Impact of breakfast skipping compared with dinner skipping on regulation of energy balance and metabolic risk[1,2]

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ABSTRACT

Background: Meal skipping has become an increasing trend of the modern lifestyle that may lead to obesity and type 2 diabetes.

Objective: We investigated whether the timing of meal skipping impacts these risks by affecting circadian regulation of energy balance, glucose metabolism, and postprandial inflammatory responses.

Design: In a randomized controlled crossover trial, 17 participants [body mass index (in kg/m^2): 23.7 ± 4.6] underwent 3 isocaloric 24-h interventions (55%, 30%, and 15% carbohydrate, fat, and protein, respectively): a breakfast skipping day (BSD) and a dinner skipping day (DSD) separated by a conventional 3-meal-structure day (control). Energy and macronutrient balance was measured in a respiration chamber. Postprandial glucose, insulin, and inflammatory responses in leukocytes as well as 24-h glycemia and insulin secretion were analyzed.

Results: When compared with the 3-meal control, 24-h energy expenditure was higher on both skipping days (BSD: +41 kcal/d; DSD: +91 kcal/d; both $P < 0.01$), whereas fat oxidation increased on the BSD only (+16 g/d; $P < 0.001$). Spontaneous physical activity, 24-h glycemia, and 24-h insulin secretion did not differ between intervention days. The postprandial homeostasis model assessment index (+54%) and glucose concentrations after lunch (+46%) were, however, higher on the BSD than on the DSD (both $P < 0.05$). Concomitantly, a longer fasting period with breakfast skipping also increased the inflammatory potential of peripheral blood cells after lunch.

Conclusions: Compared with 3 meals/d, meal skipping increased energy expenditure. In contrast, higher postprandial insulin concentrations and increased fat oxidation with breakfast skipping suggest the development of metabolic inflexibility in response to prolonged fasting that may in the long term lead to low-grade inflammation and impaired glucose homeostasis. This trial was registered at clinicaltrials.gov as NCT02635139. *Am J Clin Nutr* 2017;105:1351–61.

Keywords: energy balance, insulin sensitivity, macronutrient oxidation, meal skipping, meal frequency

INTRODUCTION

Eating in misalignment with the biological clock (e.g., skipping breakfast and consuming bigger meals in the evening or eating late at night) is associated with an increased risk of obesity and type 2 diabetes (1, 2). On the other hand, popular trends such as breakfast or dinner skipping are advertised for weight management; however, conclusive scientific evidence to support these suppositions is lacking (3).

Previous tightly controlled room calorimetry studies that investigated the impact of meal frequency on the regulation of energy balance under isocaloric conditions did not find a difference in energy expenditure between large (1–2 meals/d), normal (3 meals/d), or small, frequent (>5 meals/d) patterns (4–8). Although no effect of consumption frequency on mean 24-h energy expenditure and respiratory quotient (RQ)[7] was observed in these studies, a lower frequency of 2 or 3 compared with 6–14 meals increased sleeping or resting metabolic rate (5, 6) and diet-induced thermogenesis (DIT) (4) and changed the diurnal pattern of nutrient partitioning to increased fat oxidation until noon (4, 8). The timing of meal consumption has also been shown to affect DIT, with higher levels in the morning than in the afternoon and night (9). A 44% lower DIT in the evening than in the morning (10) argues against a beneficial effect of breakfast skipping on energy balance and suggests a favorable impact of dinner skipping. We therefore hypothesized that breakfast skipping compared with dinner skipping leads to lower total energy expenditure.

[1] Supported by budgetary resources of the University of Hohenheim.

[2] Supplemental Figures 1–3 are available from the "Online Supporting Material" link in the online posting of the article and from the same link in the online table of contents at http://ajcn.nutrition.org.

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[7] Abbrevations used: BSD, breakfast skipping day; DIT, diet-induced thermogenesis; DSD, dinner skipping day; ECG, electrocardiographic; FFA, free fatty acid; FMI, fat mass index; HF, high frequency; HOMApp, postprandial homeostasis model assessment; HRV, heart rate variability; iAUC, incremental AUC; LF, low frequency; MAGE, mean amplitude of glycemic excursions; NLRP3, NOD-like receptor protein 3; npRQ, nonprotein respiratory quotient; RMSSD, root-mean-square difference in successive normal-to-normal intervals; RQ, respiratory quotient; SDRR, SD of normal-to-normal intervals; SNS, sympathetic nervous system; tAUC, total AUC; TLR, Toll-like receptor; VCO_2, carbon dioxide production; VO_2, oxygen consumption.

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Diurnal differences in energy expenditure and nutrient partitioning can be mediated by sympathetic nervous system (SNS) activity and endocrine factors. With lower meal frequency, higher peaks and subsequently lower troughs of insulin might lead to increased fat oxidation (6). In addition to meal frequency, circadian rhythms in insulin sensitivity are known to affect blood glucose concentrations and insulin secretion in response to meal timing. Thus, the same meal consumed in the evening not only leads to a lower metabolic rate but also increases glycemic and insulinemic responses, which suggests circadian variations in energy expenditure as well as the metabolic pattern in healthy individuals (11). A nocturnal lifestyle with breakfast skipping and a delayed eating pattern thus can lead to increased 24-h glycemia (8) and impairment of insulin response to glucose (1) and could therefore contribute to an increased risk of type 2 diabetes. We therefore hypothesized that breakfast skipping compared with dinner skipping leads to impaired glucose metabolism. As a possible underlying mechanism, increased low-grade inflammation induced by a sudden shift to postprandial conditions after prolonged fasting was investigated.

The primary aim of the present study was to compare the effects of breakfast skipping with dinner skipping on 24-h energy expenditure and substrate partitioning as well as (secondary aims) on 24-h SNS activity, the inflammatory response of blood cells, and insulin, glucose, and appetite profiles by using a 3-meal control day as a reference and applying well-controlled energy balance conditions in a metabolic chamber.

METHODS

Study population

Seventeen healthy adults (9 women, 8 men) were recruited by notice board postings at the universities of Hohenheim and Stuttgart between October 2015 and April 2016. Exclusion criteria were food allergies or intolerances, alternative nutrition habits, smoking, chronic diseases, or regular use of medications. The CONSORT (Consolidated Standards of Reporting Trials) flowchart shows the passage of participants through the different stages of the present trial, including enrollment, allocation to the interventions, and analysis (**Supplemental Figure 1**). Thirteen participants were regular breakfast eaters and 4 were occasional breakfast skippers. The terms "breakfast eaters" and "skippers" were not further defined to participants or by investigators. The study protocol was approved by the ethics committee of the Medical Council of Baden-Württemberg, Germany. The trial was registered at clinicaltrials.gov as NCT02635139. All of the participants provided written informed consent before participation.

Study protocol

A randomized crossover nutrition intervention was conducted at the Institute of Nutritional Medicine at the University of Hohenheim. An outline of the study protocol is given in **Figure 1**. A 3-d run-in period with a controlled diet preceded the intervention phase to adapt macronutrient oxidation to macronutrient intake (12). On the intervention days, participants consumed isocaloric diets (55% carbohydrate, 30% fat, 15% protein) with three 24-h conditions: *1*) a conventional 3-meal-structure day (control), *2*) a breakfast skipping day (BSD), and *3*) a dinner skipping day (DSD). The BSD and DSD were randomly assigned, and the first skipping day was followed by a washout day to again obtain a constant fasting period of 18 h before the next intervention day. Thus, the sequence of the intervention days was either BSD-washout-control-DSD or DSD-washout-control-BSD. Participants were randomly assigned by using block randomization to begin with the BSD or DSD intervention in a 1:1 allocation ratio that was based on a computer-generated list of random numbers by an independent scientist. The study team enrolled and assigned the participants to the interventions. During the entire caloric chamber period, participants followed a constant daily routine: wake up at 0600; meals at 0700, 1300, and 1900; and bedtime at 2200. On the day before the first intervention day, participants were admitted to the institute at 1830 to install a continuous glucose-monitoring sensor. Participants spent the night before each intervention day in the caloric chamber and left the morning after the intervention day. On the washout day, participants were allowed to go home for 12 h.

During the intervention days, blood samples were collected frequently from 0700 to 2100 to measure free fatty acid (FFA), ghrelin, and cortisol concentrations (ghrelin and cortisol were determined in a subsample of 8 participants and ghrelin was measured on the skipping days only). The first blood sample at 0700 was taken in a fasting state. After lunch on the skipping days, blood was sampled every 30 min for 2 h for the determination of glucose and insulin concentrations as well as for the assessment of immune cell activity.

Control of energy intake and physical activity

During the whole study period participants all foods were provided from the Institute of Nutritional Medicine's metabolic

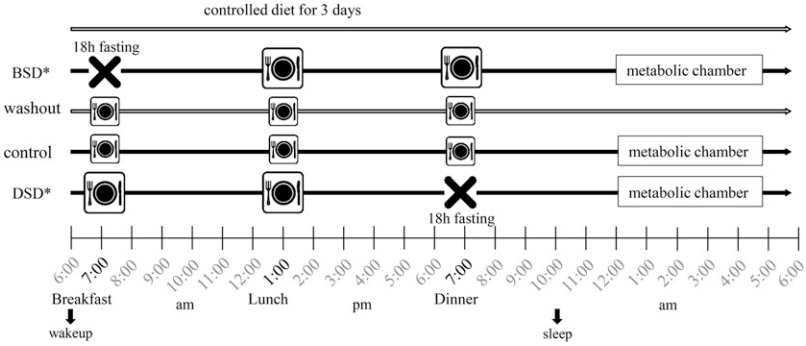

FIGURE 1 Schematic overview of the study protocol. *Randomly assigned. BSD, breakfast skipping day; DSD, dinner skipping day.

kitchen. Participants were instructed to only consume the provided food, water, and unsweetened tea and to refrain from vigorous physical activity. During the first 2 d of the 3-d run-in period, participants ate ad libitum and leftovers were backweighed to calculate dietary intake. On the other study days, all of the provided food was consumed and participants were required to remain sedentary. Macronutrient composition was kept constant throughout the entire study period and for each meal. On intervention days, participants received the same food items on each day. Individual diet composition was calculated by using Prodi6 software (Wissenschaftliche Verlagsgesellschaft). Energy intake was based on individual energy requirements to obtain energy balance. Skipped meals were therefore compensated for by an equally increased energy content of the other 2 meals on this day (equal energy content of both remaining meals). On the control day, each of the 3 meals had the same energy content. Individual energy requirement was calculated on the basis of resting metabolic rate measured by open-circuit indirect calorimetry on the morning after an overnight fast (ventilated hood system, Quark RMR; COSMED) before the study period and multiplied by a physical activity level of 1.35 as estimated for the days in the respiratory chamber. Physical activity was continuously measured by using a triaxial activity monitor (ActivPAL; Paltechnologies Ltd.).The time spent sitting or lying, standing, and stepping and the step numbers were analyzed. The ActivPAL was worn at the midline of the thigh, one-third of the way between the hip and knee and fixed with a waterproof tape according to the recommendation of the manufacturer.

Body-composition analysis

Examinations took place before the 3-d run-in period after an overnight fast. Height was measured with a stadiometer (Seca 274; Seca GmbH & Co. KG). Body weight was measured on a calibrated impedance scale (Seca mBCA 515; Seca GmbH & Co. KG). Fat mass was assessed by using air-displacement plethysmography via the BodPod Body Composition System (COSMED). Fat mass index (FMI) was calculated as fat mass divided by the square of height (kg/m^2).

Twenty-four-hour energy expenditure and substrate oxidation

The 2 respiratory chambers at the Institute of Nutritional Medicine at the University of Hohenheim measure 9 m^2 and have a total volume of 21,000 L (D&S Consulting Services, Inc.). They are furnished with a day bed, chair and desk, computer with Internet access, telephone, toilet, and sink. Air locks are used for the exchange of food and equipment. Macronutrient oxidation was determined by measuring oxygen consumption (VO_2) and carbon dioxide production ($\dot{V}CO_2$) continuously by the Promethion (model GA-3m2/FG-250) integrated whole-room indirect calorimeter system (Sable Systems International). This was accomplished by flowing a fixed 80 L fresh air/min through the metabolic chamber and obtaining a sample on the exhaust side of the system for measurement of oxygen and carbon dioxide concentrations (%). The rates of oxygen consumption and carbon dioxide production were then calculated by using equations derived by Brown et al. (13). The research-grade instrumentation is accurate to 0.001% for mass airflow (liters) and oxygen and carbon dioxide concentrations. Moreover, water vapor pressure (kilopascals) of the sample gas stream was measured directly to 0.001 kpa and used to continuously correct $\dot{V}O_2$ and $\dot{V}CO_2$, along with mass airflow (liters). This eliminates the need for any type of desiccant to dry the sample gas stream during metabolic measurements. The lack of desiccants eliminates any potential errors due to incomplete removal of moisture before analysis of sample gases and mass airflow (14). Data acquisition and processing were performed after completion of each metabolic test by using Sable Systems ExpeData software (Sable Systems International).

Mean values were obtained from minute-to-minute intervals. To correct the measured RQ for protein oxidation, nonprotein RQ (npRQ) was calculated by using nitrogen excretion in 24-h urine. Macronutrient oxidation was computed according to Jéquier and Felber (15). During the stay in the respiratory chamber, 24-h urine samples were collected in polyethylene containers. Nitrogen excretion in 24-h urine was calculated from photometrically measured urea concentration, and obligate nitrogen losses by feces and skin were assumed to be +2.5 g N/d (16).

Energy expenditure for 24 h was calculated from $\dot{V}O_2$ and $\dot{V}CO_2$ and nitrogen excretion to correct for protein metabolism by using the Weir equation ($3.941 \times \dot{V}O_2 + 1.106 \times \dot{V}CO_2 - 2.17 \times$ g urinary nitrogen) (17). Twenty-four-hour npRQ and 24-h macronutrient oxidation were assessed from 0600 to 0600 on the following day. Macronutrient and energy balance was determined by subtracting macronutrient oxidation and energy expenditure from the respective intake. On 2 days, technical problems with the power supply occurred. The respiratory chamber data for these 2 participants were excluded for all days because of the crossover study design with intraindividual comparisons. FFAs were measured photometrically, and total AUC (tAUC) was calculated for 14 h (0700–2100).

Assessment of glucose metabolism and hormonal measurements

Interstitial glucose concentrations were measured continuously by using the Dexcom G4 glucose-monitoring device (Dexcom G4 Platinum; Nintamed GmbH & Co. KG) during the entire room-calorimeter phase. The sensor was applied to the back of the upper arm to measure interstitial glucose concentrations in the subcutaneous tissue. Sensor readings were reported every 5 min. The device was calibrated twice a day by using capillary blood samples. AUC was calculated as tAUC for the entire intervention days (0600–0600) by using trapezoidal rule (18). Glucose variability was assessed by the Mean Amplitude of Glycemic Excursions (MAGE) (19) by using a published macro (20).

Glucose was measured with the use of a hexokinase method, and serum insulin was determined by electrochemiluminescence. Incremental AUC (iAUC) was calculated by using trapezoidal rule (18) for 2 h postprandially after lunch on BSD and DSD. HOMA-IR (21) and the postprandial homeostasis model assessment index (HOMApp) after lunch (22) on BSD and DSD was determined.

Postprandial iAUCs and HOMApp after lunch were only assessed on BSD and DSD because of the smaller energy content of lunch on the control day. Twenty-four-hour insulin secretion was obtained by 24-h urinary C-peptide excretion by using the luminescence immunoassay method.

Cortisol secretion was determined by luminescence immunoassay, and tAUC was calculated for 22 h (0700–0500). Ghrelin was examined by radioimmunoassay, and tAUC was calculated for 14 h (0700–2100).

SNS activity and autonomic function

Heart rate variability (HRV) was assessed in a continuous electrocardiographic (ECG) recording by using an autonomic nervous system recorder (ANS-Recorder Flex BT; Neurocor Ltd. & Co. KG). Measurements were conducted in a sitting position for 5 min every 2 h throughout the intervention days and every 30 min after the scheduled meal time for 2 h, even when the meal was skipped. ECG recordings were made under a nonstressful situation that we defined as quietly resting in an armchair in the respiratory chamber with dimmed lighting. The ECG signal was inspected for artifacts and analyzed by using corresponding NeurocorV R software (ANS-Explorer V3.5.11; Neurocor Ltd. & Co. KG). Time-domain variables included the SD of normal-to-normal intervals (SDRR; a global measure of overall HRV) and the root-mean-square difference in successive normal-to-normal intervals (RMSSD; a measure of parasympathetic activation). As a marker of sympathovagal balance, the ratio of low frequency (LF; 0.04–0.15 Hz) to high frequency (HF; 0.15–0.4 Hz; LF:HF) was analyzed. Adrenaline and norepinephrine excretions in 24-h urine were measured by using liquid chromatography–mass spectrometry.

Postprandial inflammatory responses in blood cells

To investigate if breakfast skipping changed the responsiveness of peripheral leukocytes, we used standardized full-blood assays. These overcome limitations associated with the classical analysis of cell subpopulations and are highly reliable and reproducible and less prone to contamination and variability than the analysis of isolated blood cells (23).

Heparin blood samples were collected before and 30 min, 1 h, 1.5 h, 2 h, and 4 h after lunch on BSD and DSD. Blood was diluted in culture medium (Roswell Park Memorial Institute 1640; Gibco) and stimulated for 16 h with the T cell mitogen phytohemagglutinin (Sigma-Aldrich) and the NOD-like receptor protein 3 (NLRP3) inflammasome activators LPS and nigericin (both from Invivogen). Cytokine release into the supernatant was measured by ELISA (IL-6 Duo Set; R&D Systems, Bio-Techne) and AlphaLISA [IL-1β, interferon γ (IFN-γ); PerkinElmer], according to the manufacturers' protocols. Measurements were conducted on an Enspire Multimode reader (PerkinElmer).

To analyze the effect of insulin on blood cells, blood of 1 donor was either incubated for 1 or 2 h with 100 or 200 μU insulin/mL before cells were stimulated with 10 μg phytohemagglutinin/mL or mock-treated for 16 h. Subsequently, IL-6 release into the medium was measured by ELISA (IL-6 Duo Set; R&D Systems, Bio-Techne).

T cell composition of the peripheral blood was analyzed by fluorescence-activated cell sorting staining with CD45-Peridinin-Chlorophyll-protein, CD3–fluorescein isothiocyanate, CD4-Phycoerythrin, and CD8-Allophycocyanin (all from Miltenyi Biotec) by using a FACSCanto (BD) device. Briefly, erythrocytes were lysed (BD FACS Lysing Solution), leukocytes were washed with fluorescence-activated cell sorting buffer (phosphate-buffered saline with 2% fetal calf serum, 2 mmol EDTA/L, and 0.01% sodium azide) and stained with the antibodies.

Statistical analyses

Together with total energy expenditure, fat oxidation was the primary outcome variable of the study. It was therefore used for power analysis. According to the main hypothesis, fat oxidation was compared between BSD or DSD and the control day. Power analysis was conducted by using G-Power 3.1.9.2 software (written by Faul F., University of Kiel, Germany) and a 2-sided t test for difference between 2 dependent means and an α level of 0.05. Means ± SDs for fat oxidation (61.9 ± 4.6 g/d) were based on the data of Munsters and Saris (6). To show a 6% difference in fat oxidation with a power of 80%, a total sample size of $n = 13$ was required. Data are reported as means ± SDs unless otherwise specified. Normal distribution was checked by Kolmogorov-Smirnov test. Repeated-measures ANOVA was used to examine differences in the variables of energy and macronutrient balance, glucose metabolism, HRV data, and catecholamine and cortisol concentrations between the 3 intervention days. Significant effects were followed with pairwise comparisons and Bonferroni post hoc tests. Differences between the skipping days in insulin, glucose, and ghrelin concentrations were analyzed by paired t test, and Wilcoxon's test was used if data were not distributed normally. Sex differences in baseline characteristics were analyzed by using independent-samples t test. Differences between regular breakfast eaters and occasional breakfast skippers as well as between participants with a low and high FMI were tested by Mann-Whitney U test. Correlations between npRQ and C-peptide, FFA tAUC, RMSSD, adrenaline, or norepinephrine as well as correlations between 24-h energy expenditure and adrenaline or norepinephrine were tested by Spearman's ρ and included the data for all intervention days. Cytokine data were tested by using ANOVA with Tukey's post hoc test and were analyzed and visualized by using GraphPad Prism 7.0 (GraphPad Software). All other analyses were conducted by using SPSS statistical software (version 23; SPSS, Inc.). Significance was set at $P < 0.05$.

RESULTS

Baseline characteristics of the study population are shown in **Table 1**. Eight women and 9 men aged 20–31 y participated in this study. BMI (in kg/m^2) and percentage of body fat mass ranged between 18.3 and 35.0 and 7.4% and 33.9%, respectively. According to WHO criteria, 3 participants were overweight, 2 were obese, and 1 was underweight.

Dividing men and women into 2 groups according to their mean FMI showed that there were no differences in meal skipping–induced changes between the 2 groups in postprandial glucose iAUC, insulin iAUC, and HOMApp after lunch; 24-h glycemia; C-peptide excretion; 24-h energy expenditure; or fat oxidation. However, our study was not powered to detect differences between participants with lower and higher FMI. Therefore, these analyses should be interpreted with caution.

Energy and macronutrient balances

Energy intake (kilocalories) was similar by design for the 3 intervention days (**Table 2**). Twenty-four-hour energy expenditure (kilocalories) was higher on both skipping days than on the control day. Energy balance (kilocalories per day) on the control day was therefore slightly positive and differed from that on the

TABLE 1
Baseline characteristics of the study population[1]

	Women (n = 9)	Men (n = 8)	Total (n = 17)	P[2]
Age, y	23.7 ± 2.5	25.6 ± 3.9	24.6 ± 3.3	NS
Height, m	1.64 ± 0.06	1.83 ± 0.08	1.73 ± 0.11	<0.001
Body weight, kg	57.7 ± 7.9	88.0 ± 17.3	71.9 ± 20.1	<0.001
BMI, kg/m^2	21.2 ± 1.9	26.6 ± 5.2	23.7 ± 4.6	0.022
FMI, kg/m^2	5.7 ± 1.6	6.2 ± 3.6	6.0 ± 2.6	NS

[1] Values are means ± SDs. FMI, fat mass index.
[2] P values for sex differences tested by using independent-samples t test.

skipping days. However, physical activity did not differ between the skipping days and the control day (number of steps—BSD: 655 ± 247; DSD: 710 ± 238; and control: 644 ± 207 steps/d; time spent sitting or lying—BSD: 22.3 ± 0.7; DSD: 22.1 ± 1.1; and control: 22.3 ± 0.8 h/d; time spent standing—BSD: 1.5 ± 0.7; DSD: 1.7 ± 1.0; and control: 1.6 ± 0.8 h/d; time spent stepping—BSD: 0.2 ± 0.1; DSD: 0.2 ± 0.1; and control: 0.2 ± 0.1 h/d; all n = 15; $P > 0.05$).

No difference in fasting npRQ was observed between the 3 intervention days (BSD: 0.82 ± 0.04; DSD: 0.81 ± 0.05; control: 0.83 ± 0.05; $P > 0.05$). Components of macronutrient balance are presented in Table 2. Macronutrient intake was similar between the 3 intervention days. When compared with the control day, 24-h fat oxidation was higher and 24-h carbohydrate oxidation was lower on BSD, whereas both variables did not differ from the control on DSD. FFA tAUC was higher on BSD and DSD than on the control day. No association was observed between FFA tAUC and 24-h npRQ. **Figure 2** shows the profiles of 24-h fat and carbohydrate oxidation. Even after lunch, postprandial fat oxidation on the BSD (12.05 ± 4.37 g/2 h or 0.1 g/min) was higher than on the control day (9.64 ± 4.14 g/2 h or 0.08 g/min; $P > 0.001$) or on the DSD (9.92 ± 4.67 g/2 h or 0.08 g/min; $P > 0.01$).

Fat balance was more negative and carbohydrate balance more positive on the BSD than on the control day (Table 2). On the DSD, fat and carbohydrate balances did not significantly differ from that on the control day. Protein oxidation and balance were both similar between all intervention days.

Impact of meal skipping on autonomic nervous system activity

Although 24-h adrenaline and norepinephrine excretion did not differ between the intervention days, significant differences in diurnal autonomic nervous system activity were shown by heart rate monitoring, with a higher RMSSD in the morning with breakfast skipping than with dinner skipping (data not shown). Overall HRV was higher on the BSD than on the DSD (SDRR; $P < 0.05$) but not compared with the control day (Table 2). Mean parasympathetic tone (RMSSD) and mean sympathovagal balance (LF:HF) did not differ between the intervention days (both $P > 0.05$).

TABLE 2
Comparison of the components of energy and macronutrient balance and SNS activity between BSD, DSD, and the 3-meal control[1]

	Control	BSD	DSD
Energy balance,[2] kcal/d			
Energy intake	2283 ± 487	2248 ± 486	2249 ± 486
24-h Energy expenditure	2154 ± 441	2195 ± 461**	2245 ± 463***
Energy balance	130 ± 158	53 ± 143***	5 ± 130***
Macronutrient intake,[2] g/d			
Fat	75 ± 17	74 ± 16	74 ± 16
Carbohydrate	307 ± 67	303 ± 66	303 ± 66
Protein	84 ± 18	83 ± 18	83 ± 18
Macronutrient oxidation			
24-h npRQ[2]	0.85 ± 0.05	0.83 ± 0.05**	0.84 ± 0.05
24-h Protein oxidation,[2] g/d	81 ± 18	86 ± 17	84 ± 20
24-h Carbohydrate oxidation,[2] g/d	237 ± 60	205 ± 53*	233 ± 56
24-h Fat oxidation,[2] g/d	97 ± 45	110 ± 46***	106 ± 48
tAUC FFAs, mg/dL × 14 h	79 ± 21	163 ± 32***	136 ± 36***
Macronutrient balance,[2] g/d			
Fat balance	−22 ± 33	−36 ± 33***	−32 ± 35
Carbohydrate balance	70 ± 70	97 ± 78*	69 ± 76
Protein balance	3 ± 22	−3 ± 14	−1 ± 21
SNS activity			
Adrenaline, μg/d	11 ± 3	10 ± 3	10 ± 3
Norepinephrine, μg/d	37 ± 13	44 ± 12	44 ± 14
SDRR, ms	55 ± 15	59 ± 18	53 ± 14†
RMSSD, ms	38 ± 16	41 ± 19	36 ± 14
LF:HF,[2] ms	2.8 ± 3.1	2.7 ± 2.5	2.4 ± 2.1

[1] Values are means ± SDs; n = 17 unless otherwise indicated. Repeated-measures ANOVA with Bonferroni adjustments was used. *******BSD or DSD compared with control: *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. †DSD compared with BSD, $P < 0.05$. BSD, breakfast skipping day; DSD, dinner skipping day; FFA, free fatty acid; HF, high-frequency domain; LF, low-frequency domain; npRQ, nonprotein respiratory quotient; RMSSD, root-mean-square successive difference; SDRR, SD of all normal-to-normal intervals; SNS, sympathetic nervous system; tAUC, total AUC.
[2] n = 15.

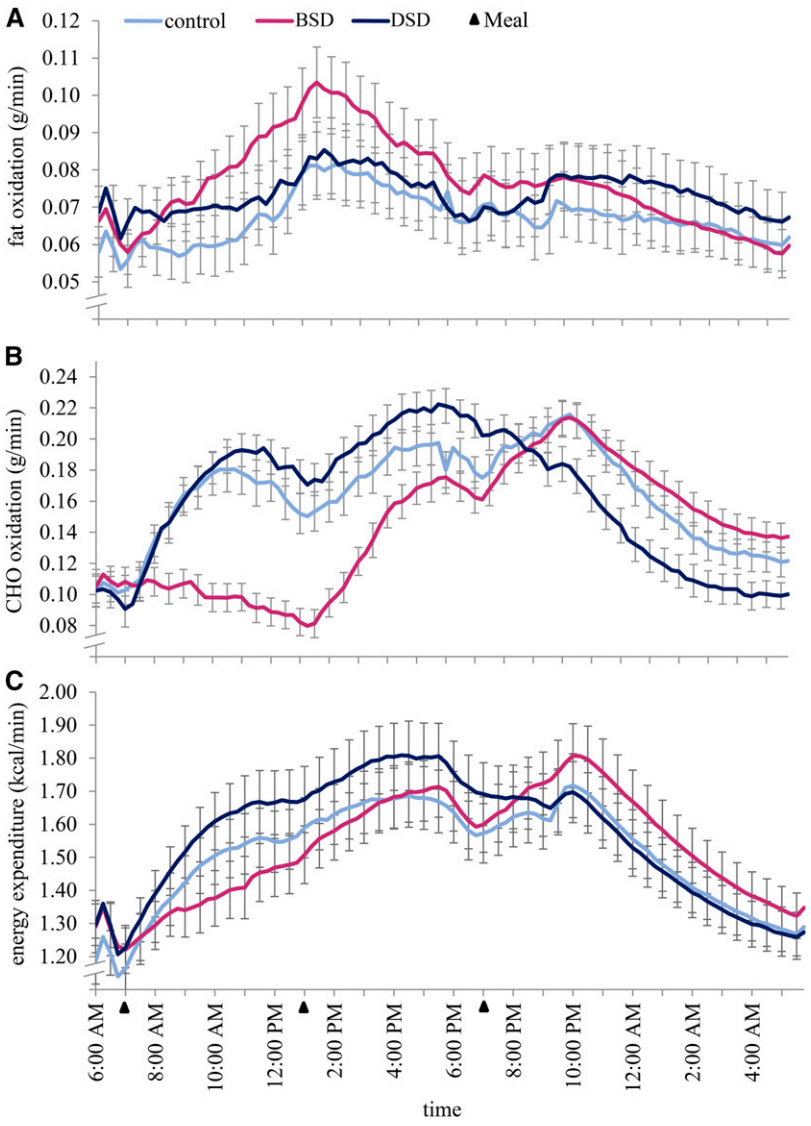

FIGURE 2 Twenty-four-hour fat oxidation (A), carbohydrate oxidation (B), and energy expenditure (C) for control, BSD, and DSD groups (n = 15). Mean values of 15 min were plotted, and SEs are shown only at every 30 min for clarity. Differences in the corresponding 24-h cumulative oxidations and 24-h energy expenditure are reported in Table 2. BSD, breakfast skipping day; CHO, carbohydrate; DSD, dinner skipping day.

Twenty-four-hour npRQ showed a positive correlation with parasympathetic tone (r = 0.52, $P < 0.001$) and an inverse association with norepinephrine excretion (r = −0.46, P = 0.002; **Supplemental Figure 2**A). A higher norepinephrine excretion also correlated with a higher 24-h energy expenditure (r = 0.41, P = 0.005; Supplemental Figure 2B).

Impact of meal skipping on appetite regulation

Ghrelin concentrations were higher in the morning on the BSD and in the evening on the DSD, equalizing one another to a similar ghrelin tAUC between the BSD and DSD (**Figure 3**).

Impact of meal skipping on 24-h and postprandial glucose metabolism and cortisol concentrations

Variables of fasting and 24-h and postprandial glucose metabolism are shown in **Table 3**. Fasting insulin sensitivity (HOMA-IR), 24-h glycemia (tAUC by continuous glucose monitoring data), and glucose variability (MAGE) as well as 24-h insulin secretion (24-h C-peptide excretion) were all similar between the intervention days. However, when compared with dinner skipping, breakfast skipping resulted in higher postprandial iAUCs of glucose and insulin as well as a higher HOMApp after lunch. No correlation was observed between C-peptide and 24-h npRQ. Cortisol tAUC and 24-h cortisol profile did not differ between intervention days (control: 174 ± 57; BSD: 181 ± 45; DSD: 156 ± 40 μg/dl × 22h; $P > 0.05$).

Impact of meal skipping on postprandial inflammatory response in blood cells

Mitogenic stimulation of full-blood cultures for 16 h with phytohemagglutinin significantly induced the secretion of the proinflammatory cytokine IL-6 in fasting blood. When comparing the kinetics of the response toward the fasting condition, a reduction in IL-6 release in blood drawn within 1 h after the lunch

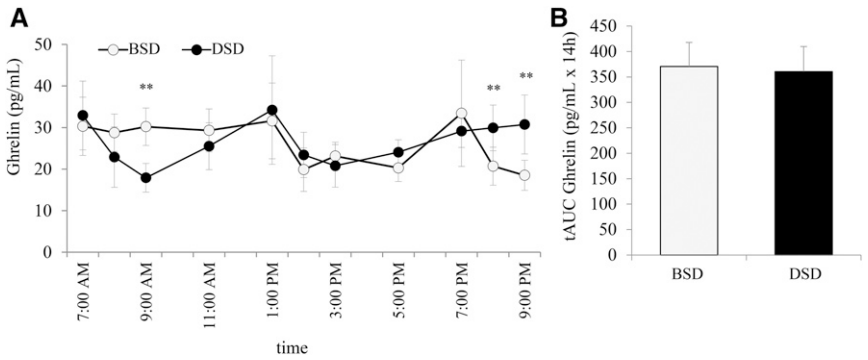

FIGURE 3 Profile of ghrelin concentrations (A) and comparison of tAUCs (B) between BSD and DSD (n = 8). Values are means ± SDs. $^{**}P < 0.01$ (paired t test). BSD, breakfast skipping day; DSD, dinner skipping day; tAUC, total AUC.

was observed, which was significant for 30 min (BSD: $P < 0.0001$; DSD: $P = 0.0038$). By contrast, at later time points, and significantly at 4 h postprandially (BSD: $P < 0.0001$; DSD: $P = 0.0013$), the cells were more responsive toward phytohemagglutinin (**Figure 4**A). When comparing BSD with DSD, IL-6 responses were significantly higher at 4 h after lunch when the participants did not receive a breakfast ($P = 0.0042$). An overall similar postprandial response was obtained for the T cell cytokine IFN-γ, albeit no significant differences between BSD and DSD were shown (Figure 4A).

Because food intake was shown to induce a higher release of IL-1β on NLRP3 stimulation in human peripheral blood mononuclear cells and monocytes 3 h postprandially (24), we decided to include the NLRP3 inflammasome activator nigericin (+LPS) in our study for a subsample of the study group. Significantly higher IL-1β responses ($P = 0.0246$) were observed 4 h postprandially with breakfast skipping, whereas this difference was not significant with dinner skipping (Figure 4B). Notably, for all responses, we observed a trend toward lower cytokine release from blood cells drawn at earlier time points (30 min to 1 h), which was significant in some cases.

Next, we addressed whether differences in the abundance of lymphocyte populations explained our observations because these were reported to occur after food intake (25–27). We therefore measured T cell composition of the peripheral blood used for the stimulation assays from 2 representative donors. No significant change in the proportion of $CD3^+$ cells from total lymphocytes was observed (data not shown), which strongly suggests that the changes in the cytokine concentrations on phytohemagglutinin stimulation were due to changes in the cell-intrinsic signaling pathways.

We also tested if insulin might be directly responsible for this effect by treating blood from a fasting donor at different times for ≤4 h with human insulin before stimulating the cells with phytohemagglutinin. This did not result in any significant change in IL-6 secretion (**Supplemental Figure 3**). Moreover, the kinetics of insulin concentrations did not correlate with the responsiveness of the leukocytes, suggesting that insulin alone is not the responsible stimulus for the change in the postprandial immune response.

DISCUSSION

Contrary to our primary hypothesis, breakfast and dinner skipping led to a small but significant increased 24-h energy expenditure (+41 and +91 kcal/d) compared with a conventional 3-meal pattern and thus improved energy balance under conditions of fixed energy intake. Our results are in contrast to previous controlled studies that used metabolic chambers, which found no effect of breakfast skipping on energy expenditure compared with a conventional 3-meal pattern (8) or a high consumption frequency of 6 (5) or 7 (4) meals. The discrepant results may be due to methodologic differences between the studies. Taylor and Garrow (5) examined overweight and obese subjects under

TABLE 3
Comparison of the fasting, 24-h, and postprandial glucose metabolism variables between the skipping days and the 3-meal control[1]

	Control	BSD	DSD
HOMA-IR[2]	1.96 ± 0.82	2.07 ± 0.91	1.96 ± 1.05
24-h $Glycemia_{tAUC}$,[2] mg/dL × 24 h	2360 ± 111	2425 ± 131	2374 ± 165
MAGE[2]	3.90 ± 1.32	3.65 ± 1.52	3.28 ± 1.75
C-peptide,[2] μg/d	74 ± 38	86 ± 40	75 ± 42
Postprandial variables after lunch			
iAUC insulin, μU/mL × 2 h	—	211 ± 74	144 ± 74**
iAUC glucose, mg/dL × 2 h	—	114 ± 41	62 ± 40***
HOMApp	—	59 ± 44	27 ± 23**

[1] Values are means ± SDs; n = 17. $^{**}P < 0.01$ (Wilcoxon's test); $^{***}P < 0.001$ (paired t test). BSD, breakfast skipping day; DSD, dinner skipping day; HOMApp, postprandial homeostasis model assessment; iAUC, incremental AUC; MAGE, mean amplitude of glycemic excursions; tAUC, total AUC.

[2] Repeated-measures ANOVA with Bonferroni adjustments was used.

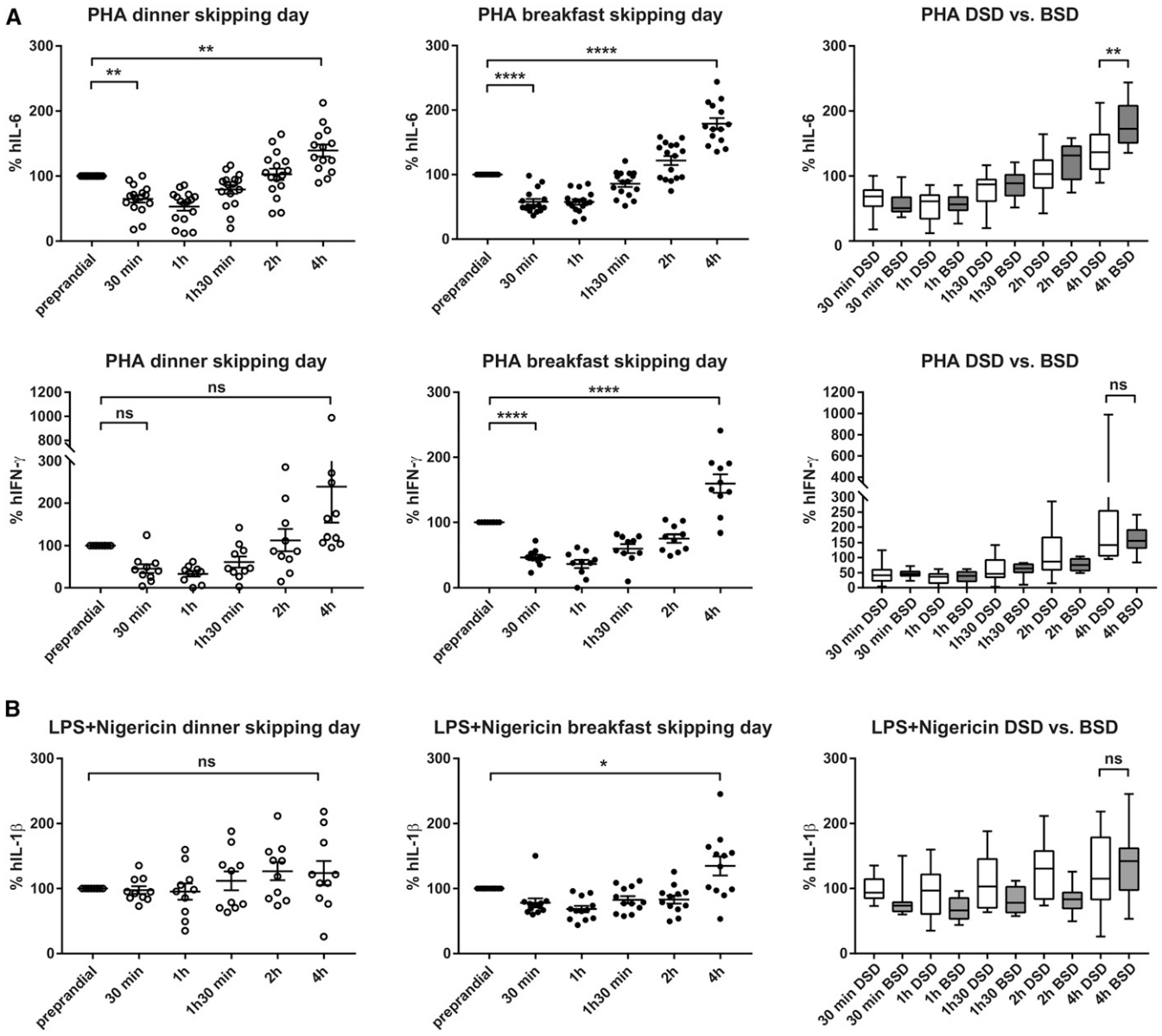

FIGURE 4 Cytokine responses in the fasted and postprandial state. (A) IL-6 (n = 16; upper panels) and IFN-γ (n = 10; lower panels) cytokine release from human blood cells after stimulation with 10 μg phytohemagglutinin/mL for 16 h. Blood was sampled in the fasted state (preprandial) and at indicated time points after lunch (postprandial). The cytokine response for each time point is shown as a percentage compared with the fasted state and was measured after a normal breakfast on DSD (left panels) or BSD (middle panels). The comparison between the kinetics of the cytokine response after BSD and a normal breakfast is shown in the right-hand panels. Values for the dot plots are means ± SEMs; the box-and-whisker plots show medians with upper and lower quartiles, with the maximum and minimum values represented by the whiskers. (B) IL-1β cytokine release from human blood cells after stimulation with 1 ng LPS/mL for 16 h followed by stimulation with 5 μmol nigericin/L for 1.5 h (NLRP3 inflammasome activation). Blood was sampled in the fasted state (preprandial) and at indicated time points after lunch (postprandial). The cytokine response for each time point is shown as a percentage compared with the fasted state and was measured after a normal breakfast on DSD (n = 10; left panels) or BSD (n = 12; middle panels). The comparison between the kinetics of the cytokine response after BSD and a normal breakfast is shown in the right-hand panels. Values for the dot plots are means ± SEMs; the box-and-whisker plots show the medians with upper and lower quartiles, with the maximum and minimum value represented by the whiskers. Statistical testing for differences between indicated groups was performed by using ANOVA with Tukey's post hoc testing. *$P < 0.05$, **$P < 0.01$, ****$P < 0.0001$. BSD, breakfast skipping day; DSD, dinner skipping day; hIFN-γ, human IFN-γ; hIL-6, human IL-6; hIL-1β, human IL-1β; NLRP3, NOD-like receptor protein 3; PHA, phytohemagglutinin.

negative energy balance, and Kobayashi et al. (8) investigated a small number of participants who had higher 24-h glycemia with breakfast skipping; in the study by Verboeket-van de Venne and Westerterp (4), RQ and energy expenditure were calculated over 3-h intervals only. However, in both of the latter studies breakfast skipping was found to increase fat oxidation during the prolonged fasting period until the first meal at 1200. In the present study, the timing of meal skipping was important for inducing a change in macronutrient partitioning, because the increase in 24-h fat oxidation and the corresponding negative fat balance was significant only for breakfast skipping but not for dinner skipping.

Both breakfast and dinner skipping led to a longer duration of the overnight fasting period. Prolonged fasting can be considered

to be a state of stress that leads to increased adrenergic activity and thus to higher lipolysis and increased energy expenditure (28). In line with this finding, concentrations of FFAs (Table 2) and 24-h energy expenditure were higher on both skipping days. Twenty-four-hour excretions of adrenaline and norepinephrine were, however, similar between both intervention days and the 3-meal control day. Nevertheless, we found that norepinephrine excretion inversely correlated with npRQ and positively with 24-h energy expenditure when data from all intervention days were combined. The individual propensity of meal skipping to increase norepinephrine concentrations could therefore explain the interindividual variance in fat oxidation and energy expenditure.

In addition to the duration of fasting, the timing of energy intake could also impact autonomic function and thus affect diurnal changes in substrate partitioning and energy expenditure. In line with this assumption, later timing of breakfast and dinner has been found to cause a phase delay in the diurnal 24-h rhythm of cardiac autonomic nervous system activity assessed by HRV (29). Although autonomic regulation assessed by heart rate monitoring differed between breakfast and dinner skipping (Table 2) a higher SDRR with breakfast skipping argues against a higher sympathetic tone and rather suggests improved autonomic regulation with breakfast skipping.

Lower 24-h insulin secretion due to a prolonged fasting period with meal skipping could contribute to increased lipolysis-induced fat oxidation. However, although concentrations of FFAs and 24-h energy expenditure were higher with both of the meal skipping days than with the 3-meal control day, 24-h insulin secretion did not differ between any of the intervention days (Table 3). Of note, insulin excursions rather than cumulative 24-h insulin secretion are more important for the regulation of nutrient partitioning. Although a high frequency of 6, compared with 3, meals was associated with lower 24-h insulin AUC, at the same time it caused a marked suppression in 24-h FFA concentrations between meals. This was due to the fact that frequent eating prevents a decrease in insulin, which facilitates lipolysis (7). In addition, a low meal frequency resulted in elevated energy expenditure during the postprandial hours, indicating a greater contribution of DIT to 24-h energy expenditure (4).

A limitation to our study protocol is that we cannot examine the effects of meal skipping on voluntary energy intake. Although ghrelin concentrations were higher in the morning with breakfast skipping and in the evening with dinner skipping, we found no differences in the AUC of 24-h ghrelin concentrations between meal skipping days and the 3-meal control day (see Results). However, a compensation of a higher energy expenditure and fat oxidation by a higher spontaneous energy or fat intake under ad libitum conditions cannot be ruled out. Interestingly, a higher meal frequency of 14 or 6 meals compared with 3 meals led to increased ghrelin concentrations (6) and ratings of hunger and "desire to eat" (6, 7). On the other hand, extending morning fasting until lunch caused incomplete energy compensation with an ad libitum lunch (30, 31). Increased hunger and decreased satiety in response to breakfast skipping were found primarily in habitual breakfast eaters (32). This may suggest that the effect of meal skipping on appetite regulatory systems is enhanced in habitual breakfast eaters. In the present study, no differences were observed between regular breakfast eaters and occasional breakfast skippers on meal skipping–induced changes in ghrelin concentrations, glucose regulation, and 24-h energy expenditure or fat oxidation (data not shown). An additional limitation of the present study is the fact that only responses to the first day of breakfast skipping or dinner skipping were measured and therefore the metabolic consequences of habitual breakfast or dinner skipping remain unclear.

In patients with type 2 diabetes, habitual breakfast skipping was associated with a later chronotype (a preference for later bed and wake times) that contributed to poorer glycemic control (33). A disrupted circadian clock provides a mechanistic explanation for the relation between a disturbed diurnal eating pattern and alterations in glucose metabolism (34). Glucose metabolism is highly circadian (35) and depends largely on the timing and composition of nutrient ingestion. Because the body uses nutrient input to set circadian rhythms (36), it is possible that both timing and nutrient composition of the diet might be important for the prevention of metabolic disturbances.

In line with impaired metabolic function with breakfast skipping, randomized controlled trials support higher glucose variability in lean subjects and impaired insulin sensitivity in obese participants who skip breakfast when compared with those who eat breakfast but found no effect on body weight or fat mass over a 6-wk period (31, 37). In support of impaired glucose homeostasis with breakfast skipping, HOMApp and glucose concentrations after lunch were higher with breakfast skipping than with dinner skipping (Table 3). Compared with dinner skipping, higher postprandial fat oxidation at lunchtime after breakfast skipping occurred despite increased insulin concentrations and suggests metabolic inflexibility after prolonged fasting. The mitochondrial capacity to switch freely between oxidative fuels in the transition from fasting to feeding is therefore lost (38). In a healthy, metabolically flexible state, the consumption of a high-carbohydrate meal results in an increase in blood insulin concentrations and respiratory quotient, indicative of a robust shift from fatty acid to glucose oxidation. Increased fat oxidation, despite higher postprandial insulin concentrations with breakfast skipping, suggests the development of metabolic inflexibility in response to prolonged fasting that may increase metabolic risk over time.

We also found that a longer fasting period with breakfast skipping increased inflammasome activity and inflammatory responses of peripheral leukocytes after lunch at later time points (Figure 4). Our data thereby showed reduced inflammatory activity at earlier times after food intake and higher responses at later time points. Higher postprandial NLRP3-dependent IL-1β secretion has also been reported recently by others (24). Because chronic low-grade inflammation is known to impair insulin sensitivity, enhanced postprandial inflammation could contribute to metabolic impairment with breakfast skipping. It is known that peripheral monocytes are activated after food intake and secrete more inflammatory cytokines than with fasting conditions (for review see reference 39), which might be even more pronounced in diabetic patients (40). The underlying stimuli for this activation, however, remain largely elusive. The generation of reactive oxygen species by leukocytes (41) and higher gut microbiota–derived LPS in the serum after feeding (42) have been discussed as possible mechanisms. Most available experimental data, however, support that FFAs from macronutrient uptake might be responsible for postprandial inflammation. Although some concepts are emerging, SFAs can activate Toll-like receptor (TLR) 4 (43) and FFAs synergize with high glucose

concentrations to amplify reactive oxygen species generation and inflammatory responses mediated by TLR2/6 and myeloid differentiation factor 2 (MD2)/TLR4 in vitro (44). Moreover, G protein–coupled FFA receptors, such as GPR43, can induce inflammatory responses in leukocytes (45). Increased lipolysis and FFA concentrations with extended fasting with breakfast skipping may therefore be causal for the observed effects.

Altogether, the present results support the association between breakfast skipping and disturbed glucose homeostasis, which is not explained by a positive energy balance. On the contrary, both breakfast skipping and dinner skipping increased total energy expenditure. In conclusion, a causal role of breakfast skipping for the development of obesity is not supported by the present data.

The authors' responsibilities were as follows—AB-W: designed the research study; AN, NM, FH, J Kahlhöfer, J Keller, and AB-W: performed the research; RR: provided technical support for the metabolic chamber; AN, FH, and NM: analyzed the data; AN, A-BW, NM, TAK, and RR: wrote the manuscript; AN and AB-W: had primary responsibility for the final content; and all authors: read and approved the final manuscript. None of the authors reported a conflict of interest related to the study.

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