Impact of sleep restriction on metabolic outcomes induced by overfeeding: a randomized controlled trial in healthy individuals

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ABSTRACT

Background: Overconsumption of energy-dense foods and sleep restriction are both associated with the development of metabolic and cardiovascular diseases, but their combined effects remain poorly evaluated.

Objective: The aim of this study was to assess whether sleep restriction potentiates the effects of a short-term overfeeding on intrahepatocellular lipid (IHCL) concentrations and on glucose homeostasis.

Design: Ten healthy subjects were exposed to a 6-d overfeeding period (130% daily energy needs, with 15% extra energy as sucrose and 15% as fat), with normal sleep (8 h sleep opportunity time) or sleep restriction (4 h sleep opportunity time), according to a randomized, crossover design. At baseline and after intervention, IHCL concentrations were measured by proton magnetic resonance spectroscopy, and a dual intravenous [6,6-$^2H_2$]-, oral ^{13}C-labeled glucose tolerance test and a polysomnographic recording were performed.

Results: Overfeeding significantly increased IHCL concentrations ($P_{overfeeding} < 0.001$; overfeeding + normal sleep: +53% ± 16%). During the oral glucose tolerance test, overfeeding significantly increased endogenous glucose production ($P_{overfeeding} = 0.034$) and the oxidation of ^{13}C-labeled glucose load ($P_{overfeeding} = 0.038$). Sleep restriction significantly decreased total sleep time, and the duration of stages 1 and 2 and rapid eye movement sleep (all $P < 0.001$), whereas slow-wave sleep duration was preserved ($P_{overfeeding \times sleep} = 0.809$). Compared with overfeeding, overfeeding + sleep restriction did not change IHCL concentrations ($P_{overfeeding \times sleep} = 0.541$; +83% ± 33%), endogenous glucose production ($P_{overfeeding \times sleep} = 0.567$), or exogenous glucose oxidation ($P_{overfeeding \times sleep} = 0.118$). Sleep restriction did not significantly alter blood pressure, heart rate, or plasma cortisol concentrations (all $P_{overfeeding \times sleep}$ = NS).

Conclusions: Six days of a high-sucrose, high-fat overfeeding diet significantly increased IHCL concentrations and increased endogenous glucose production, suggesting hepatic insulin resistance. These effects of overfeeding were not altered by sleep restriction. This trial was registered at clinicaltrials.gov as NCT02075723. Other study ID numbers: SleepDep 02/14. *Am J Clin Nutr* 2018;108:1–12.

Keywords: intrahepatocellular lipids, hepatic insulin resistance, glucose tolerance, polysomnography, slow wave sleep, stress response, cardiovascular risk factors

INTRODUCTION

Consumption of energy-dense foods is known to play an important role in the development of abdominal obesity, insulin resistance, nonalcoholic fatty liver disease, and the metabolic syndrome (1). Sugars have been suggested to be particularly involved in the development of these disorders. Several short-term intervention studies have indeed demonstrated that hypercaloric diets containing high amounts of sugars (i.e., sucrose, glucose, or fructose) can increase intrahepatocellular lipid (IHCL) concentrations and plasma triglyceride (TG) concentrations, and decrease hepatic insulin sensitivity over a short period of time (2, 3). In real-life conditions, sugars and lipids are often consumed together and in excess, and these 2 nutrients may have additive effects on IHCL concentrations (4).

Several observational studies have demonstrated that poor sleep quality and short sleep duration are also associated

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Supplemental Figure 1 and Supplemental Table 1 are available from the "Supplementary data" link in the online posting of the article and from the same link in the online table of contents at https://academic.oup.com/ajcn/.

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Abbreviations used: D, experimental day; EGP, endogenous glucose production; FFA, free fatty acids; IHCL, intrahepatocellular lipid; OGTT, oral-glucose-tolerance test; PSG, polysomnography; REM, rapid eye movement; SNS, sympathetic nervous system; SO, sleep opportunity; SWS, slow-wave sleep; TG, triglyceride; TST, total sleep time; ^{1}H-MRS, proton magnetic resonance spectroscopy.

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with an increased risk of obesity (5) and type 2 diabetes (6). This observation was further supported by intervention studies showing that a few days of sleep restriction could impair glucose tolerance (7, 8) and cause muscle and adipose tissue insulin resistance (9, 10). The mechanisms underlying this effect remain speculative. Short-term intervention studies have reported an activation of the sympathetic nervous system (SNS) and increased glucocorticoid secretion in sleep-deprived subjects (11, 12), which may decrease insulin-mediated glucose disposal (13, 14). Chronically elevated glucocorticoids also favor the deposition of intra-abdominal fat, thus further increasing cardiometabolic risk (15). In addition, sleep restriction–induced stress responses stimulate adipose tissue lipolysis and increase plasma free-fatty acid (FFA) concentrations (16), which may promote ectopic lipid deposition, notably in the liver (17). Some authors have therefore proposed that sleep restriction may act as a stressor, and that the activation of SNS and the hypothalamic-pituitary-adrenal axis would secondarily lead to the development of abdominal obesity, insulin resistance, and increased cardiometabolic risk (18).

In real life, overfeeding and sleep restriction may often occur simultaneously. In prospective studies, sleep restriction has indeed been associated with weight gain (19), which implies the presence of a positive energy balance. Furthermore, several studies have shown that daily caloric intake was increased during sleep restriction periods (20, 21), mainly from the consumption of energy-dense foods (22, 23). To date, the combined effects of these 2 factors remain, however, poorly evaluated. We therefore compared the effects of a short-term, hypercaloric, high-sucrose, high-fat diet associated with normal sleep and with sleep restriction in healthy volunteers. We selected IHCL concentration [measured by proton magnetic resonance spectroscopy (^{1}H-MRS)] as our primary outcome because this has been shown to be a reliable, early marker of metabolic dysfunction (24). We hypothesized that the increase in IHCL concentrations would be more pronounced when overfeeding was associated with sleep restriction.

METHODS

Out of 62 volunteers screened, 16 were included and 10 volunteers [5 women and 5 men; mean ± SEM age: 24 ± 1 y; BMI (kg/m^2): 21.6 ± 0.5] completed the study (**Supplemental Figure 1**). Subjects dropped out for various reasons: 2 for gastrointestinal complaints, 2 for voluntary withdrawal of consent, 1 for allergic reaction, and 1 for personal reasons unrelated to the nature of the study. All subjects were nonsmokers, had been weight stable (±3 kg) for ≥3 mo, performed <3 h of light to moderate exercise per week, were not currently taking any medication, reported no family history of diabetes, and had a habitual sleep opportunity (SO) >7 h, no sleep complaints, and no night-shift work. Before inclusion, a physical examination was carried out to confirm the good health of the subjects. Subjects who scored >5 on the Pittsburgh Sleep Quality Index questionnaire (25) or with extreme values (i.e., 16–30 and 70–86) on the self-assessment morningness-eveningness questionnaire (26) were not included. Finally, subjects exhibiting poor sleep quality or sleep disturbances (i.e., characterized by a sleep efficiency <85%, an apnoea-hypopnoea index >15/h, and periodic leg movements during sleep index >15/h), as assessed during a screening outpatient overnight polysomnographic recording, were also excluded. All women reported being not pregnant throughout the experimental period. The protocol was approved by the Human Research Ethics Committee of Canton de Vaud and was registered at clinicaltrials.gov as NCT02075723. Before inclusion, all participants gave written informed consent.

Study design

The study design is illustrated in **Figure 1**A. The subjects of this randomized, crossover design were studied on 2 occasions, separated by a 4- to 8-wk washout period. Briefly, after a 3-d weight-maintenance diet [i.e., experimental day (D)–3 to D–1], participants underwent a 6-d overfeeding period (i.e., D1–D6; 130% of daily energy requirement, with 15% extra energy as sucrose and 15% extra energy as fat), with either normal sleep (8 h SO; the overfeeding + normal sleep condition) or sleep restriction (4 h SO; the overfeeding + sleep restriction condition). The metabolic effects induced by overfeeding with normal sleep or sleep restriction were assessed by measuring the accumulation of IHCLs by ^{1}H-MRS (primary outcome), by the alteration of hepatic glucose production, and by the metabolic fate of exogenous glucose during a dual, intravenous [6,6-$^2H_2$]-, oral ^{13}C-labeled-glucose-tolerance test (OGTT). The effects of both interventions on sleep parameters were assessed by polysomnography (PSG).

Baseline (D–3 to D–1)

In both conditions, subjects received instructions to consume an energy-balanced, weight-maintenance diet 3 d prior to the baseline metabolic evaluations and sleep recordings (i.e., D–3). During the last 2 d of baseline (i.e., D–2 to D–1), they then received a controlled, weight-maintenance diet containing 55% carbohydrates (45% as starch and 10% as sugar), 30% lipids and 15% proteins, and 36 g dietary fiber (**Table 1**). Individual energy requirements were calculated from the Harris-Benedict equation multiplied by a physical activity factor of 1.5. Diets were prepared accordingly by research staff and provided daily as prepacked meals and snacks for home consumption. Volunteers were asked to consume prepacked items at specific times of the day (i.e., 0800, 1000, 1200, 1500, and 1900), and to refrain from consuming any other foods or drinks with the exception of water. Throughout the baseline period, subjects were also asked to respect regular sleep times, with a minimum of 8 h of SO/night (i.e., from 2230 to 0630), and to refrain from consuming alcohol and performing structured physical activity. Compliance was checked through the use of dietary and sleep records.

Overfeeding (D1–D6)

At the end of the baseline period, volunteers entered a 6-d overfeeding (i.e., D1–D6). They received instructions to consume an energy-balanced, weight-maintenance diet during the first 4 intervention days (i.e., D1–D4), and they received exactly the same controlled, weight-maintenance diet as the one provided during the baseline period during the next 2 d (i.e., D5–D6). Throughout the 6-d intervention period, overfeeding was achieved by adding the daily consumption of 2 drinks,

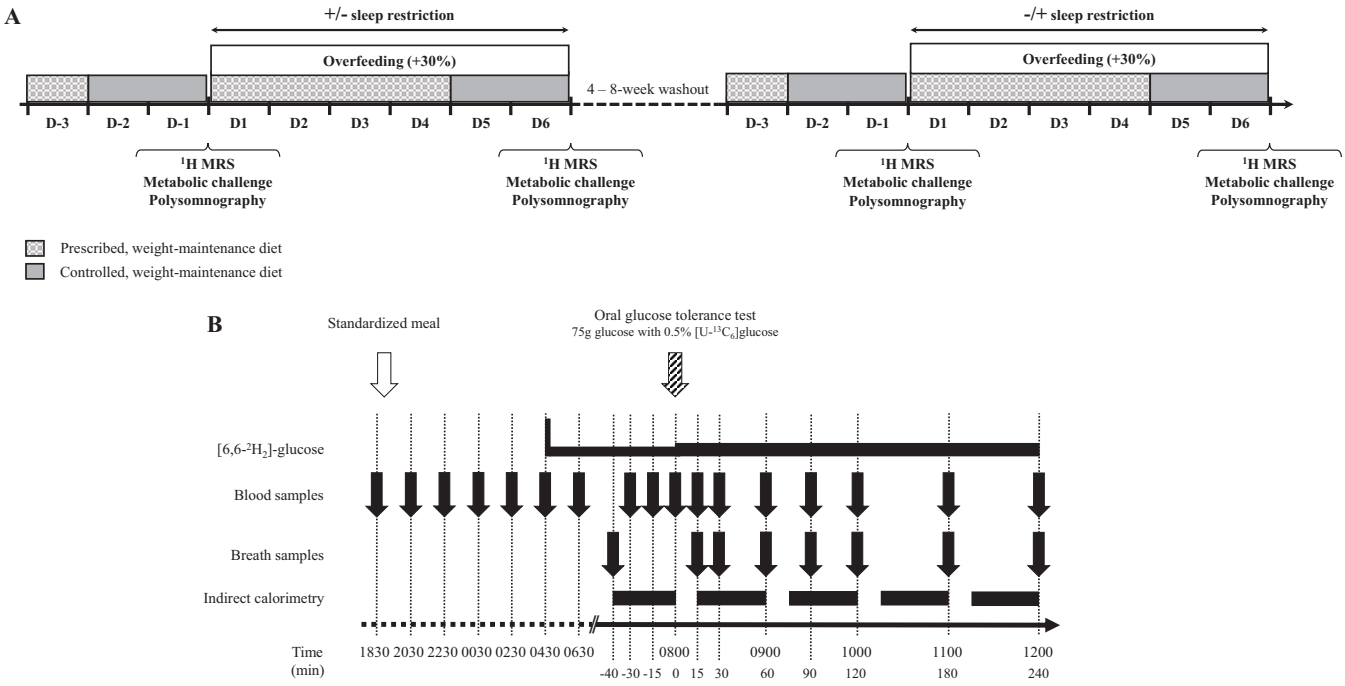

FIGURE 1 Experimental design of the study (A) and description of the metabolic challenge (B). After a 3-d weight-maintenance diet (i.e., D–3 to D–1), participants underwent a 6-d high-sucrose, high-fat overfeeding period (i.e., D1–D6; 130% of daily energy requirement), with either normal sleep (8 h SO time) or sleep restriction (4 h SO time), in a randomized, crossover design. At baseline and after both interventions, participants performed a quantification of IHCL concentrations by ^{1}H-MRS, a metabolic challenge, and a PSG (A). The metabolic challenge (B) included an overnight metabolic evaluation (i.e., 1830–0630), followed by a dual intravenous [6,6-$^{2}H_2$]-, oral ^{13}C-labeled glucose tolerance test (i.e., 0800–1200). Briefly, participants received at 1900 a standardized meal, covering 30% of their calculated daily energy requirement (white arrow). Blood samples were collected at 2-h intervals from 1830 to 0630 for the determination of night-time profiles of plasma metabolites and hormones (black arrows). The light was turned off from 2230 to 0630 (8 h SO time during the baseline evaluations and the overfeeding + normal sleep condition) or from 0200 to 0600 (4 h SO time) in the overfeeding + sleep restriction condition. At 0430, a primed (2 mg/kg), continuous (0.02 mg · kg^{-1} · min^{-1}) infusion of [6,6-$^{2}H_2$]-glucose was started. From 0720 to 0800, breath and blood samples were collected for the measurement of baseline breath $^{13}CO_2$ isotopic natural abundance, as well as fasting plasma substrate concentrations and isotopic enrichments. In parallel, respiratory exchange measurements (indirect calorimetry; black rectangles) were also performed for the assessment of resting energy expenditure and fasting substrate oxidation rates. At 0800, the perfusion rate of [6,6-$^{2}H_2$]-glucose was raised to 0.03 mg · kg^{-1} · min^{-1} and subjects ingested 75 g of glucose labeled with 0.5% [U-$^{13}C_6$]-glucose, dissolved in 300 mL water (striped arrow). The parameters of glucose metabolism were investigated over 4 h by the repeated collection of blood and breath samples (0815, 0830, 0900, 0930, 1000, 1100, and 1200), and by the continuous monitoring of respiratory gas exchanges. D, experimental day; IHCL, intrahepatocellular lipid; PSG, polysomnography; SO, sleep opportunity; ^{1}H-MRS, proton magnetic resonance spectroscopy.

providing a total of 30% extra energy, to regular meals. Each drink contained 7.5% energy requirement as sucrose (Hänseler AG) and 7.5% as fat (provided by a mixture of commercial products including fresh full cream, soy cream, and soy milk), and was consumed at snack times (i.e., at 1000 and 1500). The composition of the drinks was calculated for each volunteer by a research nutritionist, and was prepared by the investigators involved in the metabolic investigations. The overall composition of the overfeeding diet (solid diet + supplementary drinks) was 54% carbohydrate (35% as starch and 19% as sugar), 35% lipids, and 12% proteins (Table 1).

Intervention: sleep restriction

The overfeeding protocol was accompanied by either normal sleep (8 h of SO, from 2230 to 0630; the overfeeding + normal sleep condition) or sleep restriction (4 h of SO, from 0200 to 0600; the overfeeding + sleep restriction condition). More specifically, the first 2 experimental nights were spent at home. On the following 3 nights, subjects reported to the Center for Investigation and Research in Sleep, Lausanne University Hospital, where they slept under supervision. During both conditions, subjects had to refrain from any structured physical activity. Daytime naps were not allowed. Compliance with the prescribed sleeping pattern and physical activity was objectively controlled throughout both interventions by continuous sleep-wake cycle recording by wrist actigraphy (Actiwatch, Cambridge Technology).

Metabolic challenge

Description of the metabolic challenge is illustrated in Figure 1B. Subjects reported to the Clinical Research Center of Lausanne University Hospital at 1800. Upon arrival, volunteers were settled into a bed. A first catheter was then inserted into a vein of the right forearm for repeated collection of blood samples. The catheter was kept patent by continuous infusion of a saline solution. To achieve partial arterialization of venous blood, this hand was placed under a heating pad (Solis). A second catheter was inserted into a vein of the left forearm for infusion of [6,6-$^{2}H_2$]-glucose (Cambridge Isotope Laboratories). From 1830 to 0630, blood samples were collected at 2-h intervals for

TABLE 1
Nutrient content of the weight-maintenance and the high-sucrose, high-fat overfeeding diets[1]

	Weight-maintenance diet	Overfeeding diet
Energy, kcal	2333 ± 80	3033 ± 102
Carbohydrate, kcal	1282 ± 43	1629 ± 54
Starch,[2] kcal	1048 ± 36	1048 ± 36
Dietary sugar,[3] kcal	234 ± 7	234 ± 7
Sucrose,[4] kcal	—	347 ± 11
Lipids, kcal	700 ± 24	1053 ± 36
Dietary saturated fatty acids,[5] kcal	300 ± 12	300 ± 12
Dietary unsaturated fatty acids,[5] kcal	401 ± 13	401 ± 13
Added saturated fatty acids,[6] kcal	—	132 ± 4
Added unsaturated fatty acids,[6] kcal	—	220 ± 7
Protein,[7] kcal	350 ± 12	350 ± 12
Fiber,[8] g	36 ± 3	36 ± 3

[1]Data are expressed as means ± SEMs and are based on $n = 10$ volunteers.
[2]For example, bread, pasta, whole-grain rice cake, whole-grain cereals.
[3]For example, jam, fiber-rich fruit bar.
[4]Drinks.
[5]For example, olive oil, butter, cheese.
[6]Drinks: fresh full cream, soy cream, soy milk.
[7]For example, dried meat, cheese.
[8]For example, fiber-rich fruit bar, fiber crispy bread, whole-grain cereals.

the determination of night-time profiles of plasma metabolites and hormones. At 1900, participants received a standardized meal, covering 30% of their calculated daily energy requirement (containing 55% carbohydrates, 30% lipids, and 15% proteins). The light was progressively dimmed and then turned off from 2230 to 0630 (8 h of SO; baseline evaluations and the overfeeding + normal sleep condition) or from 0200 to 0600 (the overfeeding + sleep restriction condition). Free time before sleep was spent watching television, computer working, or talking with other research participants and staff. The subjects were asked to stop all activities 30 min prior to the scheduled bedtime. The investigators were present throughout each metabolic assessment to ensure that the protocol was properly followed. At 0430, a primed (2 mg/kg), continuous (0.02 mg · kg^{-1} · min^{-1}) infusion of [6,6-$^2H_2$]-glucose (10 mg/mL) was started. Upon awakening, volunteers were asked to void. They were then weighed (Seca 708, Seca GmbH) and their body composition was measured by bioelectrical impedance (Biacorpus RX 400, Medi Cal HealthCare GmbH). At 0720, 4 expired air samples were collected for the measurement of baseline breath $^{13}CO_2$ isotopic natural abundance. Three blood samples were then drawn at 0730, 0745, and 0800 for determination of fasting metabolites, hormones, [6,6-$^2H_2$]-glucose isotopic enrichment, and the natural ^{13}C abundance of plasma glucose. Resting energy expenditure and fasting substrate oxidation rates were assessed over 40 min from respiratory gas exchanges, monitored by open-circuit indirect calorimetry (Cosmed Quark RMR, Cosmed). Starting at 0800, a 4-h OGTT with dual glucose isotopes was performed to assess fasting and postprandial endogenous glucose production and the metabolic fate of exogenous glucose. Subjects ingested 75 g of glucose labeled with 0.5% [U-$^{13}C_6$]-glucose (Cambridge Isotope Laboratories), and dissolved in 300 mL water. To account for the increase in whole-body glucose turnover, the perfusion rate of [6,6-$^2H_2$]-glucose was raised to 0.03 mg · kg^{-1} · min^{-1} from 0800 until 1200. Blood and breath samples were collected at 15, 30, 60, 90, 120, 180, and 240 min following glucose ingestion. Throughout the 240-min glucose challenge period, respiratory gas exchanges were continuously monitored by indirect calorimetry. Urine was collected throughout the metabolic investigation to quantify the urea nitrogen excretion rate.

At the end of the metabolic evaluation, subjects received a standardized lunch (35% daily energy requirements, with 55% as carbohydrates, 30% lipids, and 15% proteins) and then transferred to Bern University Hospital by public transportation for the assessment of IHCLs by ^{1}H-MRS. All IHCL assessments were performed between 1500 and 1700, but at exactly the same time for the 2 visits of each subject. In the postintervention period, subjects received their last high-sucrose, high-fat drink 1 h before the measurement of IHCL concentrations.

^{1}H-MRS

IHCL concentration was determined by ^{1}H-MRS on a clinical 3-T MR system (TIM Trio, Siemens Medical) through the use of a method that closely matches the one described in the supplemental text of Bucher et al. (27). In short, magnetic resonance spectra from a region of interest of 25 × 25 × 30 mm^3 were localized with a short echo time-stimulated echo acquisition mode sequence (echo time 13 ms, middle time 15 ms, repetition time ~5 s depending on the respiratory rate) preceded by a navigator part (Siemens' 2D "prospective acquisition correction" module) that timed signal acquisition to occur in expiration based on the position of the diaphragm. Radiofrequency field strength was calibrated for the specific region of interest in expiration and optimization of field homogeneity was also performed in breath hold (28). Water-suppressed (24 acquisitions) and nonwater-suppressed (16 acquisitions) spectra were acquired with all acquisitions frequency aligned in postprocessing to

minimize effects of residual motion and thus improve linewidths. The medians of the water-suppressed and nonwater-suppressed spectra were fitted in FiTAID (29) with the use of prior knowledge constraints. The quantification of IHCLs was based on the total signal of water and lipids from the same region of interest. The transverse relaxation time T_2 of hepatic water needed for relaxation correction was determined from separate scans obtained in a single breath hold in expiration (5 spectra with echo times of 13, 20, 30, 50, and 75 ms, and a repetition time of 1500 ms). This relaxation time turned out to be independent of the intervention conditions and the same subject-specific T_2 value was used for all spectra from the same subject based on the median value determined from the 4 measurements in each subject. Absolute quantification of IHCLs was further based on assumptions on hepatic water content and fat composition, as described earlier (30).

Analytic procedures

Arterialized venous blood was collected on lithium heparin for measurement of isotopic enrichments and with EDTA-coated tubes for measurement of glucose, lactate, FFAs, TGs, insulin, and cortisol. After blood collection, samples were immediately centrifuged at 4°C for 10 min at 3600 × *g* to separate plasma from blood. Plasma aliquots were then stored at −20°C until analysis. Plasma glucose, lactate, FFA, TG, and urinary nitrogen concentrations were measured by enzymatic methods (RX Monza, Randox Laboratories Ltd), and plasma insulin and cortisol concentrations were measured by radioimmunoassay kits (Merck Millipore).

Breath $^{13}CO_2$ isotopic enrichments were determined by isotope-ratio mass spectrometry (SerCon Ltd, Crewe, United Kingdom). Plasma [6,6-$^2H_2$]- and [$^{13}C_6$]-glucose isotopic enrichments were measured by gas chromatography-mass spectrometry (Hewlett-Packard Instruments), in chemical ionization mode with selective monitoring of mass-to-charge ratios (*m/z*) 337, 333, and 331. For technical reasons, plasma samples for [6,6-$^2H_2$]-glucose isotopic enrichments were not available for 2 volunteers. For this reason, the parameters relating to glucose metabolism during the OGTT are based on $n = 8$ subjects [expected exogenous glucose oxidation ($n = 10$ subjects), which is independent from plasma [6,6-$^2H_2$]-glucose isotopic enrichments].

PSG

PSG was performed in order to rule out sleep disorders during the screening visit, and to investigate the redistribution of sleep architecture with sleep restriction. Subjects were equipped with a PSG recorder (Titanium, Embla Flaga) between 1700 and 2000 at the Center for Investigation and Research in Sleep, Lausanne University Hospital. The screening night took place in the patient's home environment, and sleep recordings during the experimental period took place in the sleep laboratory under continuous surveillance, following the American Academy of Sleep Medicine 2007 recommended setup specifications (31). Breathing was recorded with the use of nasal pressure sensors. A trained sleep technician scored the PSG recordings manually with the use of Somnologica software version 5.1.1 (Embla Flaga). Each recording was reviewed by an expert sleep physician (JH-R). Sleep stages and arousals were scored according to the American Academy of Sleep Medicine 2007 recommendations (31), and the total sleep time (TST), and the time spent in the different sleep stages [i.e., stage 1, stage 2, slow-wave sleep (SWS) stage and rapid-eye movement (REM) sleep] were obtained.

Calculations

The rates of net substrate oxidations and energy expenditure were evaluated from respiratory gas exchanges. The rates of appearance and disappearance of total glucose was calculated from plasma [6,6-$^2H_2$]-glucose isotopic enrichments according to Steele's equations for nonsteady-state conditions, as adapted by Debodo et al. (32). The metabolic clearance rate of glucose was calculated by dividing the rate of disappearance of total glucose by plasma glucose concentration. The systemic rates of exogenous [$^{13}C_6$]-glucose appearance and endogenous glucose production (EGP) were calculated as initially established in rats (33), and adapted for stable isotopes in humans (34). Exogenous glucose oxidation was estimated as previously described (34). The suppression of EGP was calculated by dividing the rate of EGP following the glucose load by its fasting value (i.e., time 0) and was used as an index of hepatic insulin sensitivity. Changes in metabolic responses were assessed by calculating the area under the curve according to the trapezoidal rule; this was performed over 720 min for the overnight assessment (i.e., 1830–0630), and over 240 min after glucose ingestion (i.e., 0800–1200).

Statistical analysis

The sequence of treatment allocation was determined by random generation of blocks with Microsoft Excel software (Microsoft Corp., Redmond, WA). The primary outcome was IHCL concentration. Previous data from our laboratory showed that a 6- to 7-d overfeeding diet can significantly increase the amounts of IHCLs (2), especially when fructose and fat are combined (4). By assuming the same effect size in IHCL accumulation after overfeeding, a sample size of 10 volunteers was required ($1 - \beta$: 90%; $\alpha = 0.05$). Prior to analysis, the normality and homoscedasticity of data distribution were inspected visually and checked by Shapiro-Wilk and Bartlett tests, respectively. When necessary, variables were normalized through the use of the Box-Cox transformation [^{1}H-MRS: IHCL ($\lambda = -0.34$); night-time metabolic parameters: TG ($\lambda = -0.59$), FFA ($\lambda = -0.26$), lactate ($\lambda = -0.95$), cortisol ($\lambda = -0.63$); fasting parameters: insulin ($\lambda = -1.99$), TG ($\lambda = -0.75$); OGTT: lactate ($\lambda = -1.27$), TG ($\lambda = -0.51$), suppression of EGP ($\lambda = 1.84$); PSG: stage 1 ($\lambda = 0.30$)]. Baselines were compared by Student's paired *t* tests (2-tailed). All baseline comparisons were similar ($P > 0.05$), except for resting heart rate ($P = 0.046$) and stage 2 sleep ($P = 0.016$). The significant effect of overfeeding ($P_{overfeeding}$), and its interaction with SO time ($P_{overfeeding \times sleep}$) were investigated by the use of mixed-model analysis, with overfeeding and sleep as fixed effects and subject-specific intercepts and slopes as random effects. Baseline values for resting heart rate and stage 2 sleep were incorporated into their respective models, because they were significantly different between both conditions ($P < 0.05$). Furthermore, the effects of

TABLE 2
Clinical characteristics after a 3-d weight-maintenance diet and after a 6-d high-sucrose, high-fat overfeeding period with normal sleep (8 h SO) or sleep restriction (4 h SO)[1]

					P	
	Weight maintenance	Overfeeding + normal sleep	Weight maintenance	Overfeeding + sleep restriction	Overfeeding	Overfeeding × sleep
Weight, kg	63.7 ± 2.5	64.4 ± 2.5	64.2 ± 2.4	64.7 ± 2.5	0.050	0.626
Fat mass, %	14.1 ± 1.7	13.6 ± 1.9	14.9 ± 1.5	14.4 ± 1.8	0.713	0.518
Systolic blood pressure, mm Hg	114 ± 3	115 ± 3	114 ± 3	114 ± 5	0.906	0.723
Diastolic blood pressure, mm Hg	70 ± 2	68 ± 3	66 ± 3	71 ± 4	0.454	0.113
Resting heart rate, beats/min	68 ± 3	74 ± 3	73 ± 4	70 ± 4	0.731	0.423

[1] Values are means ± SEMs. For all variables, $n = 10$ volunteers. The normality and homoscedasticity of the data distribution were inspected visually and checked by Shapiro-Wilk and Bartlett tests, respectively. No transformations were necessary for statistical analyses. The significant effect of overfeeding ($P_{overfeeding}$), and its interaction with SO time ($P_{overfeeding \times sleep}$), were investigated by the use of mixed models. $P < 0.05$ was considered as statistically significant. SO, sleep opportunity.

sex, order body weight change, and washout period were initially incorporated into each model, and were removed as soon as they did not improve the goodness of fit. Post-hoc paired *t* tests (2-tailed) were performed when $P_{overfeeding \times sleep}$ values were found to be significant. Statistical analyses were performed with R software version 3.3.1 (R Foundation for Statistical Computing, Vienna, Austria). All values are expressed as means ± SEMs. Differences were considered as significant when *P* values were <0.05.

RESULTS

The experimental protocol was carried out between March 2014 and December 2015. All participants declared having carefully followed the dietary and sleep instructions, as specified in the protocol. Sleep compliance was confirmed by actigraphy data analysis, with 367 ± 29 min as the mean daily rest time with normal sleep, and 220 ± 5 min with sleep restriction (Student's paired *t* test, $P = 0.001$).

Clinical characteristics

Body weight, body fat mass, blood pressure, and resting heart rate during the weight-maintenance diet and after both interventions are presented in **Table 2**. Overall, body weight was significantly increased by 0.6 ± 0.1 kg after the 6-d overfeeding intervention. However, body composition, blood pressure, and resting heart rate did not change significantly (all P = NS).

IHCL concentrations

Overfeeding caused a marked increase in IHCL concentrations (**Figure 2**). However, sleep restriction did not exacerbate the

TABLE 3
Impact of a 6-d high-sucrose, high-fat overfeeding period with normal sleep (8 h SO) or sleep restriction (4 h SO) on the parameters of glucose metabolism following the ingestion of a 75-g oral glucose load[1]

					P	
	Weight maintenance	Overfeeding + normal sleep	Weight maintenance	Overfeeding + sleep restriction	Overfeeding	Overfeeding × Sleep
Total glucose appearance (g over 240 min, $n = 8$)	69.6 ± 3.6	75.2 ± 3.8	69.6 ± 2.6	72.0 ± 2.6	0.003	0.191
Exogenous glucose appearance (g over 240 min, $n = 8$)	58.8 ± 3.7	60.3 ± 3.5	58.6 ± 2.1	58.7 ± 1.8	0.689	0.693
EGP (g over 240 min, $n = 8$)	10.8 ± 0.9	14.9 ± 2.5	10.9 ± 1.1	13.3 ± 1.0	0.034	0.567
Suppression of EGP (%, $n = 8$)	−75 ± 2	−70 ± 4	−74 ± 2	−69 ± 3	0.006	0.793
Total glucose disappearance (g over 240 min, $n = 8$)	67.0 ± 3.7	72.0 ± 4.2	68.1 ± 2.7	72.2 ± 2.5	0.012	0.789
Exogenous glucose oxidation (g over 240, $n = 10$)	24.3 ± 0.6	26.4 ± 0.9	24.9 ± 1.0	25.2 ± 0.8	0.038	0.118
Glucose clearance (L/min, $n = 8$)	0.29 ± 0.03	0.29 ± 0.02	0.26 ± 0.02	0.32 ± 0.04	0.144	0.188

[1] Values are means ± SEMs. For technical reasons, plasma samples for [6,6-$^2H_2$]-glucose isotopic enrichments were not available for 2 volunteers. For this reason, the parameters relating to glucose metabolism during the oral-glucose-tolerance test are based on $n = 8$ subjects [expected exogenous glucose oxidation ($n = 10$ subjects), which is independent from plasma [6,6-$^2H_2$]-glucose isotopic enrichments]. The normality and homoscedasticity of data distribution were inspected visually and checked by Shapiro-Wilk and Bartlett tests, respectively. For statistical analyses, the Box-Cox transformation was applied to the suppression of EGP ($\lambda = 1.84$). The significant effect of overfeeding ($P_{overfeeding}$), and its interaction with SO time ($P_{overfeeding \times sleep}$) were investigated by the use of mixed models. $P < 0.05$ was considered as statistically significant. EGP, endogenous glucose production;SO, sleep opportunity.

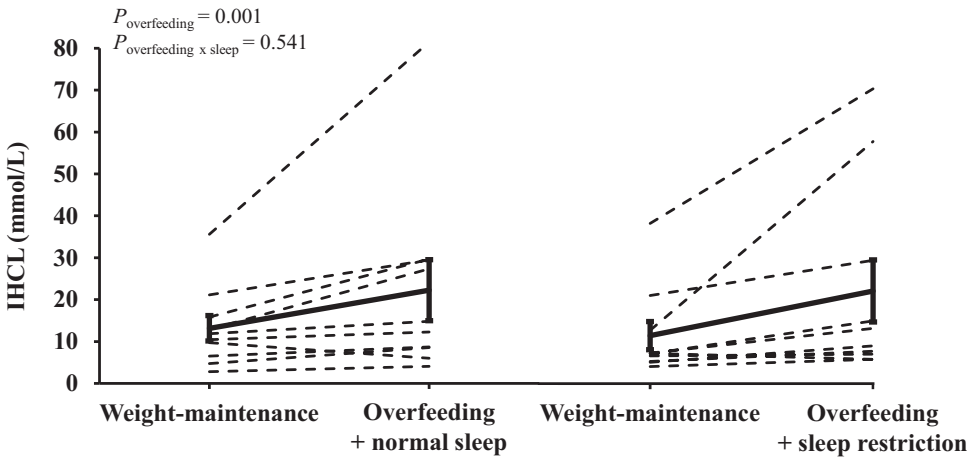

FIGURE 2 Individual (dashed lines) and mean ± SEM (solid lines) changes in IHCL concentrations after a 6-d high-sucrose, high-fat overfeeding period with normal sleep (8 h SO) or sleep restriction (4 h SO). For each condition, $n = 10$ volunteers. The normality and homoscedasticity of data distribution were inspected visually and checked by Shapiro-Wilk and Bartlett tests, respectively. For statistical analysis, IHCL concentration was transformed by the use of the Box-Cox transformation ($\lambda = -0.34$). The significant effect of overfeeding ($P_{overfeeding}$) and its interaction with SO time ($P_{overfeeding \times sleep}$) were investigated by the use of a mixed model. $P < 0.05$ was considered as statistically significant. IHCL, intrahepatocellular lipid; SO, sleep opportunity.

increased IHCL concentrations induced by overfeeding. Compared with the weight-maintenance diet, IHCL concentrations were significantly increased by 53% ± 16% in overfeeding + normal sleep (from 13.2 ± 3.0 to 22.2 ± 7.3 mmol/L) and by 83% ± 33% in overfeeding + sleep restriction (from 11.2 ± 3.3 to 21.6 ± 7.2 mmol/L; $P_{overfeeding} < 0.001$; $P_{overfeeding \times sleep} = 0.541$). Results were not altered when the statistical analysis was redone after removing one outlier ($P_{overfeeding} = 0.003$; $P_{overfeeding \times sleep} = 0.503$).

Night-time metabolic parameters

Night-time profiles of plasma insulin, lactate, FFA, TG, and cortisol concentrations are illustrated in **Figure 3**. Overfeeding did not change plasma insulin concentrations (Figure 3A), but significantly increased plasma lactate concentrations (Figure 3B). Plasma FFA concentrations increased modestly after sleep onset (Figure 3C). Compared with the weight-maintenance diet, the increase in plasma FFA concentrations was significantly blunted in overfeeding + normal sleep, but was preserved in overfeeding + sleep restriction. Plasma TG concentrations showed no major change throughout the night, and were not altered by overfeeding + normal sleep (Figure 3D). However, plasma TG concentrations were significantly decreased after overfeeding + sleep restriction. Plasma cortisol concentrations slightly decreased over time, reaching their nadir around 0030, and then exponentially increased until the end of the night (Figure 3E). Sleep restriction did not alter plasma cortisol concentrations.

Fasting parameters

Fasting parameters at 0800 (i.e., the last time point before OGTT) are given in **Supplemental Table 1.** Overfeeding significantly increased fasting plasma insulin and lactate concentrations, and significantly decreased fasting plasma FFA concentrations. It did not alter fasting plasma glucose and TG concentrations, or endogenous glucose production. Basal energy expenditure and lipid oxidation rates were not changed, but basal carbohydrate oxidation rates were significantly higher after overfeeding. None of the fasting parameters were differently altered with sleep restriction (all $P_{overfeeding \times sleep}$ = NS).

OGTT

The kinetics of plasma glucose, insulin, lactate, FFA, and TG concentrations during the OGTT are illustrated in **Figure 4**. After the ingestion of the glucose load, plasma glucose, insulin, and lactate concentrations peaked (Figure 4A–C) within the first hour, whereas plasma FFA concentrations were markedly suppressed (Figure 4D). Plasma TG concentrations remained stable throughout the test (Figure 4E). Overfeeding significantly increased plasma lactate concentrations during the OGTT and significantly decreased plasma glucose and FFA concentrations. However, it did not change plasma insulin and TG concentrations. Overfeeding significantly increased net carbohydrate oxidation after the glucose load (overfeeding + normal sleep, from 35.2 ± 2.2 to 40.7 ± 2.4 g; overfeeding + sleep restriction, from 37.3 ± 1.6 to 41.3 ± 1.9 g over 240 min; $P_{overfeeding} = 0.002$; data not shown), whereas no change was observed in both net lipid oxidation (overfeeding + normal sleep, from 9.0 ± 1.2 to 7.9 ± 0.8 g; overfeeding + sleep restriction, from 8.3 ± 1.0 to 8.7 ± 0.5 g over 240 min; $P_{overfeeding} = 0.618$; data not shown) and energy expenditure (overfeeding + normal sleep, from 271 ± 10 to 274 ± 11 kcal; overfeeding + sleep restriction, from 270 ± 10 to 275 ± 11 kcal over 240 min; $P_{overfeeding} = 0.173$; data not shown).

The parameters related to glucose metabolism in response to the ^{13}C-labeled glucose challenge are depicted in **Table 3**. Overall, overfeeding significantly increased total glucose appearance. No change was observed on exogenous glucose appearance after overfeeding, whereas EGP was significantly increased. Moreover, the suppression of EGP was significantly altered by overfeeding, suggesting impaired hepatic insulin

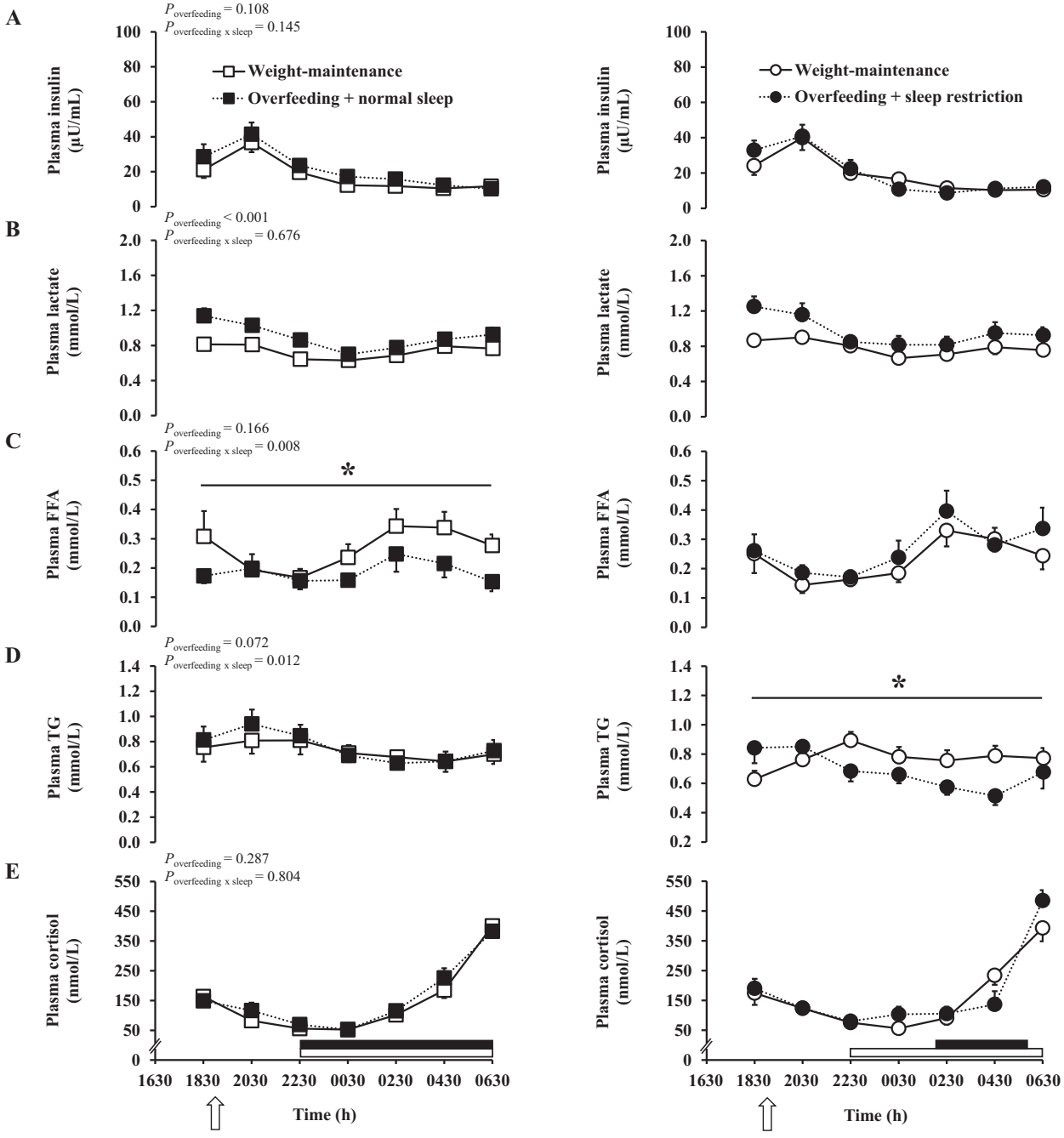

FIGURE 3 Mean ± SEM changes in night-time plasma insulin (A), lactate (B), FFA (C), TG (D), and cortisol (E) concentrations after a 6-d high-sucrose, high-fat overfeeding period with normal sleep (8 h SO; left panels) or sleep restriction (4 h SO; right panels). For all variables, $n = 10$ volunteers. The normality and homoscedasticity of data distribution were inspected visually and checked by Shapiro-Wilk and Bartlett tests, respectively. For statistical analyses, the Box-Cox transformation was applied to night-time plasma TG ($\lambda = -0.59$), FFA ($\lambda = -0.26$), lactate ($\lambda = -0.95$), and cortisol ($\lambda = -0.63$) concentrations. The significant effect of overfeeding ($P_{overfeeding}$) and its interaction with SO time ($P_{overfeeding \times sleep}$) were investigated by the use of mixed models. Post-hoc paired *t* tests (2-tailed) were performed when $P_{overfeeding \times sleep}$ were <0.05 (i.e., night-time plasma FFA and TG concentrations). Compared with the weight-maintenance diet, plasma FFA concentrations were significantly blunted in overfeeding + normal sleep ($P = 0.017$) but were preserved in overfeeding + sleep restriction ($P = 0.209$). Plasma TG concentrations were not altered by overfeeding + normal sleep ($P = 0.332$) but were significantly decreased after overfeeding + sleep restriction ($P = 0.011$). $^{*}P < 0.05$ compared with the weight-maintenance diet. White arrow: ingestion of a standardized meal at 1900; white rectangles: SO time at baseline (8 h, from 2230 to 0630); black rectangles: SO time during overfeeding + normal sleep (8 h, from 2230 to 0630; left panels) or overfeeding + sleep restriction (4 h, from 0200 to 0600; right panels). FFA, free-fatty acid; SO, sleep opportunity; TG, triglyceride.

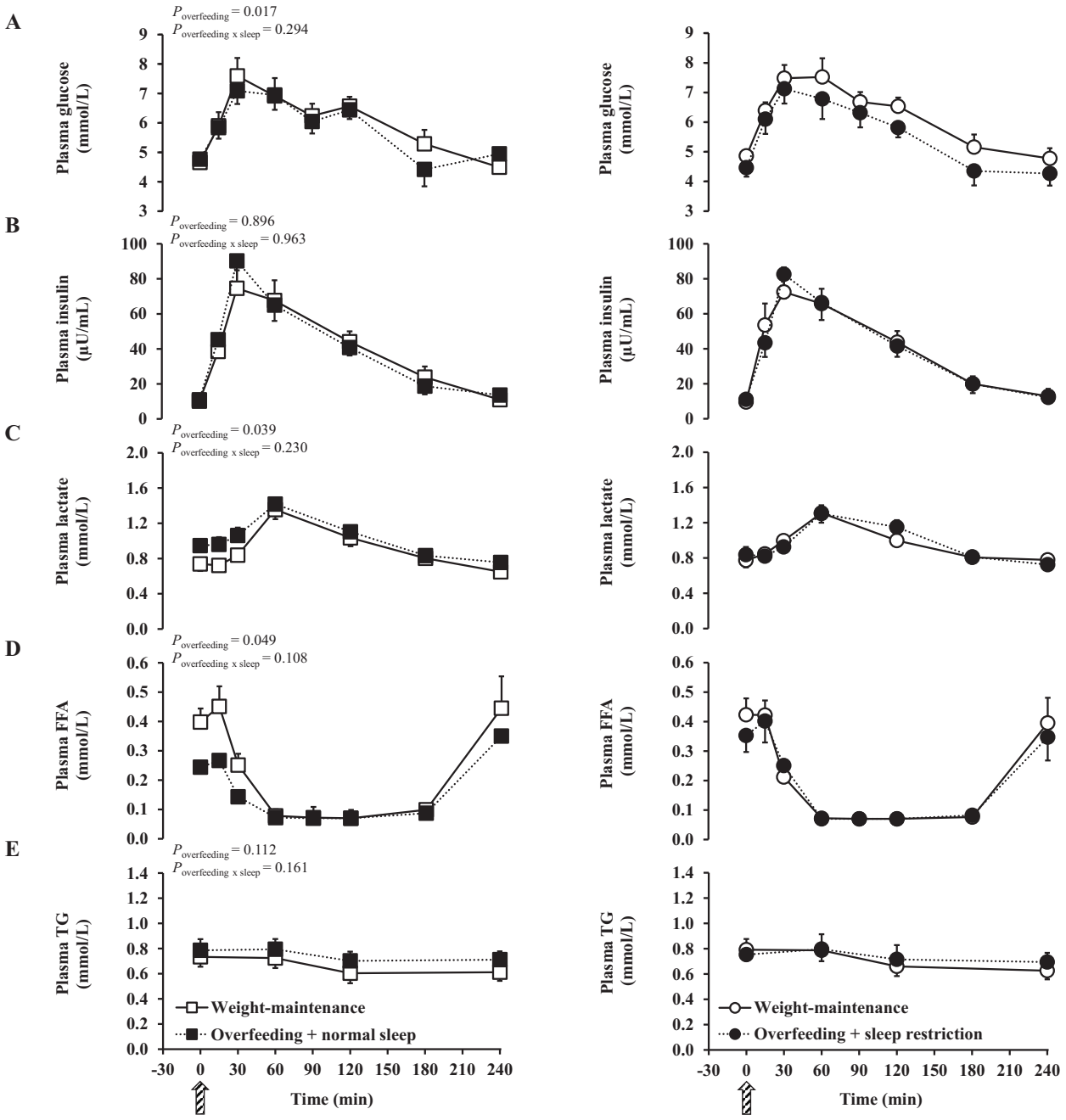

FIGURE 4 Mean ± SEM changes in plasma glucose (A), insulin (B), lactate (C), FFA (D), and TG (E) concentrations during a 75-g oral-glucose-tolerance test after a 6-d high-sucrose, high-fat overfeeding period with normal sleep (8 h SO; left panels) or sleep restriction (4 h SO; right panels). For all variables, $n = 10$ volunteers. The normality and homoscedasticity of data distribution were inspected visually and checked by Shapiro-Wilk and Bartlett tests, respectively. For statistical analyses, the Box-Cox transformation was applied to plasma lactate ($\lambda = -1.27$) and TG ($\lambda = -0.51$) concentrations. The significant effect of overfeeding ($P_{overfeeding}$) and its interaction with SO time ($P_{overfeeding \times sleep}$) were investigated by the use of mixed models. $P < 0.05$ was considered as statistically significant. Striped arrow: ingestion of 75 g of glucose labelled with 0.5% [U-$^{13}C_6$]-glucose, and dissolved in 300 mL water. FFA, free-fatty acid; SO, sleep opportunity; TG, triglyceride.

sensitivity. Overfeeding significantly increased total glucose disappearance and exogenous glucose oxidation, but did not change glucose clearance. All the parameters evaluated during OGTT were not significantly changed by sleep restriction (all $P_{overfeeding \times sleep}$ = NS).

Polysomnographic recording

TST and stage 1, stage 2, SWS and REM sleep are shown in **Figure 5**. After overfeeding + normal sleep, no change was observed regarding TST, and time spent in each of the sleep stages (all P = NS). In contrast, TST was significantly reduced with

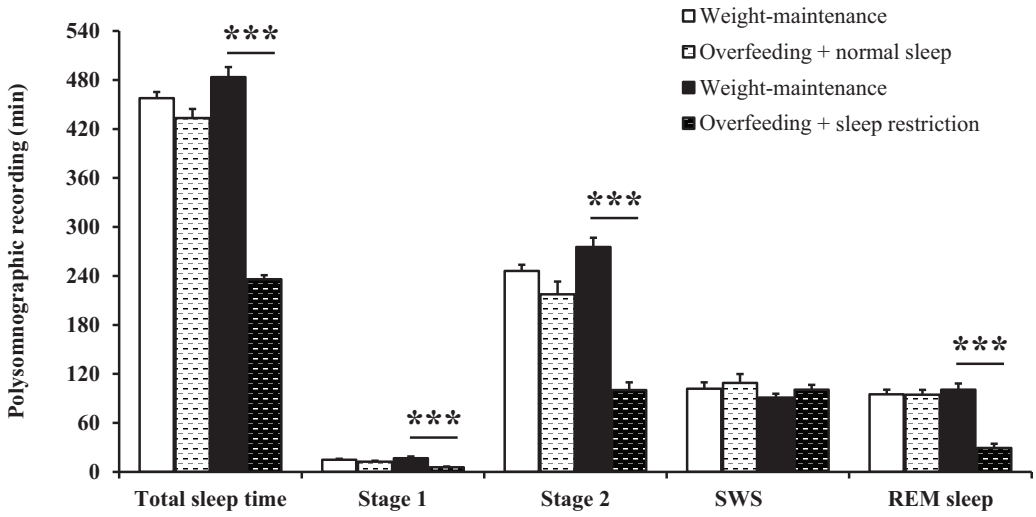

FIGURE 5 Mean ± SEM changes in total sleep time and sleep stage durations after a 6-d high-sucrose, high-fat overfeeding period with normal sleep (8 h SO) or sleep restriction (4 h SO). For all variables, $n = 10$ volunteers. The normality and homoscedasticity of data distribution were inspected visually and checked by Shapiro-Wilk and Bartlett tests, respectively. For statistical analyses, the Box-Cox transformation was applied to stage 1 ($\lambda = 0.30$). The significant effect of overfeeding ($P_{overfeeding}$) and its interaction with SO time ($P_{overfeeding \times sleep}$) were investigated by the use of mixed models. Post-hoc paired *t* tests (2-tailed) were performed when $P_{overfeeding \times sleep} < 0.05$ (i.e., total sleep time, stage 1, stage 2, and REM sleep, all $P < 0.001$; SWS, $P = 0.809$). $^{***}P < 0.001$, post-hoc paired *t* tests (2-tailed). REM, rapid eye movement; SO, sleep opportunity; SWS, slow-wave sleep.

overfeeding + sleep restriction. It was associated with reduced time spent in stage 1, stage 2, and REM sleep, whereas time spent in SWS remained unaffected ($P_{overfeeding \times sleep} = 0.809$).

DISCUSSION

The purpose of this study was to investigate whether sleep restriction would exacerbate the adverse metabolic effects induced by overfeeding. To test this hypothesis, we exposed healthy volunteers to either 8 or 4 h of SO time during a 6-d high-sucrose, high-fat overfeeding. IHCL concentration was selected as a primary outcome because it is considered to be an early and sensitive marker of metabolic dysfunction (24). As expected, our results showed that overfeeding significantly increased IHCL concentration. However, this effect of overfeeding was not significantly altered by sleep restriction.

Metabolic effects of overfeeding

Our short-term mixed overfeeding significantly increased IHCL concentration by +53% ± 16% compared with the baseline weight-maintenance diet. This is consistent with other studies that report significant IHCL concentration increases with 30% excess energy as fructose (2, 4) or fat (2, 35). It was, however, not associated with an increase in fasting plasma TG concentrations as observed with fructose overfeeding (36). This may be due to the fact that the ~45-g total daily fructose intake used in our overfeeding protocol remained below the threshold 50-g/d value above which postprandial hypertriglyceridemia occurs (37). In addition, fructose and fat overfeeding may have additive effects on IHCL concentration, but opposite effects on fasting plasma TG concentration (4), which may account for divergent net effects of our mixed overfeeding on IHCL and plasma TG concentrations. Overfeeding also significantly increased fasting insulin concentrations and EGP during an OGTT, indicating that hepatic insulin sensitivity was impaired. This result is in accordance with several reports documenting the occurrence of impaired hepatic insulin sensitivity early after exposure to hypercaloric, high-fructose diets (2, 36, 38). Overfeeding also significantly decreased fasting FFA concentrations, as previously reported after short-term hypercaloric diets enriched with simple sugars (3) and fat (39). These effects are concomitant with increased adipose expression of key genes involved in fatty acid synthesis [e.g., sterol regulatory element binding protein-1c (*SREBP-1c*), fatty acid synthase (*FAS*), acetyl-CoA carboxylase (*ACC*)] and TG synthesis [e.g., long-chain acyl-CoA synthetase (*ACSL*), diacylglycerol acyltransferase (*DGAT2*)], whereas genes involved in lipolysis and lipid oxidation pathways remained either unchanged (35, 40, 41) or decreased over time (39). In addition, overfeeding slightly decreased plasma glucose responses and increased exogenous glucose oxidation during OGTT. This occurred independently of any change in plasma insulin, and most likely reflects an upregulation of genes involved in glucose transport and in modifying intracellular glucose metabolism (40, 42).

Effects of sleep restriction on metabolic responses to overfeeding

Sleep restriction did not significantly alter the effects of overfeeding on IHCL and diurnal plasma TG concentrations. It also did not alter the effects of overfeeding on fasting and post-prandial EGP, or on plasma glucose and insulin concentrations. These observations strongly argue against our hypothesis that sleep restriction would increase the cardiometabolic risk associated with a short-term overfeeding. This appears at odds with other studies that have demonstrated that sleep restriction impaired glucose tolerance (7, 8) or decreased whole-body

insulin sensitivity (8, 9). The maintenance of the SWS duration in our sleep-restriction protocol may have contributed to the preservation of glucose homeostasis. Experimental studies have indeed demonstrated that glucose tolerance and insulin sensitivity can be drastically impaired by a selective suppression of SWS stage by acoustic stimuli, independently of any change in total sleep time (43, 44). In contradiction with this explanation, however, the aforementioned studies reported that sleep restriction impaired glucose tolerance or decreased whole-body insulin sensitivity, and sometimes caused both effects independently of any change in SWS (7–9). Another explanation may be that our experimental protocol involved an overfeeding, whereas most studies used weight-maintenance diets. One other study performed in subjects during a period of positive energy balance reported that sleep restriction induced a modest 5% increase in the area under the glucose curve in response to an OGTT, but no increase in insulin responses (45). Chronic overconsumption of carbohydrate or fat is associated with an upregulation of many pathways involved in glucose and fat utilization in insulin-sensitive cells, and it is therefore possible that the adverse effects of sleep restriction on glucose tolerance were hidden by the more powerful effects of overfeeding to promote energy substrate use. Discrepancies may also be related to differences in the methods used to assess glucose homeostasis (e.g., OGTT compared with clamp studies).

We had hypothesized that sleep restriction would act as a stressor, and hence expected to observe signs of an activation of the hypothalamic-pituitary-adrenal axis and of the SNS. We observed, however, no change in night-time cortisol concentrations after sleep restriction. Although we did not measure plasma catecholamine concentrations, no change in heart rate and blood pressure were observed. Therefore, our data do not provide any direct or indirect evidence that sleep restriction elicited substantial stress responses. One study that used a similar protocol had already reached the same conclusions (46), and other studies have succeeded in documenting significant stress responses (7–9, 16). The reasons for these discrepancies remain unclear, but may possibly be related to differences in the protocols used for sleep restriction or the methods used to assess stress responses.

In this study, sleep restriction induced a significant decrease in night-time TG concentration and blunted the decrease in night-time FFA concentrations. An increase in night-time FFA concentrations after sleep restriction had already been reported by others (16). The mechanism responsible for this effect cannot be identified in our present data. It is possible that sleep restriction elicited low-grade stress responses, or stress responses specifically targeted at adipose tissue, which eluded our crude evaluation based on heart rate, blood pressure, and plasma cortisol concentrations. Alternatively, the maintenance of night-time FFA concentrations may merely be due to the higher night-time metabolic rate associated with sleep restriction. An elevation of 24-h energy expenditure was indeed reported during sleep-restriction periods, in part due to the longer time spent awake (20). Whatever the explanation, the maintenance of night-time FFA release was not associated with a significant increase in IHCL concentration.

Limitations

Our protocol presents several limitations that need to be mentioned. First, we investigated the effects of sleep restriction associated with an overfeeding intervention. However, we did not assess the effects of sleep restriction alone (i.e., during a weight-maintenance diet). Second, the duration of overfeeding was short, and it is possible that a longer duration would have been required to elicit a significant stress response and cause whole-body insulin resistance. Third, our protocol was performed under free-living conditions, and we cannot exclude that the effects of sleep restriction may have been mitigated by changes in daily physical activity and energy expenditure (20). Fourth, we did not measure visceral fat mass, which is an important determinant of cardiometabolic risk. Finally, this protocol was performed in healthy volunteers, and our results cannot be extrapolated to subjects with sleep disorders, such as sleep apnoea syndrome, which is associated with reduced SWS and recurrent episodes of intermittent hypoxia, and may stimulate stress responses (47).

Conclusions

Our findings indicate that a short-term, high-sucrose, high-fat overfeeding significantly increased IHCL concentration and impaired hepatic insulin sensitivity, whereas glucose tolerance remained unaffected. These effects of overfeeding were not, however, potentiated by sleep restriction.

The authors' contributions were as follows—VL, LT, LE, JH-R, and RH: designed the study; VL: generated the random generation of blocks; JC and VL: assigned subjects to interventions; BP, RK, and CB: performed MR scanner measurements, IHCL quantification, and interpretations; JC, VL, EP, RR, LE, and PS: recruited subjects and performed metabolic tests; FC, JH-R, and RH: performed actigraphy and polysomnographic recordings and interpretations; JC and RR: analyzed the data and performed statistical analysis; JC, LT, and VL: wrote the manuscript; VL: had primary responsibility for the final content; and all authors: read, commented on, and approved the manuscript. LE is currently an employee of Nestec SA. RH is member of the medical advisory board of NightBalance, and received personal fees from this company. LT has previously received research support from Soremartec Italia, Srl for research unrelated to this article, speaker fees from Nestlé AG, Switzerland, and the Gatorade Sport Science Institute, and consultant fees from Takeda Pharma. None of the other authors reported conflicts of interest related to the study.

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