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The prebiotic inulin improves substrate metabolism and promotes short-chain fatty acid production in overweight to obese men

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ABSTRACT

Background and Aims: Human gut microbiota play an important role in maintaining human health. Dietary fibers, i.e. prebiotics, are fermented by human gut microbiota into the short-chain fatty acids (SCFAs) acetate, propionate, and butyrate. SCFAs promote fat oxidation and improve metabolic health. Therefore, the prebiotic inulin might be an effective dietary strategy to improve human metabolism. We aimed to investigate the acute metabolic effects of ingesting inulin compared with digestible carbohydrates and to trace inulin-derived SCFAs using stable isotope tracer methodology.

Methods: In a double-blind, randomized, placebo-controlled crossover design, 14 healthy, overweight to obese men consumed a high-fat milkshake containing A) 24 g inulin of which 0.5 g was U-^{13}C-inulin (INU) or B) 24 g maltodextrin placebo (PLA), with a wash-out period of at least five days. Fat oxidation was measured via an open-circuit ventilated hood and blood samples were collected up to 7 h after ingestion. Plasma, breath, and fecal samples were collected, and appetite and satiety scores were assessed.

Results: Fat oxidation increased in the early postprandial phase (0−3 h), and both plasma glucose and insulin were lower after INU ingestion compared with PLA (all $P < 0.05$). Plasma free fatty acids were higher in the early, and lower in the late postprandial period after INU ingestion. Inulin was fermented into SCFAs as indicated by higher plasma acetate concentrations after INU compared with PLA ($P < 0.05$). In addition, we found continuous increases in plasma ^{13}C-SCFA enrichments ($P < 0.05$ from $t = 120$ onwards) and breath $^{13}CO_2$ enrichments after INU intake. There were no effects on plasma triglycerides, free glycerol, satiety hormones GLP-1 and PYY, and appetite and satiety scores.

Conclusions: Ingestion of the prebiotic inulin improves fat oxidation and promotes SCFA production in overweight to obese men. Overall, replacing digestible carbohydrates with the fermentable inulin may favor human substrate metabolism.

Clinical Trial Registry: The trial was registered at clinicaltrials.gov under number NCT02009670.

Abbreviations: AMPK, adenosine monophosphate (AMP)-activated protein kinase; CHO, carbohydrate; DP, degree of polymerization; FFA, free fatty acid; GLP-1, glucagon-like peptide-1; GPR, G-coupled protein receptor; iAUC, incremental area under the curve; INU, inulin treatment (0.5 g ^{13}C-labeled inulin combined with 23.5 g unlabeled inulin); MPE, mole percent excess; PLA, placebo treatment; PYY, peptide YY; SCFA, short-chain fatty acid; VAS, Visual Analogue Scale.

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1. Introduction

Obesity and the metabolic syndrome are rapidly expanding health threats [1]. Body weight, insulin sensitivity, and glucose metabolism in humans are found to be related to the gut microbiota [2–4]. Gut microbial composition and activity can be influenced by ingesting dietary fibers, also called prebiotics. A prebiotic is defined as a non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacterial species in the colon [5]. One important activity of the human gut microbiota is fermentation of dietary fibers into short chain fatty acids (SCFAs), mainly acetate, propionate, and butyrate. Recent evidence suggests that increasing colonic and plasma SCFA concentrations could be used as a strategy to prevent and treat obesity and obesity-induced insulin resistance [3, 6, 7]. In addition, indigestible prebiotics have a lower blood glucose-raising potential, i.e. lower glycemic index, and a lower caloric value than digestible carbohydrates, thereby promoting glucose homeostasis [8]. On the contrary, a recent study in rats showed that continuous intragastric acetate infusions decreased insulin sensitivity [9]. Therefore, human studies investigating metabolic effects concerning prebiotic ingestion and subsequent SCFA formation are warranted.

Inulin is a prebiotic that targets gut microbiota and thereby influences microbial composition and activity. Studies applying in vitro models have indicated that the human gut microbiota can ferment inulin into acetate, propionate, and butyrate in considerable amounts [10–13]. In addition, several human studies have shown that daily inulin supplementation increases fecal bifidobacteria, indicating an alteration in gut microbial composition [14, 15]. A longer-term inulin supplementation caused reductions in body weight, BMI, as well as energy and fat intake in patients with type 2 diabetes mellitus [16]. In addition, replacing digestible carbohydrates with inulin for three to eight weeks in healthy subjects reduced plasma lipid concentrations, mostly triglycerides [15, 17–20] but also LDL-cholesterol and total cholesterol [21], and lowered fasting glucose and insulin concentrations [16, 20, 22].

Only few human studies have investigated underlying mechanisms contributing to the positive effects of inulin on human metabolism [7, 23]. It has been shown that 24 g of oral inulin intake increases serum concentrations of acetate, propionate, and butyrate 4 to 6 h after ingestion, supporting the hypothesis that fermentation of inulin into SCFAs may lead to metabolic improvements [23]. In addition, a recent study from our research group has shown that colonic infusion of ~22 mmol of the SCFA acetate in overweight to obese males caused an acute increase in fasting fat oxidation and peptide YY (PYY), a satiety hormone reducing appetite [7]. The exact mechanism by which inulin influences the host's substrate metabolism and whether inulin-derived SCFAs contribute to the observed metabolic effects in humans remains to be investigated.

This study was designed to compare the acute effects of a mixed high-fat liquid meal containing digestible carbohydrates with a mixed high-fat liquid meal where a considerable amount of the digestible carbohydrates was replaced by the fermentable carbohydrate inulin. We hypothesized that dietary inulin is fermented by the microbiota into SCFAs in the late (3–7 h) postprandial period [23]. The primary aim of the current study was to investigate the early and late postprandial effects of oral inulin intake on fat oxidation and energy expenditure in overweight and obese subjects. Secondary outcomes include the effects of inulin on carbohydrate oxidation, circulating metabolites (glucose, triglycerides, free fatty acids (FFAs), free glycerol), plasma hormones (insulin, Glucagon-Like Peptide-1 (GLP-1), PYY), plasma and fecal SCFA concentrations and enrichments, as well as appetite. The application of a unique stable isotope technique allowed us to assess whether inulin is fermented into SCFAs and to explore the relation between SCFAs and metabolic parameters in humans.

2. Materials and Methods

2.1. Study Population

Fifteen healthy, normoglycemic, Caucasian men aged 20–50 y with a body mass index between 25 and 35 kg/m^2 were recruited between January 2014 and July 2014 from the vicinity of Maastricht, The Netherlands. Subjects were excluded for any of the following reasons: fasting plasma glucose >7.0 mmol/L, gastroenterological diseases or major abdominal surgery in the past, cardiovascular diseases, cancer, liver or kidney malfunction, life expectancy of <5 y, alcohol or drug abuse, excessive smoking (>20 cigarettes per day), being on a weight loss intervention, use of prebiotics, probiotics, or antibiotics for 3 months prior to the study or during the study, vigorous exercise training, use of any medication that influences glucose or fat metabolism and inflammation, and regular use of laxatives. All procedures were approved by the Medical Ethics Committee of Maastricht University Medical Center+ (MUMC+) and conducted in accordance with the Declaration of Helsinki (revised version, October 2008, Seoul, South Korea). Written informed consent was obtained from all subjects. This clinical study was monitored by clinical research associates of the Clinical Trial Center Maastricht (CTCM, Maastricht, The Netherlands).

2.2. Study Design

The study was a double-blind, randomized, placebo-controlled crossover trial. An independent researcher performed randomization and gave the randomization key to the principle investigator after completion of the analyses. Subjects were investigated during two investigation days with at least 5 days of washout period in between. Previous trials have shown that this period is long enough to exclude potential carry-over effects [7]. At the start of each investigation day, the subjects consumed a standardized high-fat milkshake containing: a) 24 g inulin (0.21 MJ; Sensus, Roosendaal, The Netherlands, average degree of polymerization (DP) 8) of which 0.5 g was U-^{13}C-labeled inulin (DP 3–60, IsoLife, Wageningen, The Netherlands), referred to as INU, or b) 24 g maltodextrin (0.42 MJ; Avebe, Veendam, The Netherlands) as placebo (PLA). The milkshake provided 2.3 MJ energy and consisted of 46 En% fat (28 g), 42 En% carbohydrates (58 g), and 12 En% protein (17 g).

Fig. 1 shows the design of the investigation day. On the evening before the investigation day, subjects were provided with a standardized meal (Macaroni Bolognese, Euroshopper, Albert Heijn, Zaandam, The Netherlands) composed of 65 En% carbohydrates, 20 En% fat, 15 En% protein. Furthermore, subjects were asked to refrain from ingesting naturally ^{13}C-enriched food products such as corn, sugar cane, or pineapple. Every investigation day started with baseline measurements of indirect calorimetry, blood and breath sampling, and Visual Analogue Scale (VAS) assessment for appetite and satiety. After baseline measurements, the subjects ingested a high-fat milkshake containing INU or PLA. Thereafter, on various time points for 7 h, blood and breath samples were collected, indirect calorimetry was performed, and VAS for appetite and satiety were obtained. In addition, the subjects were asked to sample stool the first day after each investigation day.

2.3. Calculations

The primary outcome parameter, fat oxidation, was measured using an open-circuit ventilated hood system (Omnical, MUMC+, The Netherlands). CO_2 production (VCO_2 in L/min) and O_2 consumption (VO_2 in L/min) were measured during eight time periods: $t = -30$–0, 15–40, 60–85, 120–145, 180–205, 250–300, 320–360, and 380–420 min (Fig. 1). The equations of Weir [24] and Frayn [25] were used to calculate resting energy expenditure and the total rate of fat and carbohydrate oxidation. The estimated nitrogen (N) excretion was

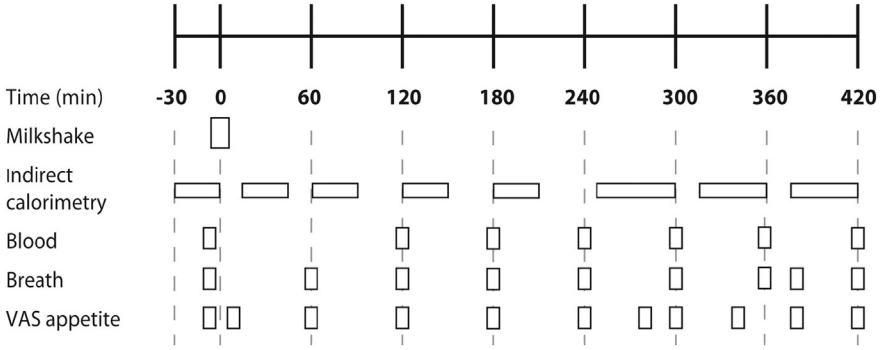

Fig. 1. Protocol of the investigation day. Abbreviation: VAS: Visual Analogue Scale.

calculated based on the assumption that protein oxidation represents 15% of total energy expenditure.

2.4. Analyses

Blood samples were collected from an antecubital vein at 13 different time points on each investigation day (Fig. 1). Blood was collected into pre-chilled 0.2 M EDTA tubes (Sigma Dorset, UK) for analysis of FFAs, triglycerides, free glycerol, glucose, insulin, and SCFAs. For GLP-1 analysis, blood was collected in a 2 mL EDTA tube in which 20 μL of dipeptidyl peptidase (DPP)-4 inhibitor was added. For PYY analysis, blood was collected in 2 mL aprotinin-EDTA tubes in which 20 μL of DPP-4 inhibitor was added. The samples were centrifuged at 1300*g* at 4 °C for 10 min. Plasma was aliquoted, directly snap-frozen in liquid nitrogen, and stored at −80 °C until analysis.

Plasma FFAs, triglycerides, and glucose were measured with enzymatic assays on an automated spectrophotometer (ABX Pentra 400 autoanalyzer, Horiba ABX, Montpellier, France). Plasma free glycerol was measured after precipitation, using an enzymatic assay (Enzytec™ Glycerol, Roche Biopharm, Switzerland) on a Cobas Fara spectrophotometric auto-analyzer (Roche Diagnostics, Basel, Switzerland). The concentrations of insulin and PYY were determined with commercially available radioimmunoassay (RIA) kits (Human Insulin specific RIA and Human PYY (3-36) RIA, Millipore Corporation, MA, USA). Plasma samples were assayed for total GLP-1 immunoreactivity using an antiserum, which reacts equally with intact GLP-1 and the primary (N-terminally truncated) metabolite as previously described [26]. Deproteinization and subsequent preparation of plasma samples for analysis of SCFA concentrations was performed as reported before [27]. Briefly, plasma samples were deproteinized using acidified methanol and centrifuged at 50,000*g* for 10 min at 4 °C. The clear supernatant was transferred to a 300 μL glass micro-insert, spring loaded in a 4 mL vial, equipped with a self-sealing septum. Analysis was performed using liquid chromatography-mass spectrometry. SCFA concentrations of acetate, butyrate, and propionate were determined using calibration curves of standards with known SCFA concentrations. The detection limits for acetate, propionate, and butyrate of this method were 0.1, 0.05, and 0.05 μmol/L, respectively. Plasma ^{13}C-labeled SCFA were analyzed as described before [28]. Plasma samples were deproteinized and extracted according to Morrison et al. [29] The extracted samples for analysis of ^{13}C enrichment of SCFAs were injected (4 μL) in splitless mode at 240 °C into a Delta XP isotope ratio mass spectrometer (Thermo Fisher Scientific, Bremen, Germany) equipped with a Trace gas chromatograph and a combustion interface type 3 (GC-C-IRMS). An AT Aquawax-DA column (30 m × 0.53 mm i.d. and 1.00 μm film thickness; Grace, Lokeren, Belgium) was used. The initial oven temperature was 80 °C (3 min) and ramped to 140 °C at a rate of 4 °C/min followed by an increase to 240 °C (10 min) at a rate of 16 °C/min. Helium was used as carrier gas in a constant flow mode of 2.5 mL/min. After elution from the GC column, the compounds were oxidized into CO_2 in the combustion reactor after which the enrichment was measured in the IRMS detector. To calculate the enrichment of the SCFAs from the measured $^{13}CO_2$ enrichment, the number of labeled carbon atoms per molecule and the enrichment of the administered substrate (^{13}C-SCFAs) were taken into account. Data were processed using Isodat NT (version 2.0, Finnigan™, Thermo Fisher Scientific).

Breath samples were collected via a mixing chamber in 12 mL exetainers at 10 different time points (Fig. 1). These were analyzed for $^{13}CO_2$ using gas chromatography isotope ratio mass spectrometry (GC-IRMS). Scores for appetite and satiety were measured using VAS from 0 to 100 mm. VAS scores were collected at 11 different time points (Fig. 1). Volunteers were asked to indicate which position on a line best reflected their feeling concerning hunger, full feeling, satiety, and desire to eat.

The day after every investigation day, the subjects collected a fecal sample from their first stool. The samples were directly stored on dry ice at home. Upon arrival in the laboratory, fecal samples were weighed and frozen at −80 °C. Fecal acetate, propionate, and butyrate were measured by gas chromatography–mass spectrometry (GC–MS, Medical laboratory 'Dr. Stein & Collegae', Mönchengladbach, Germany), according to the method described by Garciá-Villalba et al. [30] Suspensions of fecal samples (100 mg in 1 mL 4 M NaCl) for ^{13}C-labeled SCFAs were acidified with H_2SO_4 (150 μL) and extracted into diethyl ether (3 mL). The ether layer was dried over Na_2SO_4 and injected into the GC-C-IRMS. Prepared fecal samples were analyzed as described above in the section on plasma ^{13}C-labeled SCFAs.

2.5. Statistical Analyses

Values are expressed as mean ± SEM. For energy expenditure, substrate oxidation, plasma metabolites, and VAS scores, differences between INU and PLA were tested using two-way repeated measures ANOVA for time and treatment. In case of a significant time-by-treatment interaction, a post hoc test with Bonferroni correction was performed to compare treatment effects at specific time points. The iAUCs were calculated using the trapezoidal rule. Differences in incremental area under the curve (iAUC) over the early (0–3 h) and late (3–7 h) postprandial period after treatment were compared using a paired *t*-test. Relative changes in energy expenditure and substrate oxidation compared to baseline are reported as iAUC. For $^{13}CO_2$ and ^{13}C-SCFA enrichments, one-way ANOVA for time with post hoc test with Bonferroni correction and pairwise comparisons at specific time points vs. baseline are reported. A Pearson's correlation coefficient was computed to assess the relationship between the primary outcome, fat oxidation, and SCFAs. For calculation of correlations, we included all time points at which we measured both fat oxidation and plasma SCFAs. Moreover, we calculated correlations between BMI and metabolic outcome parameters (fat oxidation, carbohydrate oxidation energy expenditure, plasma lipids, glucose, insulin, satiety hormones, and plasma SCFAs). Statistical analysis was performed using SPSS version 22 and a two-sided $P < 0.05$ was considered statistically significant. The sample-size calculation, with a 2-sided significance level of 0.05 and

Table 1
Baseline values of male participants ($n = 14$).

Characteristics ($n = 14$)	
Age (y)	34 ± 3
BMI (kg/m^2)	30.4 ± 0.7
Waist-to-hip ratio	1.0 ± 0.01
Fasted glucose (mmol/L)	5.2 ± 0.02
Hemoglobin (mmol/L)	9.5 ± 0.02
ALT (U/L)	36.1 ± 4.9
HbA_1c (%)	5.2 ± 0.2
Creatinine (mmol/L)	93.4 ± 3.4

Values are means ± SEMs.
Abbreviations: ALT: alanine aminotransferase; HbA_1c: glycated hemoglobin.

power of 0.8, was based on a study by Higgins et al. [31] investigating postprandial fat oxidation after intake of a meal rich in resistant starch compared with placebo. The sample size was estimated at $n = 15$ including an expected dropout rate of 10%.

3. Results

3.1. Subject Characteristics

Fifteen healthy subjects were included in the study. One subject discontinued intervention due to having a migraine attack during one of the investigation days and therefore being unable to complete the test period (Supplemental Fig. 1). None of the subjects reported any side effects. Table 1 shows the baseline characteristics of the subjects.

3.2. Fat Oxidation, Carbohydrate Oxidation and Energy Expenditure

Baseline fasting fat oxidation was 0.056 ± 0.001 g/min and 0.070 ± 0.002 g/min for INU and PLA resp. ($P = 0.025$). Fat oxidation increased over time to a greater extent after INU treatment when compared with PLA ($P = 0.003$). Early postprandial fat oxidation was higher after INU intake when compared with PLA (iAUC 0–3 h: 0.85 ± 0.12 vs. −0.88 ± 0.12 g/min·3 h, $P = 0.011$, Fig. 2A and B). Fat oxidation was not

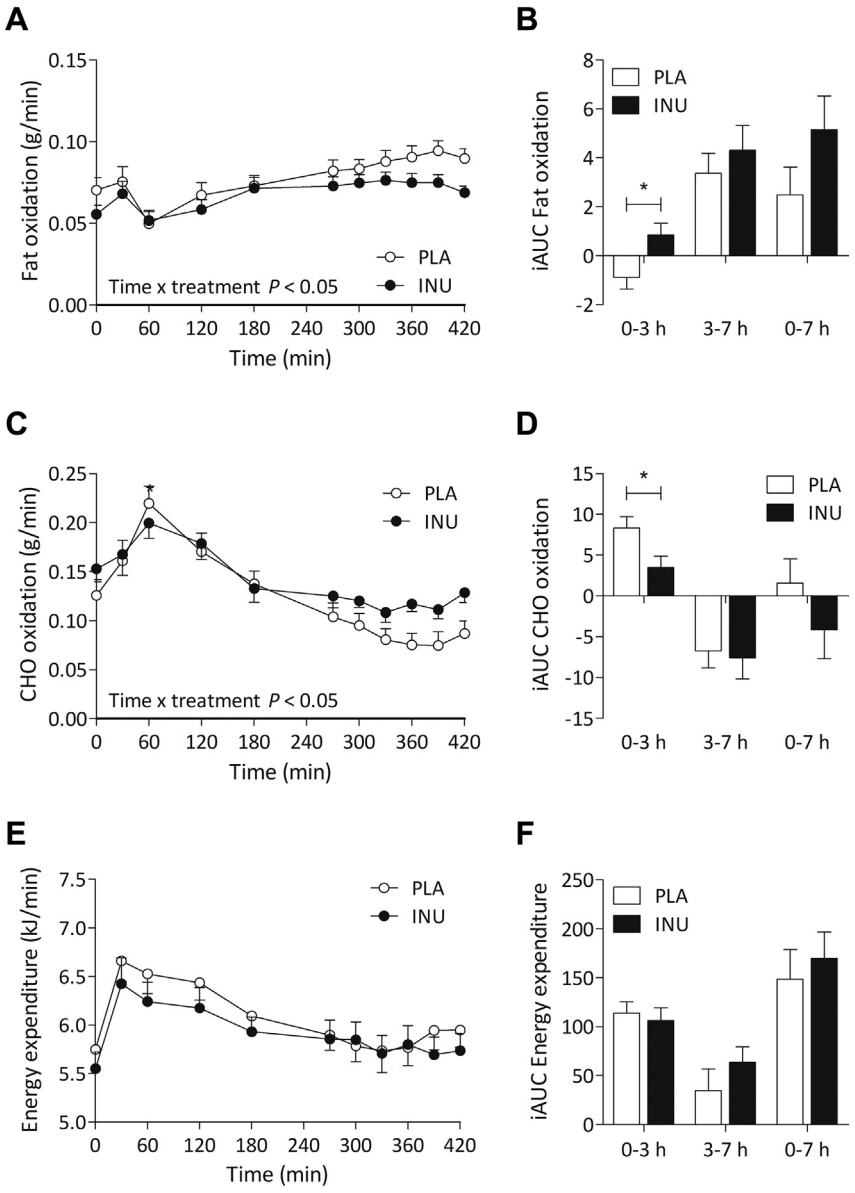

Fig. 2. Fat oxidation, carbohydrate oxidation, and energy expenditure. Relative change in mean (±SEM) fat oxidation (A and B), carbohydrate oxidation (C and D), and energy expenditure (E and F), and corresponding incremental area under the curve (iAUC) after consumption of inulin or placebo ($n = 14$) in the early and late postprandial phase. Data were analyzed with a 2-factor repeated-measures ANOVA. Data for iAUC were analyzed using a paired *t*-test. *Different from placebo, $P < 0.05$. Only significant changes ($P < 0.05$) or trends ($P < 0.1$) on time x treatment are reported. CHO: carbohydrate oxidation. INU: inulin. PLA: placebo.

significantly different between INU and PLA intake in the late postprandial phase.

Immediately after milkshake ingestion, carbohydrate oxidation increased reaching peak concentrations at $t = 60$ min in both conditions. INU intake resulted in lower carbohydrate oxidation when compared with PLA at $t = 60$ min ($P = 0.030$). Early postprandial carbohydrate oxidation was lower in the INU group when compared with PLA (iAUC 0–3 h: 3.47 ± 0.35 vs. 8.28 ± 0.35 g/min·3 h, $P = 0.009$, Fig. 2C and D). Carbohydrate oxidation was not significantly different between INU and PLA intake in the late postprandial phase.

Energy expenditure increased immediately after intake of both the INU and PLA treatment, and tended to be higher after the INU intake when compared with PLA over the entire 7 h test period ($P = 0.059$), but did not significantly differ in the early or late postprandial phase (Fig. 2E and F).

3.3. Plasma Lipids

Baseline FFA plasma concentrations before ingestion of the milkshake differed significantly between the two interventions ($P = 0.030$ at $t = 0$). Immediately after intake of the milkshake, plasma FFA concentrations decreased in both treatments, with lowest concentrations reached at 120 min, followed by a gradual increase. The INU treatment significantly reduced the early postprandial decrease in plasma FFAs (Fig. 3A, $P < 0.001$), resulting in significantly higher plasma FFAs in the early postprandial phase when compared with PLA (iAUC 0–3 h: −23,027 ± 726 vs. −39,615 ± 1356 μmol/L·3 h, $P = 0.009$, Fig. 3B). FFAs were significantly lower at $t = 360$ and $t = 420$ min after INU when compared with PLA (both $P < 0.05$). There were no significant differences in plasma free glycerol and triglycerides between INU and PLA treatment (Fig. 3C–F).

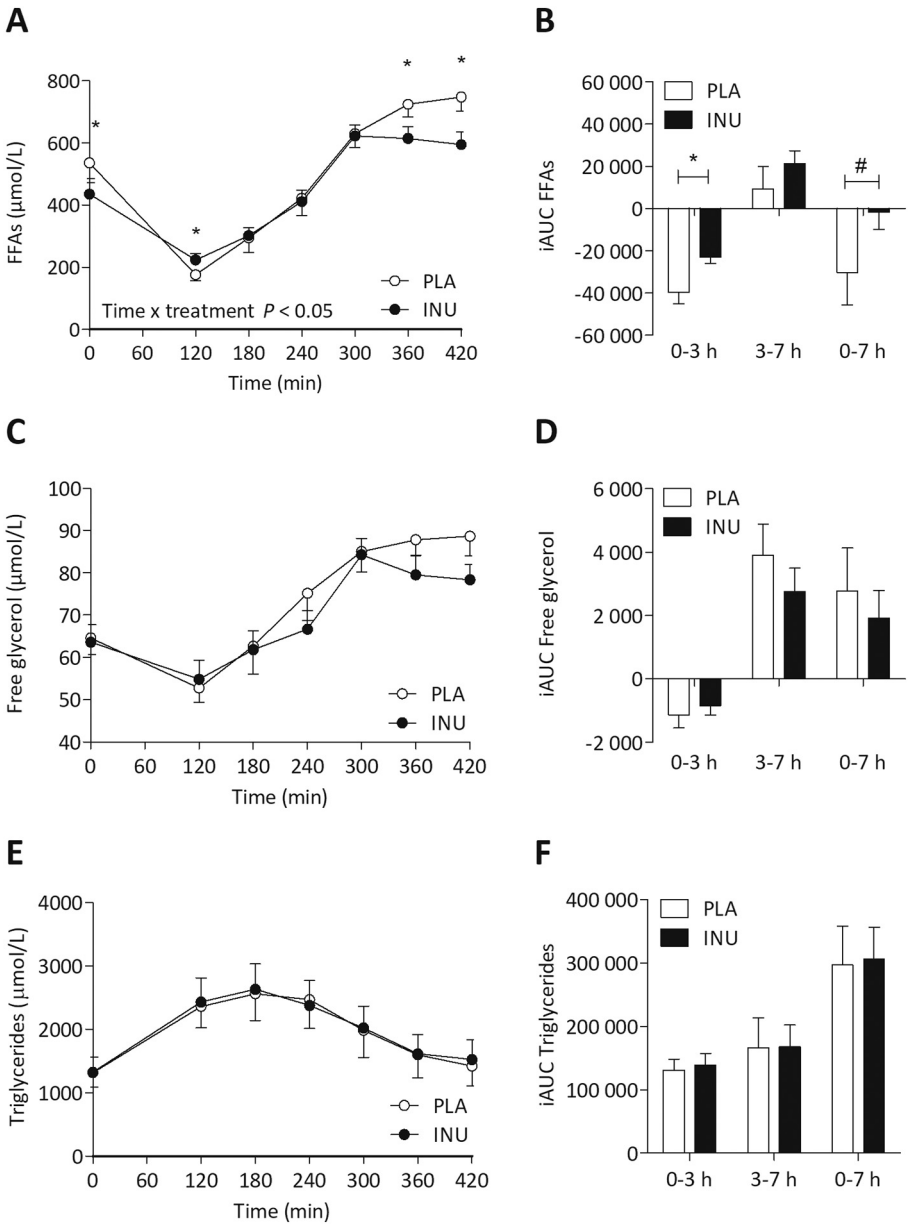

Fig. 3. Plasma lipids. Mean (±SEM) FFAs (A and B), free glycerol (C and D), and triglycerides (E and F), and corresponding incremental area under the curve (iAUC) after consumption of inulin or placebo ($n = 14$) in the early and late postprandial phase. Data were analyzed with a 2-factor repeated-measures ANOVA. Data for iAUC were analyzed using a paired t-test. *Different from placebo, $P < 0.05$. #Different from placebo, $P < 0.1$. Only significant changes ($P < 0.05$) or trends ($P < 0.1$) on time x treatment are reported. FFA: Free fatty acid. INU: Inulin. PLA: Placebo.

3.4. Plasma Glucose, Insulin and Satiety Hormones

The high-fat milkshake increased plasma glucose and insulin concentrations directly after intake, independent of the treatment. The increase in plasma glucose was, however, lower after the INU treatment when compared with PLA (5.4 ± 0.03 vs 5.8 ± 0.04 mmol/L at time point $t = 120$, $P = 0.011$, Fig. 4A). INU resulted in a lower glucose response in the early postprandial phase when compared with PLA

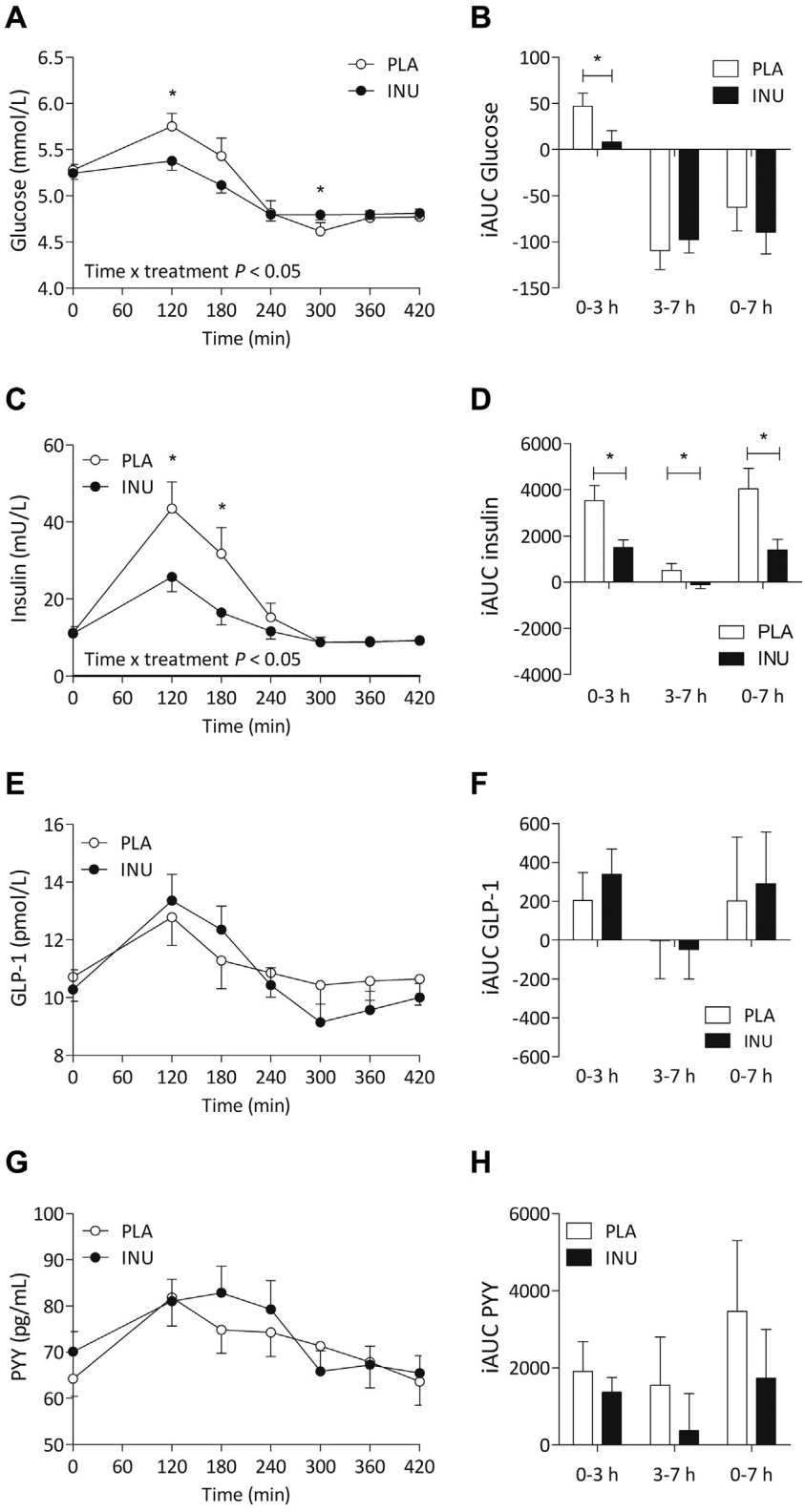

Fig. 4. Plasma glucose, insulin, and satiety hormones. Mean (±SEM) glucose (A and B), insulin (C and D), GLP-1 (E and F), and PYY (G and H), and corresponding incremental area under the curve (iAUC) after consumption of inulin or placebo ($n = 14$) in the early and late postprandial phase. Data were analyzed with a 2-factor repeated-measures ANOVA. Data for iAUC were analyzed using a paired *t*-test. *Different from placebo, $P < 0.05$. Only significant changes ($P < 0.05$) or trends ($P < 0.1$) on time x treatment are reported. GLP-1: glucose-like peptide-1. INU: inulin. PLA: placebo. PYY: peptide YY.

(iAUC 0–3 h: 8.12 ± 3.07 vs. 46.97 ± 3.57 mmol/L·3 h, $P = 0.002$, Fig. 4B). In the late postprandial phase, no difference in plasma glucose concentrations was observed between INU and PLA treatment. In line with the findings on plasma glucose, INU resulted in a lower plasma insulin concentration when compared with PLA ($P < 0.001$, Fig. 4C). In addition, INU intake resulted in a lower insulin response in both the early (iAUC 0–3 h: 1494 ± 81 vs. 3523 ± 161 mU/L·3 h, $P = 0.001$), and late postprandial phase (iAUC 3–7 h: −108 ± 43 vs. 506 ± 75 mU/L·4 h, $P = 0.002$, Fig. 4D) when compared with PLA.

There were no significant differences in plasma concentrations of satiety hormones GLP-1 and PYY between INU and PLA treatment, neither in the early nor late postprandial phase (Fig. 4E–H).

3.5. Plasma SCFA Concentrations and Enrichments

INU intake resulted in higher plasma acetate concentrations when compared with PLA ($P = 0.001$, Fig. 5A). Plasma acetate was higher in the late postprandial phase (iAUC 3–7 h: 1330 ± 358 vs. −7987 ± 559 μmol/L·4 h, $P = 0.002$, Fig. 5B), resulting in higher total plasma acetate over the 7 h test period (iAUC 0–7 h: 1310 ± 522 vs. −10,169 ± 834 μmol/L·7 h, $P = 0.007$). There was no significant difference in plasma propionate (Fig. 5C and D) or plasma butyrate (Fig. 5E and F) when compared with PLA. INU treatment, however, resulted in a trend towards higher plasma butyrate concentrations when compared with PLA over the entire test period (Fig. 5E and F, $P = 0.056$). Fat oxidation significantly correlated with plasma propionate ($r = 0.250$, $P = 0.008$) and plasma butyrate ($r = 0.231$, $P = 0.014$), but not with plasma acetate. The correlation coefficient for propionate and butyrate was, however, too low to be considered relevant. There were no significant correlations between BMI and all metabolic parameters, including plasma SCFA concentrations.

Plasma ^{13}C-SCFAs and exhaled $^{13}CO_2$ increased significantly directly after INU intake (Fig. 6). A pairwise comparison showed a significant effect from $t = 120$ onwards for ^{13}C-SCFAs (Acetate $P < 0.001$, propionate $P = 0.015$, butyrate $P = 0.011$) and from time point $t = 60$ onwards for $^{13}CO_2$ ($P < 0.05$). As shown in Fig. 6, plasma ^{13}C-butyrate enrichment (0.36 MPE) was higher when compared with plasma ^{13}C-acetate (0.17 MPE) or plasma ^{13}C-propionate enrichment (0.14 MPE).

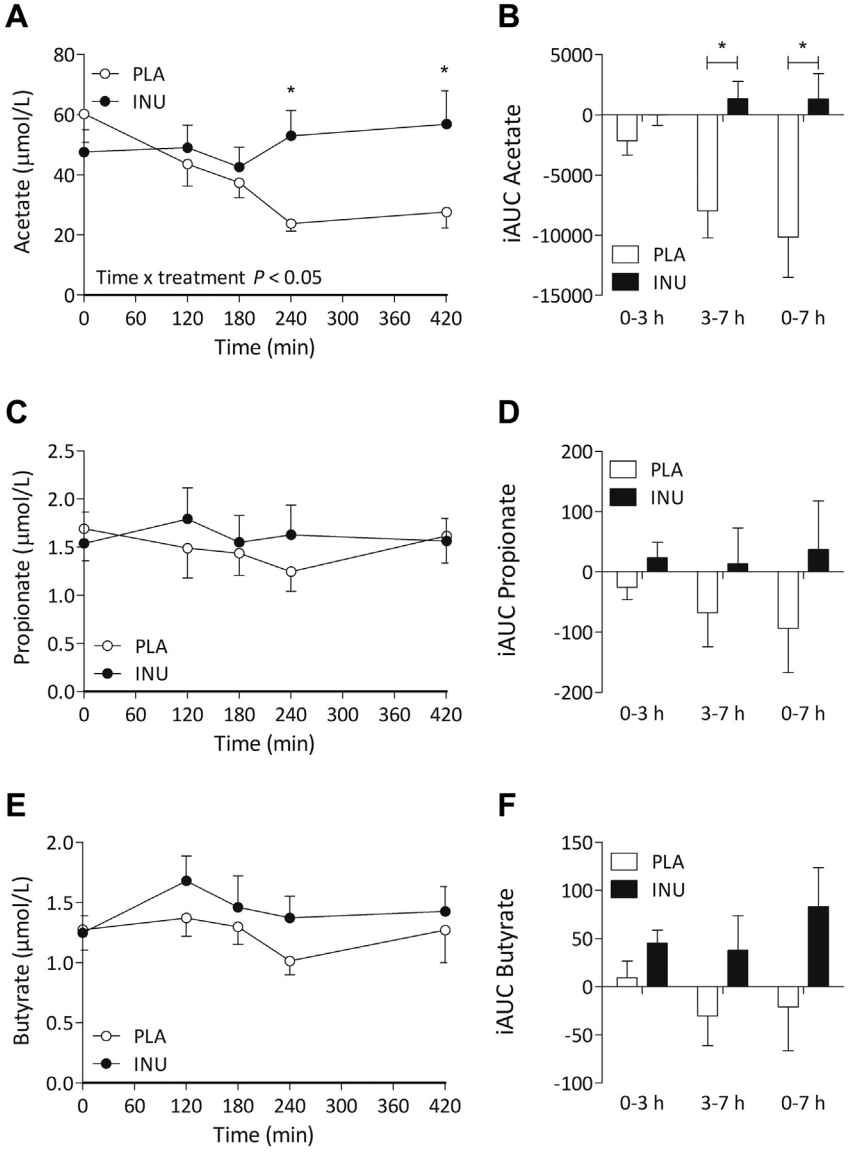

Fig. 5. Plasma SCFAs concentrations. Mean (±SEM) acetate (A and B), propionate (C and D), and butyrate (E and F), and corresponding incremental area under the curve (iAUC) after consumption of inulin or placebo ($n = 14$) in the early and late postprandial phase. Data were analyzed with a 2-factor repeated-measures ANOVA. Data for iAUC were analyzed using a paired t-test. *Different from placebo, $P < 0.05$. Only significant changes ($P < 0.05$) or trends ($P < 0.1$) on time x treatment or treatment are reported. INU: inulin. PLA: placebo.

3.6. Fecal SCFA Concentrations and Enrichments

Fecal SCFA concentrations did not differ between the two groups (Fig. 7A). ^{13}C-SCFA enrichment in the INU group showed an enrichment of 0.31 ± 0.02 MPE for acetate, 0.38 ± 0.02 MPE for propionate and 0.30 ± 0.02 MPE for butyrate (Fig. 7B). One fecal sample from the PLA condition was tested and used as background enrichment.

3.7. VAS Scoring on Appetite and Satiety

No significant differences in VAS scores on appetite and satiety were observed between treatments in the early or late postprandial phase. For both treatments, satiety and fullness increased directly after the intake of the high-fat milkshake containing the INU or PLA, while hunger and desire to eat decreased immediately after intake of the milkshake (Fig. 8A–D).

4. Discussion

The present study investigated the acute metabolic effects of the ingestion of a single 24 g dose of the prebiotic inulin compared with digestible maltodextrin in a high-fat milkshake in overweight to obese subjects. We observed increased fat oxidation and a reduced postprandial decrease in plasma FFAs in the early postprandial phase after inulin ingestion. Also, inulin ingestion resulted in lower early postprandial plasma glucose and insulin concentrations compared with the maltodextrin placebo. In addition, inulin intake resulted in higher plasma acetate concentrations and lower plasma FFA concentrations in the late (6–7 h) postprandial period compared with placebo. Tracer methodology revealed that ^{13}C-labeled inulin is fermented into SCFAs as indicated by $^{13}CO_2$ in exhaled breath as well as appearance of ^{13}C-labeled SCFAs in the systemic circulation from 2 h after inulin intake. To our knowledge, this is the first human study demonstrating that the prebiotic inulin improves substrate metabolism in overweight men.

The improvement in substrate metabolism after inulin ingestion can be related to the lower glycemic and insulinemic responses after the meal with inulin compared with maltodextrin. As a result of the reduced insulinemic response after inulin, lipolysis is inhibited to a lesser extent, resulting in an attenuated postprandial decline in plasma FFAs and a higher fat oxidation. Additionally, it has previously been shown that the intracellular glucose availability may determine the rate of skeletal muscle fat oxidation suggesting that the lower glycemic response after inulin may also have had direct effects on fat oxidation [32]. In addition, digestible carbohydrates have a higher energy content than fermentable fibers. Nonetheless, replacing maltodextrin by inulin did not affect appetite and satiety, as indicated by the VAS scores (Fig. 8). Low-glycemic index nutrients such as inulin can be used to modulate the postprandial glycemic response, and thereby may improve insulin sensitivity and obesity-related symptoms [33].

The second mechanism by which inulin may improve substrate metabolism is by microbial fermentation of inulin into SCFAs. SCFAs have been found to regulate the balance between fatty acid synthesis, oxidation, and breakdown in vitro. SCFAs activate fatty acid oxidation in liver and muscle tissue [34], while inhibiting fatty acid synthesis in the liver and lipolysis in adipose tissue [35–37]. In addition, it has been suggested that SCFAs increase oxidative metabolism in liver and muscle by activating AMPK [34, 35, 38]. Of importance, SCFAs can directly be converted into acetyl-CoA, which enters the citric acid cycle and can be used for oxidation [39]. The use of stable isotope-labeled inulin allowed us to assess the metabolic fate of ingested inulin. We demonstrated that inulin is fermented into all three SCFAs, as indicated by the postprandial appearance of ^{13}C-acetate, ^{13}C-propionate, and ^{13}C-butyrate in plasma. Inulin-derived SCFAs were partly absorbed by the intestine and reached the systemic circulation (Fig. 6). Via the systemic circulation, SCFAs become available to metabolically active organs such as the liver, muscle, and adipose tissue, where they can promote substrate metabolism. In addition, part of the SCFAs was excreted, as indicated by the presence of ^{13}C-SCFAs in the feces (Fig. 7B). The relative abundance of ^{13}C-butyrate in the systemic circulation was greater when compared with ^{13}C-acetate and ^{13}C-propionate. However, plasma concentrations of butyrate did not differ following inulin ingestion compared with placebo, while acetate concentrations were higher after inulin compared with placebo ingestion. This discrepancy between ^{13}C-SCFA enrichments and SFCA concentrations may be explained by an inulin-induced increase in endogenous acetate production that dilutes the inulin-derived (exogenous) ^{13}C-acetate enrichment. Moreover, Boets et al. [40] showed in young healthy individuals - using continuous infusions of stable isotope-labeled SCFAs - that ingestion of 15 g inulin leads to cumulative amounts of 55 ± 30 mmol acetate, 1.1 ± 0.9 mmol propionate, and 1.0 ± 0.9 mmol butyrate appearing in plasma.

It has been postulated that a different microbial composition between lean and obese volunteers accounts for a different fermentation profile of dietary fibers [41]. In the study by Boets et al., a negative correlation was found between BMI and the rate of appearance of endogenous butyrate and propionate. However, once fermentation of inulin started, no correlation between BMI and the cumulative amount of exogenous SCFA production was observed [40]. These data suggest that the capacity to ferment inulin and to produce SCFAs does not differ between healthy and overweight/obese individuals. However, future research should confirm this finding in a larger group of overweight versus non-overweight subjects.

Our research group has recently shown that colonic administration of acetate improves fat oxidation in overweight subjects [7]. In addition, consumption of inulin or colonic SCFA administration reduces plasma FFA concentrations in both healthy and hyperinsulinemic subjects [23, 42, 43]. In line, we show in the present study that ingestion of the prebiotic inulin increases fat oxidation (early phase) and reduces plasma FFAs (late phase). Though we cannot prove a causal relationship, it seems likely that the production of SCFAs from inulin ingestion is, at least partly, responsible for the improved fat oxidation and reduction in plasma FFAs. Lowering plasma FFAs may improve long-term glucose control, since high FFA concentrations are associated with peripheral and hepatic insulin resistance [44]. Moreover, SCFAs can improve glycemia by binding to colonic G protein-coupled receptors leading to release of the gut hormones PYY and GLP-1. PYY and GLP-1 promote glucose storage in muscle and adipose tissue [45, 46]. However, we observed no differential increase in plasma PYY and GLP-1 in the inulin treatment compared with placebo. Therefore, it seems unlikely that the glucose/insulin-lowering effect of inulin in the present study can

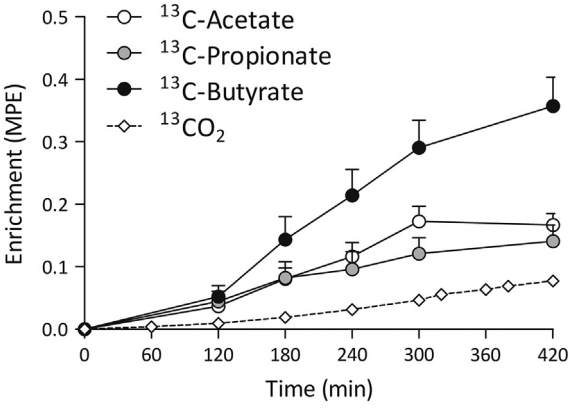

Fig. 6. Plasma SCFA and exhaled CO_2 enrichments. Mean (±SEM) plasma ^{13}C-acetate, plasma ^{13}C-propionate, plasma ^{13}C-butyrate, and exhaled $^{13}CO_2$ after consumption of inulin or placebo (n = 14). A repeated measures ANOVA showed a significant time effect ($P < 0.05$) for all three plasma ^{13}C-SCFAs, and a pairwise comparison showed this significant effect from time point t = 120 onwards (Acetate t = 120 $P < 0.001$, propionate t = 120 $P = 0.015$, butyrate t = 120 $P = 0.011$). A repeated measures ANOVA showed a significant time effect ($P < 0.05$) for exhaled $^{13}CO_2$ from time point t = 60 onwards ($^{13}CO_2$ t = 60 $P < 0.001$). INU: inulin. PLA: placebo.

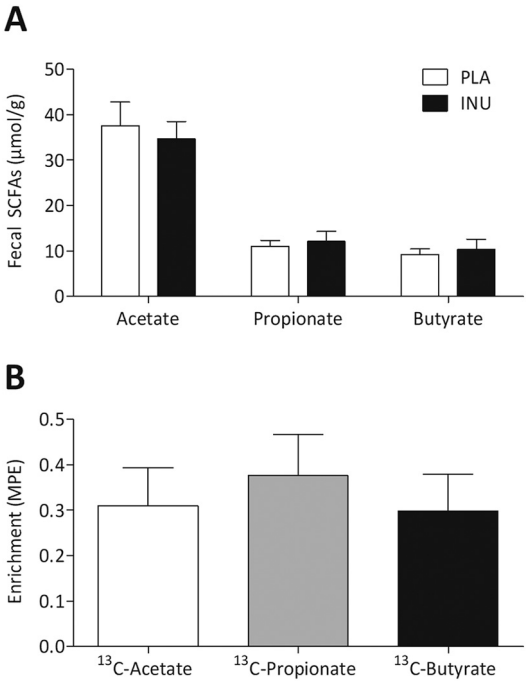

Fig. 7. Fecal SCFA concentrations and enrichments. Mean (±SEM) Fecal SCFA concentration (A), and fecal SCFA enrichment (B) after consumption of inulin or placebo ($n = 14$). There were no significant differences between the two treatments. For ^{13}C-enrichment, only the inulin group was tested. INU: inulin. PLA: placebo. SCFA: short-chain fatty acid.

mechanistically be attributed to an increase in gut hormones by SCFA stimulation, but is rather an effect of the lower glycemic index of inulin.

The early postprandial increase in fat oxidation was accompanied by an immediate increase in breath $^{13}CO_2$ and plasma ^{13}C-SCFAs. Stable isotope-labeled inulin was used to trace inulin-derived SCFAs, which revealed that fermentation of inulin is initiated in the early postprandial phase from 2 h after ^{13}C-inulin intake. Therefore, it seems likely that the combination of lower glycemic index of inulin and fermentation of inulin into SCFAs contributes to the observed improvements in substrate metabolism. Our observation is in line with a study by Rahat-Rozenbloom et al. [47], showing an increase in plasma SCFA concentrations and an increase in breath hydrogen at 2 h after inulin intake, indicating fermentation of inulin from 2 h onwards in overweight to obese subjects. Conversely, Tarini et al. [23] found an increase in plasma SCFAs between 4 and 7 h after inulin intake in a lean, healthy population. An early increase in plasma ^{13}C-SCFAs may suggest inulin fermentation in the upper gastrointestinal tract. However, in vitro and in vivo studies have shown that inulin remains intact while passing through the small intestine and is mainly fermented in the colon [48–50]. Importantly, obese individuals have an increased gastric emptying rate and faster intestinal transit [51, 52]. As such, dietary inulin might reach the colon more rapidly in obese compared with lean individuals, where it is fermented into SCFAs that are released into the circulation and subsequently modulate human metabolism.

Previous studies have investigated either the fermentation profile of inulin or chronic metabolic effects after inulin supplementation in a healthy population. We extend on this work by showing that in overweight to obese individuals a single bolus of inulin is fermented into SCFAs already 2 h after meal ingestion onwards (as indicated by the presence of ^{13}C-labeled SCFAs in blood derived from dietary ^{13}C-labeled inulin), which was accompanied by an improved fat oxidation. Although most studies report a different microbiota composition in obese compared with lean individuals, we show that obese microbiota is able to ferment inulin into SCFAs, and that inulin intake improves metabolic parameters in this population. Our data implicate that replacing digestible carbohydrates with fermentable fibers may improve metabolic health in overweight to obese individuals. Future studies could implement the use of inulin as a dietary strategy to increase SCFA availability and assess its effectiveness on markers of the metabolic syndrome. Moreover, the use of recently developed inulin-SCFA esters, whereby inulin serves as a vector to increase the amount of SCFAs, is

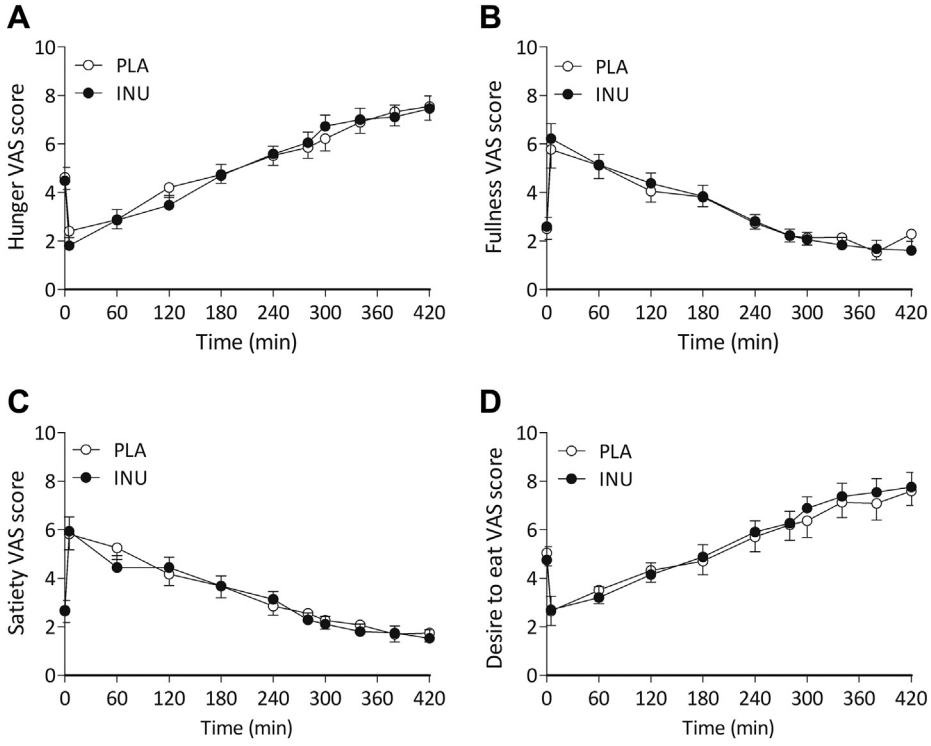

Fig. 8. VAS scores on appetite and satiety. VAS scores on hunger (A), fullness (B), satiety (C), and desire to eat (D) after consumption of inulin or placebo ($n = 14$). There were no significant differences between treatments. INU: inulin. PLA: placebo. VAS: Visual Analogue Scale.

an interesting approach for future studies to further increase fat oxidation and improve human metabolism [53]. In addition, more studies should focus on overweight and obese individuals, since this target population might have different postprandial responses than an lean population [41].

In conclusion, ingestion of the prebiotic inulin increases fat oxidation and promotes SCFA production in overweight to obese men. The lower glycemic index of inulin and its SCFA-producing property might be responsible for the improvement in human metabolism. Replacing digestible carbohydrates with the fermentable inulin may favor human substrate metabolism.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.metabol.2018.06.009.

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Statement of Authorship

CvdB, EC, EB, CD and KL designed the research project; CvdB and AK conducted the research, CvdB, SG, SOD, HvE and JH analyzed data; CvdB, EC, AK, SOD, HvE, JH, SG, EB, CD and KL wrote the paper; CvdB and KL had primary responsibility for final content. All authors read and approved the final manuscript. No conflicts of interest have been declared.

Conflict of Interest Statement

The authors report no conflicts of interest in this work.

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