, FCGR3A, which was surprisingly seen only in CON.

The present study has limitations. The two groups differed in median age by 9 years. Although not statistically significant, this difference may have introduced a bias. Since REL were older than CON, we would expect the age difference to enlarge differences in insulin resistance. In fact, there was no significant difference between the two groups. The findings in the present study do not exclude the possibility that postprandial circulating inflammatory responses may differ between

REL and CON at a greater age or after a long-term intervention with a MUFA-rich diet versus a diet high in medium-chain SFA. The secondary endpoint in the present study was gene expression, and it should be mentioned that gene expression changes cannot be directly translated into physiologically relevant changes, i.e. changes in protein content and activity. Finally, the study was underpowered for gene expression analyses. Thus, possible gene expression differences between REL and CON may not have been found.

In conclusion, IL-6 increased similarly in REL and CON in response to both a meal high in medium-chain SFA and MUFA. Leptin decreased slightly to medium-chain SFA in both groups and to MUFA in REL, whereas adiponectin and hs-CRP remained unchanged in both REL and CON. Both MUFA and medium-chain SFA elicited changes in the expression of inflammatory and adipokine genes in muscle and adipose tissue, without large differences between REL and CON. Interestingly, medium-chain SFA induced slightly more proinflammatory gene expression changes than MUFA.

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