Is the timing of caloric intake associated with variation in diet-induced thermogenesis and in the metabolic pattern? A randomized cross-over study

This is an author version of the contribution published:

Questa è la versione dell autore dell opera:

[Simona Bo, Maurizio Fadda, Andrea Castiglione, Giovannino Ciccone, Antonella De Francesco, Debora Fedele, Alice Guggino, Mirko Parasiliti Caprino, Sara Ferrara, Marina Vezio Boggio, Giulio Mengozzi, Ezio Ghigo, Mauro Maccario, Fabio Broglio. Is the Timing of Caloric Intake Associated with Variation in Diet-Induced Thermogenesis and in the Metabolic Pattern? A Randomized Cross-Over Study. Int J Obesity 2015; Dec;39(12):1689-95. doi: 10.1038/ijo.2015.138.

]

The definitive version is available at:

La versione definitiva è disponibile alla UR

[http://www.ncbi.nlm.nih.gov/]pubmed/2621941

Is the Timing of Caloric Intake Associated with Variation in Diet-Induced Thermogenesis and in the Metabolic Pattern? A Randomized Cross-Over Study.

Simona Bo[1*], Maurizio Fadda[2], Andrea Castiglione[3], Giovannino Ciccone[3], Antonella De Francesco[2], Daniela Fedele[1], Alice Guggino[1], Mirko Parasiliti Caprino[1], Stefania Ferrara[1], Marco Vezio Boggio[1], Giulio Mengozzi[4], Ezio Ghigo[1], Mauro Maccario[1], Fabio Broglio[1]

[1]Department of Medical Sciences, University of Turin, Turin, Italy

[2]Unit of Clinical Nutrition, Città della Salute e della Scienza Hospital of Turin, Turin

[3]Unit of Clinical Epidemiology, CPO, Città della Salute e della Scienza Hosp

[4]Clinical Biochemistry Laboratory, Città della Salute e della Scienza Turin, Italy

*Corresponding author: Simona Bo, Department of Medical Sciences, University of Tu

10126 Turin, Italy; Telephone +(39)(011)6336036; Email simona.bo@unito.it; Fax +(39)(011)6335

Running title: timing of caloric intake, thermogenesis and metabolism

Word Count: abstract 295, text 3885

Conflicts of Interest: The authors report no conflict of interest.

Financial support: This study was supported by a grant from the Ministry of Education, University and of Italy (60% ex 2014)

Abstract

Background/Objectives: Diet-induced thermogenesis is generally reported to be higher in the morning, although contrasting results exist due to different experimental settings related to preceding fasting, exercise, sleep, and dieting. A definitive answer to this issue is warranted. We compared the calorimetric metabolic responses to identical meals consumed at 8:00 am and at 8:00 pm by volunteers, after standardized diet, physical activity, and duration of fasting. Subjects/Methods: 20 subjects (age range 20-35 years, BMI 19-25 kg/m²) were enrolled in a randomized crossover trial. The only meal received was the same standard meal in the morning, and 7 days after, in the evening or vice versa. A 30-min basal calorimetry was performed; a 60-min calorimetry was done after 120 min from the beginning of the meal. Blood samples were drawn every 30 min for 180 min. General linear models (GLM), adjusted for period-carryover, were used to evaluate the morning effect, i.e. the difference of morning (meal minus fasting values) minus evening (meal minus fasting) delta values. Results: Resting Metabolic Rate (RMR) did not change from morning to evening; RMR delta-values were significantly higher after the morning meal (16; 95% CI [illegible] vs [illegible] kcal; p<0.001). RMR was significantly increased after the morning meal (90.5; 95% CI 40.4, 140.6 kcal; p<0.001), while differences in the area under the curve for glucose (-180; -256, -103 mg/dl×h, p<0.001), insulin (-1.9; -3.0, -0.7 µU/ml×h; p=0.001) and fatty free acid concentrations (-16; -30, -2.0 mmol/l×h; p=0.024) were significantly lower. Delayed and larger increases in glucose and insulin concentrations were found after the evening meals.
Conclusions: The same meal consumed in the evening determined a lower RMR and increased glycemic/insulinemic responses, suggesting a circadian variation in energy expenditure and metabolic pattern in healthy individuals. The timing of meals should probably be considered when nutritional recommendations are given.

ClinicalTrials.gov Identifier NCT02343380

Introduction

An increasing number of studies have shown that the timing of food intake influences en risk of weight gain and obesity, independent of total daily caloric intake, dietary composition and es energy expenditure [1].

Early insulin secretion after meal ingestion is significantly higher in the morning than in th to a more rapid glucose clearance [2], whereas insulin sensitivity decreases later during the day [3, 4]. Circadian variations in the concentrations of hormones and peptides regulating appetite, as well as genetic variants of the circadian clock genes have been implicated in the specific modificat metabolic pathways [1]. Additionally, gastric emptying seems to be more rapid in the morning than in the evening [5], and an increased efficacy of dietary carbohydrates absorption has be supported in suppertime conditions [6].

A few studies evaluated the circadian variation of the energy balance [7-12]. The thermic effect of food, also called diet-induced thermogenesis (DIT), is defined as the i metabolic rate (RMR) after the ingestion of a meal. This component ac energy expenditure (about 10%), but has been reported to be involved in the development and persistence of obesity [13]. DIT is significantly higher after the consumption of a morning meal than after the same snack at night [7], and a reduced evening response may contribute to insulin resistance [8]. Furthermore, habitual nighttime eating or snacking have been associat potentially promoting weight gain [14].

However, data in the literature show contrasting results [9-12], with differences in the level of exercise le hours of sleeping, meal time, interval between the meals, duration of fasting, presence of comorbidity, low number of the subjects enrolled and likely confounding [15]. Indeed, the ideal setting to study diurnal variation in energy expenditure is the postabsorptive RMR under the same conditions a different times. The aim of our study was therefore to compare the metabolic

responses to identical meals (high protein, low carbohydrate) consumed in the morning (8:00 am) and in the evening (8:00 pm) by healthy volunteers, after standardizing diet, physical activity and resting.

Subjects and Methods

Recruitment of participants

Twenty healthy volunteers (ten males and ten females) were enrolled among students and graduates of the Department of Medical Sciences, Turin (Italy).

Inclusion criteria were: age 20-35 years, body mass index (BMI) 19-26 kg/m^2, moderate exercise level, smoking <10 cigarettes/day. Exclusion criteria were: any acute or chronic disease, menopause, drugs or supplementary regimen, any alimentary restrictions or specific diets, night workers, unable to give a written informed consent.

Design

This was a randomized crossover clinical trial.

Outcomes

The primary outcome was evaluating changes in RMR after the morning meal consumption compared to changes in RMR after the evening meal. The secondary outcome was evaluating changes in circulating concentrations of glucose, insulin, free fatty acids (FFA) and triglycerides after morning and evening meal consumption.

Intervention

Participants were randomly allocated to receive a standard meal at 8:00 am and the same the week after the standard meal at 8:00 pm, or vice versa. Eight hours before the meal (respectively at 12:00 pm or 12:00 am), participants received the same standard meal without protein supplementation (at their home), and then asked to fast for the following 6-h. Drinking coffee, alcohol, and smoking were not allowed

permitted. The week preceding each test, participants were instructed not to change their diet and to refrain from heavy physical activity. A 24-hour urine collection was collected the day before to determine urinary nitrogen excretion.

The standard meal consisted of: 100g white bread, 100g ham, 50g cheese, 125g yog[urt], 25g protein supplement (Resource Instant Protein, Nestlé, Switzerland). The composition of the meal was 30% protein, 31% carbohydrates, 39% fat, total kcal 1168. Participants had to consume each meal in 20-30 min. Participants were taken by car to the laboratory at 7:00 am (or pm, according to randomization), then underwent the anthropometric measurements and the insertion of a 16G indwelling catheter into an antecubital vein of the forearm, subsequen[tly] 500 ml of saline solution until the end of the testing sessions. In order, the blood samples have been withdrawn from an extension line tubing.

A 30-min basal calorimetric exam was performed (Figure 1). Participants remained in a supine position awake and motionless on the hospital bed for the whole experiment, except during the meal, when they could eat and were allowed to sit. At 8:00 am (or pm), the participants consumed the meal, then returned in the supine position for 90 min, followed by a second 60-min calorimetric evaluation. In order to obtain a compliance to the experiment, the second calorimetric evaluation was from 120 to 180 min from the beginning of the meal. In fact, we have previously performed a pilot study to evaluate the tolerance to the calorimetric exam, and have found that maintaining immobility while more than 1 hour consecutive.

Blood samples were drawn every 30 min from the first calorimetry (basal) until the end of the se[cond] (post-prandial) (Figure 1). Time 0 was after the first calorimetry and before the meal. Time 150 and 180 referred to the time intervals from the beginning of the meal. The same time schedule was adopted in the case of the morning meal (at 8:00 am) and the evening meal (at 8:00 pm). The second test was carried out after 7 days from the first.

Sample size

A sample size of 20 subjects (10 in the morning-first group and 10 in the evening-first group) was required to test an effect size of 0.66 with a power of 80% and a two-tailed α value = 0.05.

Randomization

The random sequence (morning/evening or evening/morning meal) was generated, using blocks of different lengths in random order.

Measurements

The Minnesota Leisure Time Physical Activity questionnaire, previously validated in a European cohort [17], was completed by all the participants together with a 3-day food record, which consisted of written food diary [18]. Subjects were instructed to record everything they ate on 2 consecutive week days and 1 weekend day. The 3-day food record data were loaded on the Win Foo (Medimatica, Colonnella, Teramo, Italy), and the mean nutritional valu

Body weight and height were measured with subjects wearing light clothes using a mechanical scale column (SECA 711, Hamburg, Germany) to the nearest 0.1 kg and 0.1 cm, respectively. Waist circumference was measured by a plastic tape meter at the top of the iliac crest, after a norm were determined by single frequency bioelectrical impedance manufacturer (BIA 101, Akern, Italy). Indirect calorimetry by DATEX (Datex Division of Instruments Corp., Helsinki was used to measure the rate of energy expenditure, by measuring the inspired (O_2) and exp and carbon dioxide (VCO_2), which reflect nutrient metabolism. Deltatrac is one of the reference tools for reliable measurement in vivo, and its accuracy has been detailed in several studies [19]. Before measurements, the instrument was warmed up for 30 min. The canopy was placed o the subjects' face and carefully checked to prevent air leakage. After 5 min of initial ... gas sample were continuously analyzed by a paramagnetic and infrared gas analyzer for O_2 and CO_2 respectively. During the calorimetric exam, no participant talked. The exams were performed in

room with a temperature kept at 22-23 °C. The energy expenditure was calculated within 3-h from the beginning of the meal, because it has been reported that the DIT respo sufficient precision within 3 h and to prevent the movements occurring with prolonged periods of immobility [15].

Blood samples were immediately centrifuged and aliquots of plasma were stored at -80 °C until analysis. The following determinations were performed: glucose, insulin, FFA, triglycerides. Serum glucose was measured by enzymatic colorimetric assay (Menarini Diagnostics, Florence, Italy); serum determined by immunoradiometric assay (Beckman Coulter, Immunotech, Prague, Czech coefficients of variation: intra-assay coefficient of variation 3.99% and inter-assay coefficient of variation 4.8%). FFA concentrations were measured by a fluorometric assay (Sigma-Aldrich, St. Louis, MO, USA). Plasma triglycerides were assayed by enzymatic colorimetric method (Hitachi, Roche Diagnostics, Mannheim, Germany). Total urinary nitrogen excretion was determined on the 24-h urine collection of the day before each test. Tot was assessed by a kinetic assay (Hitachi, Roche Diagnostics, Mannhei

Definitions

The physical activity level was calculated as the product of the duratio hours/week), weighted by an estimate of the metabolic equivalent of th activities performed.

Both basal and after-meal RMR were calculated according to the Weir formula [20]. RMR was calculated [a in relation to fat free mass (FFM) determined by impedance as: RMR/FFM and expressed as

DIT was considered as the difference between after-meal RMR and basal RMR (after-meal RMR - basal RMR).

The Respiratory Quotient (RQ) was the ratio between VCO_2 and VO_2 (VCO_2/VO_2).

Glucose and fat oxidation were calculated according to the Frayn formulas [21]:

$$\text{Carbohydrate oxidation (CHO) (g/min)} = 4.55\ VCO_2\ (l/min) - 3.21\ VO_2\ (l/min) - 2.87\ N\ (g/min)$$

$$\text{Fat oxidation (g/min)} = 1.67\ VO_2\ (l/min) - 1.67\ VCO_2\ (l/min) - 1.92\ N\ (g/min)$$

VO_2 = oxygen consumption; VCO_2 = carbon dioxide production; N= urinary nitrogen exc Rate of N, an index of protein oxidation, was measured during the calorimetry.

Area under the curve (AUC) for glucose, insulin, FFA and triglycerides were calc trapezoidal model [21].

We defined as Delta the difference between each variable after the meal and at fasting:

Delta = variable value after the meal – variable at fasting

In the case of the calorimetric variables, the values at fasting corresponded to the results of the basal resp calorimetry; delta RMR therefore coincided with DIT. For the laboratory variables, the fasting corresponded to the values at time 0.

Blinding

Due to the nature of the intervention, blinding participants and health laboratory personnel who performed the chemical analyses was blinded to the group assignment

Ethics

The study was approved by the ethics committee of the Città della Salute e della Scienza of T procedures conformed to the principles of the Helsinki Declaration. All written informed consent to take part to the study.

Statistical methods

Clinical and laboratory variables were presented as mean (SD). Fasting triglyceride and ins values were logarithmically transformed into normal distribution. The Student's t-test for norma paired data was applied to estimate the within-subject changes of the variables at morning and at evening. Within-subject changes in the variables after the meal in the morning and the evening meal consump analyzed to estimate the effect of timing, i.e. the difference between the morning delta and the evening delta.

211 Morning effect = morning value − evening value

212 In the case of AUCs, the morning effect was the difference between mor
213 evening AUC for the variable.
214 General linear models (GLM) with patients as random effects were per
215 possible period and carryover effects and to estimate morning and evening means and 95 %
216 confidence intervals (95% CI). In order to make easier the interpretation of the
217 adjusted estimates of triglycerides and insulin AUCs were expressed b
218 In an explorative analysis GLM were analyzed for glucose, insulin, FFA and
219 values at single time points.
220 The repeated measures from 0 to 180 minutes of glucose, insulin, FFA
221 reported in tables and as means and 95 % confidence intervals at time 0
222 Statistical analyses were performed using SAS/STAT 13.1 (SAS Institute, Cary, Texas).
223
224 Results
225 Mean age, weight, height, body mass index (BMI), and waist circumfer
226 years, 67.3 ± 12.5 kg, 1.70 ± 0.1 m, 23.8 ± 3.4 kg/m^2, and 82 ± 10 cm, respectively. Fat mass an
227 determined by bioelectrical impedance analyses were 14.5 ± 6.0 kg and
228 Participants were moderately active: their median METs h/week was 4
229 low fiber diet (total kcal 1989.9 ± 523.0; fat 39.9 ± 15.7 % total kcal; saturate
230 monounsaturated fatty acids 14.9 ± 4.2 % total kcal; carbohydrates 46.6
231 There were no meaningful differences between the two periods (first morning
232 first for anthropometrics RMR values (Table 1 SI, Supplementary Information).
233 In Table 1 the morning and evening calorimetric variables that were measured and
234 reported. Fasting was slightly lower in the morning. After meal RMR and DIT values were significantly higher

after the morning meal. Fasting and post-meal RQs at morning were significantly higher than the corresponding RQs at evening. Both fasting and after-meal CHO oxidation values were significantly higher at fasting and post-meal fat oxidation significantly lower in the morning in comparison with the evening va Period and carry-over effects were tested by using GLM and resulted not significant for all variables. The crude and adjusted effects of morning meal did not differ; therefore, only the adjusted effects were reported. The difference in RMR values, the difference in DIT, indicate a higher DIT increase after the morning than after the evening meal (Table 2). On the other hand, differences in RQ values, and the differences between morning AUCs for glucose, insulin and FFA concentratio were negative, indicating a significantly higher effect of the evening meal on these varia meal.

Adjusted estimates of morning meal effects tested by GLM showed significantly lower values of glucose at time 60, 90, 120, 150; lower values of insulin at time 90, 120, 150; FFA at 120, 150, 180, and lower values of triglycerides at 60 min (Table S1, Supplementary Information).

Basal values of glucose, insulin, FFA and triglycerides did not differ f (Figures 1-2 (panels A) and the mean values according to the different time points of glucose, insul reported. In the panels B-D of the Figures 1-2, means and 95% CI variations from time 0 were presented.

Discussion

The results of the present paper indicate that the time of food intake affects both the thermogenic and the metabolic response to meals. This could have practical implications since probably the time of intake should be considered when planning a healthy diet.

Energy expenditure and metabolic responses to meals

Our data show that the meal-induced DIT after the morning meal was higher than that observed dinner, while basal RMR were only slightly lower, consistent with data available in the literature [7,15]. Furthermore, glucose, insulin and FFA AUC values resulted significantly higher at the evening meal rather than at morning.

A circadian pattern in the thermic and metabolic responses to nutrients could therefore be hypothesized. DIT consists of two components: an obligatory component, linked to the ene absorption and metabolism of nutrients, and an additional facultative component, most likely sympathetic nervous system [23,24]. A circadian rhythm of circulating noradrenaline and epinephrine has been found, with increased values in the morning [25]. Epinephrine increases metabolic rate, oxidation [26]. Other explanations for the reduced evening postprandial thermic and metabolic responses may be the slower evening gastric emptying, with a decreased efficiency of dietary carbohydrates absorption [27], and the reported decrement in insulin sensitivity in evening [3]. Possible contributors to the daily variations in insulin secretion, with higher morning β-cell function, could be the circadian fluctuations in the concentrations of ACTH, cortisol, glucagon-like peptide 1, and glucose-dependent insulinotrop polypeptide, as well as the meal-induced glucagon response, delayed after evening meals [2-3, 27-29]. Insulin resistance may determine a decreased thermic effect of glucose because of the diminis insulin-mediated glucose uptake and metabolism by skeletal muscle, and insulin-induced that r thermogenesis [8,30]. Furthermore, sympathetically mediated thermogenesis seems to be decreased hyperinsulinemia or insulin resistance [23,30]. Therefore, both circadian variation in catecholamine concentrations and insulin sensitivity might contribute to the evening reduction of DIT. Consistent with the reported reduced insulin sensitivity at evening, we found lower glucos delayed and larger increases in the concentrations of glucose and insulin at evening (Fig. 2, Tables S2 insu Supplementary Information).

A circadian control of FFA metabolism has been described, with higher levels around midnight [31 afternoon [2], and an increased activity of lipoprotein lipase during [27]. The circadian clock target genes in adipocytes may impact on lipid breakdown and ac mobilization from adipose tissue, thus acting on energy homeostasis a [32-33]. It is difficult to establish with our data if the higher increase in FFA AUC after th consequence of insulin resistance or rather the cause of the impaired effects of FFA on the reduction in glucose uptake in skeletal muscles and o phosphorylation, impairment in insulin signaling and action [34-35].

Not all authors found a lower DIT after [9-12]. Weststrate et al. did not find a significant difference between morning and afternoon DIT in 10 normal weight, but the fasting period w shorter in the afternoon than in the morning and the metabolic conditions of the participants were not the same [9]. In the morning, DIT was slightly higher, the energy balance (the difference between energy intake and expenditure) was lower, values of RQs and CHO oxida significantly increased, in line with our results [9]. Another study found a higher energy expe during enteral nutrition in the morning in patients hospitalized in a Neurologic unit on artificial therefore the results are difficult to compare with those in our healthy individuals [11]. Sato found no difference in 24h energy expenditure in a respiratory chamber between normal (7:00 pm) o (10:30 pm) evening meal in 10 young Japanese, but the measure of DIT was not available, the experimental meals were not consistent with our protocol (approximately 3 h-apart), and postprandial glucose AUCs were significantly increased in the late meal [10]. Similarly, delaying the time of an identical meal by about 3 h for a week did not change postprandial energy expendi oxidation and glucose tolerance [12].

In our study protocol, we used extreme conditions (12 h distance among meals and a high protein content, which is known to exert a greater effect on energy expenditure [31-33]). It is possible that with a mixed meal we cou[ld] have found lower values of DIT and lower differences between the morning and eveni[ng]. Nevertheless, the circadian variation in DIT might explain at least in part th[e] individual variability in DIT and CHO and fat oxidation which could not be accounted for in antecedent diets or in the method of measurement [7, 15]. The significant difference in morning-evening DIT in our sample persisted when energy expenditure was calculated in relation to the FFM (Table 2).

Respiratory Quotients

RQ values generally range within 0.70-1.00, with 100% lipid oxidation at 0.70, and 100% [CHO] oxidation at 1.00 [19]. We found reduced CHO oxidation and RQ values and incre[ased] [...] the evening, suggesting a modification of metabolic pathways toward a higher util[ization] [...] in the evening, supported by the reduction of FFA levels after the evening meal ([...] although starting from higher FFA values (Figure 3, panel A).

After the experimental infusion of glucose/insulin [23], as well as after both meals [38], an increase in CH[O] oxidation and a decrease in lipid oxidation have been reported, in line with our data. Other studies have fou[nd] higher RQ in the morning than in the evening [7, 19, 21, 53]. A significantly higher lipid oxidation [...] CHO oxidation were described at 6:00 pm with meal compared to 10:00 am in the same subjects both after a short (3 days) and prolonged (18 days) protocol, and this has been considered the time of the day in which anabolic functions take place [40], in line with the circadian rhythm [...] glycogen synthesis [41]. Even if delta RQs were slightly higher in the evening (Table 2), the increased evening fat oxidation and FFA AUC in our sample suggest, in line with the higher insulin resis[tance] [...] clock time, a preferential use of lipid substrates in our sample.

Clinical perspectives

Human studies have shown that both in adolescents and adults, consuming most of the daily energy at evening is associated with an increased risk of obesity, hyperglycemia, li syndrome, non-alcoholic fatty liver disease, and cardiovascular diseases [14, 21, 28, 42-45]. Circadian misalignment has adverse metabolic and cardiovascular consequences [46]. Examples of chronic cond of this phenomenon are shift work and sleep deprivation, both have been associated with an increa risk of obesity, metabolic syndrome and cardiovascular diseases [31, 37]. The timing of meals influences the success of weight loss: late eaters lose less weight than early eaters [47]; overweight/obese women lose significantly more weight after a low-calorie dinner weight loss program than after a high-calorie dinner program [48]. Therefore, dietary recommenda should probably include indications for the time of day of food consumption, besides advice and quantity.

Limitations

First of all, caution is needed when trying to link results from short-term studies on healthy subjects. We did not evaluate the energy expenditure 8 h after the beginning of the meal, [7], but other authors who recommend >5 h measurements [31]. However, our experiment wa consistent with studies showing that energy expenditure is lower after 3 h and that DIT response to me be assessed with sufficient accuracy for comparison across subjects in this time interval [7, 49]. Furthermore, most of the differences in the metabolic patterns were found -h after the meal. We used the glucose equation to calculate CHO oxidation after overnight fast [21], plasma glucose turnover is derived from glycogenolysis in liver, but comparisons were perfor

individuals under the same conditions [25], assuming exclusive glucose oxid
not introduce a major error.

Conclusions

Consuming a high-carbohydrate evening meal seems an unfavorable metabolically
unfavorable respect to the consumption of the same meal at morning. En
metabolism may be tightly linked to circadian rhythms; gaining further
useful to curb the current increasing rate of metabolic disorders.

Acknowledgments

The authors thank Professor Gianfranco Peaccetti for his critical reading of the
manuscript. This study was supported by a grant from the University and Res
Italy (60% 2014).

Conflicts of interest

The authors report no conflict of interest.

Supplementary information is available at journal's website.

References

1) Garaulet M, Gómez-Abellán P. Timing of food intake and obesity: a novel association. Physiol Behav. 2014;134:44-50.

2) Lindgren O, Mari A, Deacon CF, Carr RD, Winzell MS, Vikman J, Ahrén B. Differential islet and incre hormone responses in morning versus afternoon after standardized meal in healthy J Clin Endocrinol Metab. 2009;94:2887-2892.

3) Van Cauter E, Desir D, Decoster C, Fery F, Balasse EG. Nocturna constant glucose infusion. J Clin Endocrinol Metab. 1989;69:604-611.

4) Scheer FAJL, Hilton MF, Mantzoros CS, Shea SA. Adverse metabolic and cardiovascul circadian misalignment. Proc Natl Acad Sci. 2009;106:4453-4458.

5) Goo RH, Moore JG, Greenberg E, Alazraki HP. Circadian variation humans. Gastroenterology. 1987;93:515-518.

6) Tsuchida Y, Hata S, Sone Y. Effects of a late supper on digestion carbohydrates in the following morning. J Physiol Anthropol. 2013;32:9.

7) Romon M, Edme JL, Boulenguez C, Lescroart JL, Frimat P. Circadian variation of diet-induced thermogenesis. Am J Clin Nutr. 1993;57:476-480.

8) Ravussin E, Acheson KJ, Vernet O, Danforth E, Jéquier E. Eviden for the decreased thermic effect of glucose in human obesity. J Clin Invest. 1985;76:1268-1273.

9) Weststrate JA, Weys PJM, Poortvliet EJ, Deurenberg P, Hautvast JGAJ. Diurnal variation in postabsorptive resting metabolic rate and diet-induced thermogenesis. Am J Clin Nutr. 1989;50:908-914.

10) Sato M, Nakamura K, Ogata H, Miyashita A, Nagasaka S, Omi N, et al. Acute effect of late evenin on diurnal variation of blood glucose and energy metabolism. Obes Res Clin Pract. 2011;5:e220-e228.

11) Leuck M, Levandovski R, Harb A, Quiles C, Hidalgo MP. Circadian
oxygen consumption. JPEN J Parenter Enteral Nutr 2014;38:263-268.

12) Bandin C, Scheer FA, Luque AJ, Ávila-Gandía V, Zamora S, Madrid JA, et al. Meal timing affects glu
tolerance, substrate oxidation and circadian-related variables: a randomized, crossov
Obes; epub ahead of print 14 October 2014; doi:10.1038/ijo.2014

13) Reed GW, Hill JO. Measuring the thermic effect of food. Am J Clin Nutr 1996;63:164-169.

14) Hibi M, Masumoto A, Naito Y, Kiuchi K, Yoshimoto Y, Matsumoto M
whole body fat oxidation and increases LDL cholesterol in healthy young women. Am J Physiol Regul
Integr Comp Physiol 2013;304:R94-R101.

15) Weststrate JA. Resting metabolic rate and diet-induced thermogenesis: a methodologic
Am J Clin Nutr 1993;58:592-601.

16) Taylor HL, Jacobs DR Jr, Schucker B, Knudsen J, Leon AS, Debacker G. A questionnaire for the asse
of leisure time physical activities. J Chronic Dis 1978;31:741-755.

17) Elosua R, Marrugat J, Molina L, Pons S, Pujol E. Validation of the Minnesota Leisure Tim
Activity Questionnaire in Spanish men. The MARATHOM Investigat
1209.

18) Bo S, Ciccone G, Durazzo M, Gambino R, Massarenti P, Baldi I, et al. Efficacy of antioxidant trea
reducing resistin serum levels: a randomized study. PLoS Clin Trials 2007;2:e17.

19) Moreira da Rocha EE, Alves VG, da Fonseca RB. Indirect calorimetry: methodology, ins
clinical application. Curr Opin Clin Nutr Metab Care 2006;9:247-256.

20) Weir JB. New methods for calculating metabolic rate with special
109:1-9.

21) Ferrannini E. The theoretical basis of indirect calorimetry: a review. Metabolism 1988;37:287-301.

22) Allison DB, Paultre F, Maggio C, Mezzitis N, Pi-Sunyer FX. The use of areas under curves in research. Diabetes Care 1995;18:245-250.

23) Acheson KJ, Ravussin E, Wahren J, Jéquier E. Thermic effect of g facultative thermogenesis in man. J Clin Invest 1984;74:1572-1580

24) Astrup A, Simonsen L, Bülow J, Madsen J, Christensen NJ. Epinep carbohydrate-induced thermogenesis in humans. Am J Physiol 1989;257:E340-E345.

25) Linsell CR, Lightman SL, Mullen PE, Brown MJ, Causon RC. Circadian rhythms of epinephrine and norepinephrine in man. J Clin Endocrinol Metab 1985;60:1210-1215

26) Müller MJ, Acheson KJ, Piolino V, Jeanpretre N, Burger AG, Jequier E. Thermic ef role for endogenous insulin. Metabolism 1992;41:582-587.

27) Al-Naimi S, Hampton SM, Richard P, Tzung C, Morgan LM. Postpran meals and snacks eaten during simulated night and day shift work. Chronobiol Int 2004;21:937-947.

28) Van Cauter E, Shapiro ET, Tillil H, Polonsky KS. Circadian modulation of glucose and insuli meals: relationship to cortisol rhythm. Am J Physiol 1992;262:E467-E475

29) Goetz F, Bishop J, Halberg F, Sothern RB, Brunning R, Senske B, Timing of single daily meal influences relations among human circadian rhythms in urinary cyclic AMP and h and iron. Experientia 1976;32:1081-1084

30) Astrup A, Andersen T, Henriksen O, Christensen NJ, Bülow J, Madsen J, Quaade F. Impaired glucose-induced thermogenesis in skeletal muscle in obesity. The role of the sympathoadrenal system. Int J Obes 1987;11:51-66

31) Dallmann R, Viola AU, Tarokh L, Cajochen C, Brown SA. The human circadian metabolome. Proc Natl Acad Sci 2012;109:2625-2629.

32) Shostak A, Husse J, Oster H. Circadian regulation of adipose t issue. Adipocyte 2013;2:201-206

33) Huang W, Ramsey KM, Marcheva B, Bass J. Circadian rhythms, sle
2011;121:2133-2141. J Clin Invest

34) Randle PJ. Regulatory interactions between lipids and carbohydra
35 years. Diabetes Metab Rev 1998;14:263-283

35) Roden M, Prince TB, Perseghin G, Petersen KF, Rothman DL, Clin
induced insulin resistance in humans. J Clin Invest 1996;97:2859-2865

36) Westerterp KR, Wilson SA, Rolland V. Diet induced thermogenesis measured over 24h in a respiration
chamber: effect of diet composition. Int J Obes Relat Metab Disord 1999;23:287-292.

37) Johnston CS, Day CS, Swan PD. Postprandial thermogenesis is inc
diet versus a high-carbohydrate, low-fat diet in healthy, young women. J Am Coll Nutr 2002;21:55-61

38) Farshchi HR, Taylor MA, Macdonald IA. Decreased thermic effect of food after an ir
with a regular meal pattern in healthy lean women. Int J Obes 2004;28:653-660.

39) Chwalibog A, Thorbek G. Energy expenditure and oxidation of carb
day and night. Thermochimica Acta 2002;394:241-252.

40) Sensi S, Capani F. Chronobiological aspects of weight loss in obe
regimens. Chronobiol Int 1987;4:251-261.

41) Sollberger A. The control of circadian glycogen rhythms. Ann N Y Acad Sci 1964;117:519-554.

42) Bo S, De Carli L, Venco E, Fanzola I, Maiandi M, De Michieli F, et al. Impact of snacking pattern on
overweight and obesity risk in a cohort of 11- to 13-year-old adolescents. J Pediatr Gastroenterol Nutr 2014;59:465-
471.

43) Wang JB, Patterson RE, Ang A, Edmond JA, Shetty N, Arab L. Tim
associated with the risk of obesity in adults. J Hum Nutr Diet 2014;27 Suppl 2:255-262.

44) Bo S, Musso G, Beccuti G, Fadda M, Fedele D, Gambino R, et al. Consuming more of daily calor
at dinner predisposes to obesity. A 6-year population-based prospective cohort study. PLoS ONE 2014;
9: e108467.

45) Cahill LE, Chiuve SE, Mekary RA, Jensen MK, Flint AJ, Hu FB, et al. Prospective study of breakfa
and incident coronary heart disease in a cohort of male US health
Circulation 2013; 128: 337-343.

46) Gonnissen HKJ, Rutters F, Mazuy C, Martens EAP, Adam TC, Westerterp-Plantenga MS. Effect of a
phase advance and phase delay of the 24-h cycle on energy metabolism, appetite, a
hormones. Am J Clin Nutr 2012; 96: 689-697.

47) Garaulet M, Gómez-Abellán P, Alburquerque-Béjar JJ, Lee YC, Ordovás JM, Scheer FA. Timing of food
intake predicts weight loss effectiveness. Int J Obes 2013; 37: 604-611.

48) Jakubowicz D, Barnea M, Wainstein J, Froy O. High caloric intake
influences weight loss of overweight and obese women. Obesity 2013; 21: 2504-2512.

49) Segal KR, Edano A, Thomas MB. Thermic effect of a meal over 3 and 6 ho
Metabolism 1990; 39: 985-992.

Figure Legends

Figure 1

The arrows indicate the times at which samples were drawn. During the visit, p
submitted to anthropometric measurements and was peripherally cannulated. Th
to the time before the first calorimetric examination, which was performed 1 h
and before the meal. The times 30, 60, 90, 120, 150 and 180 were refe
the beginning of the meal.

The same time schedule was adopted in the case of the evening meal (at
8:00 pm).

Figure 2

Mean glucose values at the different time points (panel A). Variation of glucose from time 0
changes from the value at time 0 (black dot), evening mean). The grey dot
whiskers indicate the 95% CIs (panel

Figure 3

Mean insulin values at the different time points (panel A). Variation of
changes from the value at time 0 (black dot), evening mean). The changes fr
whiskers indicate the 95% CIs (panels A and B).

Figure 4

Mean FFA values at the different time points (panel A). Variation of FF
from the value at time 0 (black dot), evening mean (grey dot). The whiskers
indicate the 95% CIs (panels A and B).

Figure 5

Mean triglyceride values at the different time points (panel A). Variation of triglycerides from
mean changes from the value at time 0 (black dot), evening mean chan
The whiskers indicate the 95% CIs (panels A and B).

Figure 1. Time schedule of the study

ð¯ ð¯ ð¯ ð¯ ð¯ ð¯ ð¯ ð¯

Basal 0 30 60150 180 90

ç______ç______ç______ç____________l________ç___l__________l___ç________

ç visit ç calorimetry ç meal ç rest ç rest ç rest ç calorimetry ç calorimetry ç

The arrows (ð¯) indicate the time when the blood samples were drawn. During

submitted to anthropometric measurements and a venous catheter was peripherally inserted. Basal corresponded

to the time before the first calorimetric examination was performed. Th

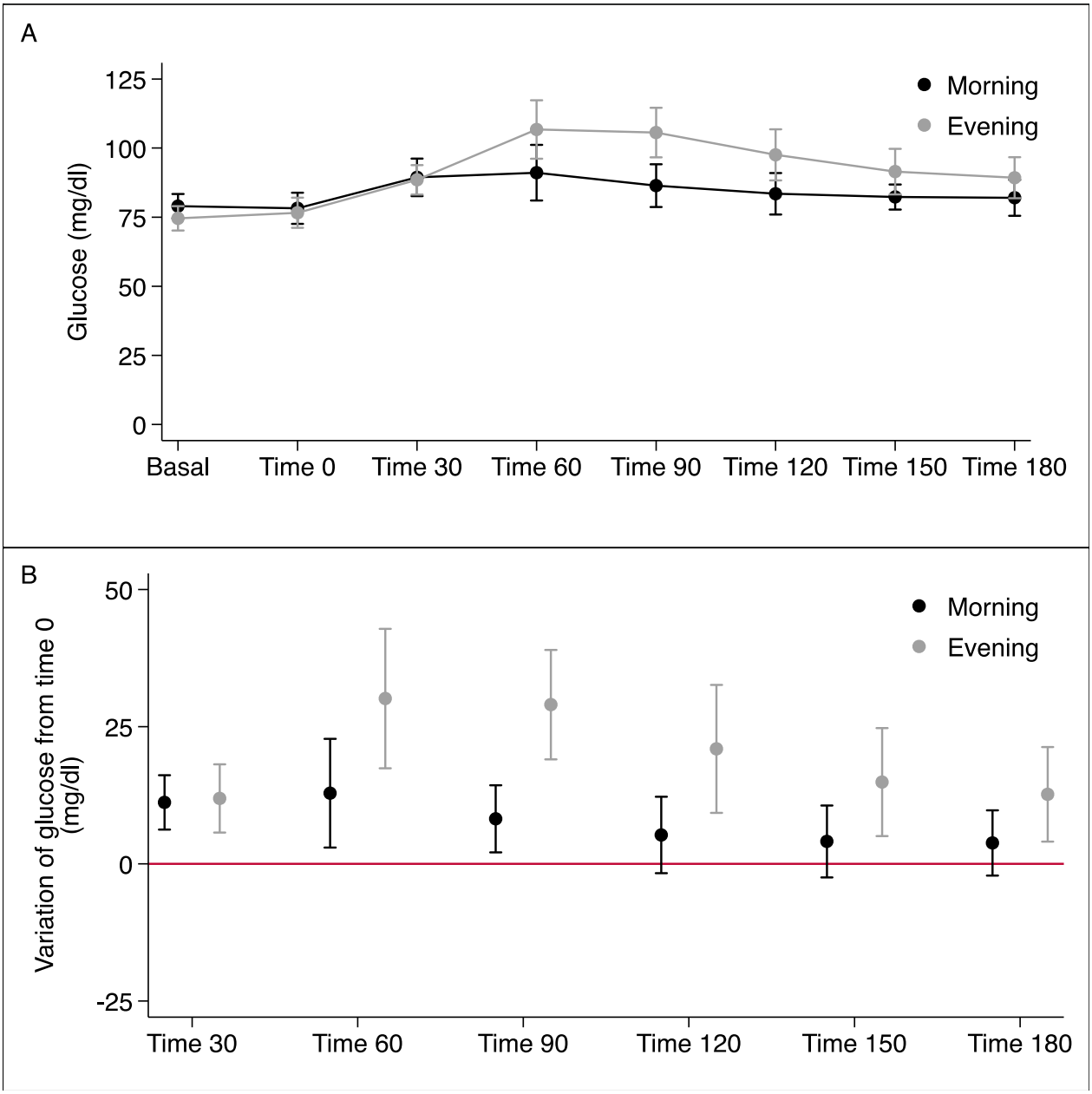
and before the meal. The times 30, 60, 90, 120, 150 and 180 were time interval in minutes after

the beginning of the meal.

The same time schedule was adopted in the case of the morning meal (

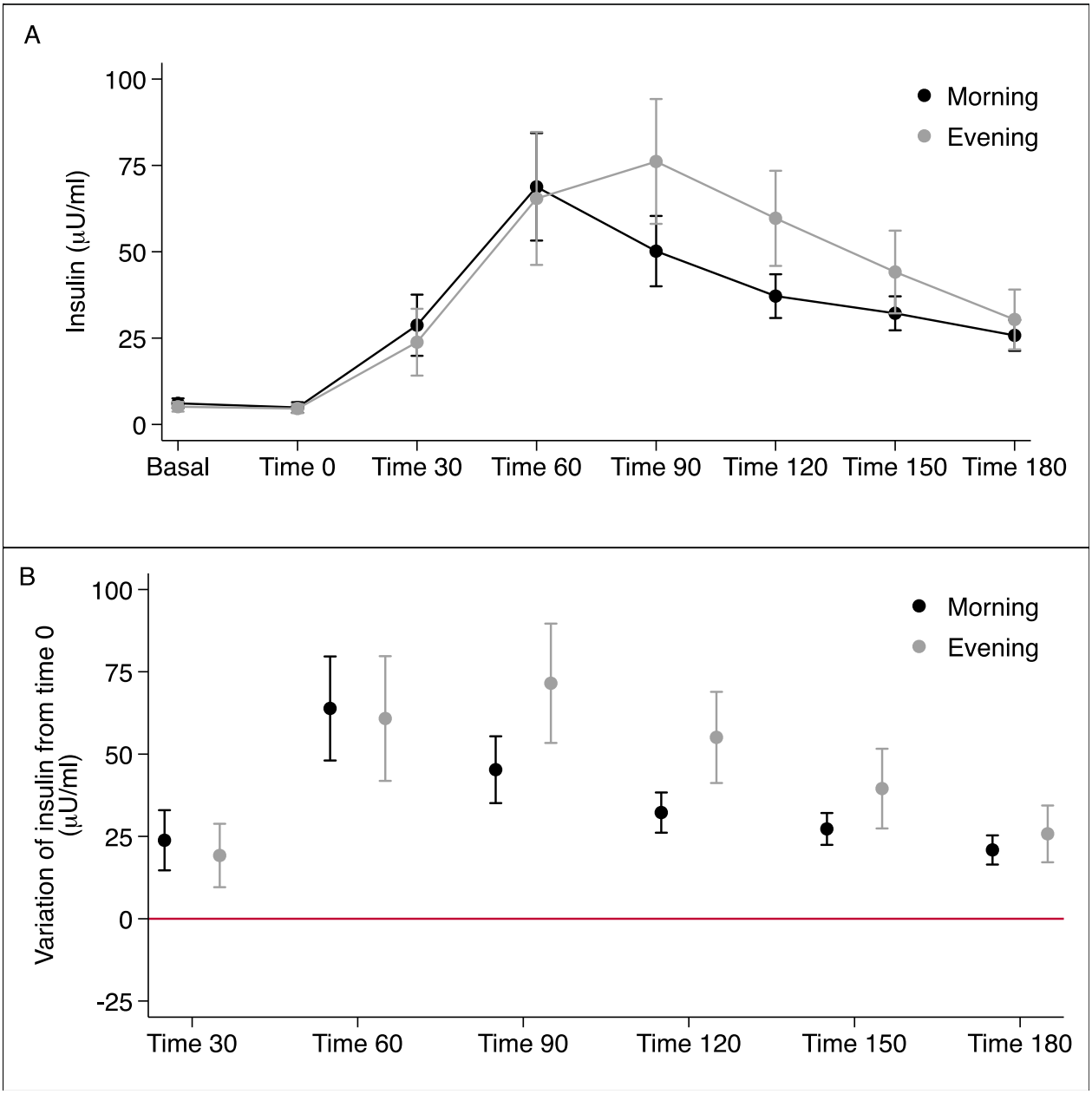
8:00 pm).

Figure 2. Mean glucose values at the different time points (panel A) and variation o
(panel B)



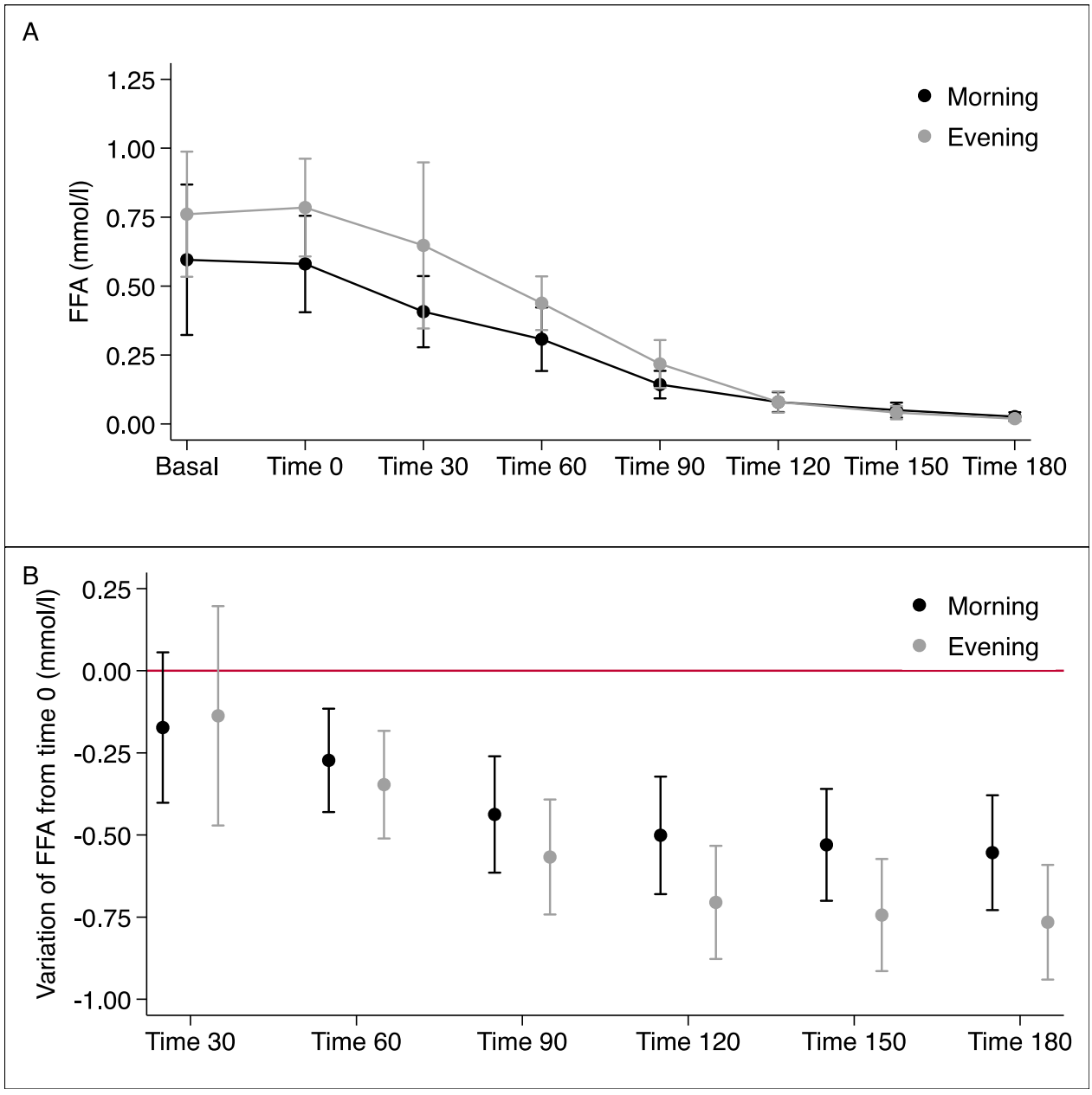
Mean glucose values at the different time points (panel A). Variation of glucose from time 0: mo
changes from the value at time 0 (black dot), evaluated at each time point (panel B). The grey dot
whiskers indicate the 95% CIs (panel

Figure 3. Mean insulin values at different time points (panel A) and variation from time 0 (panel B)



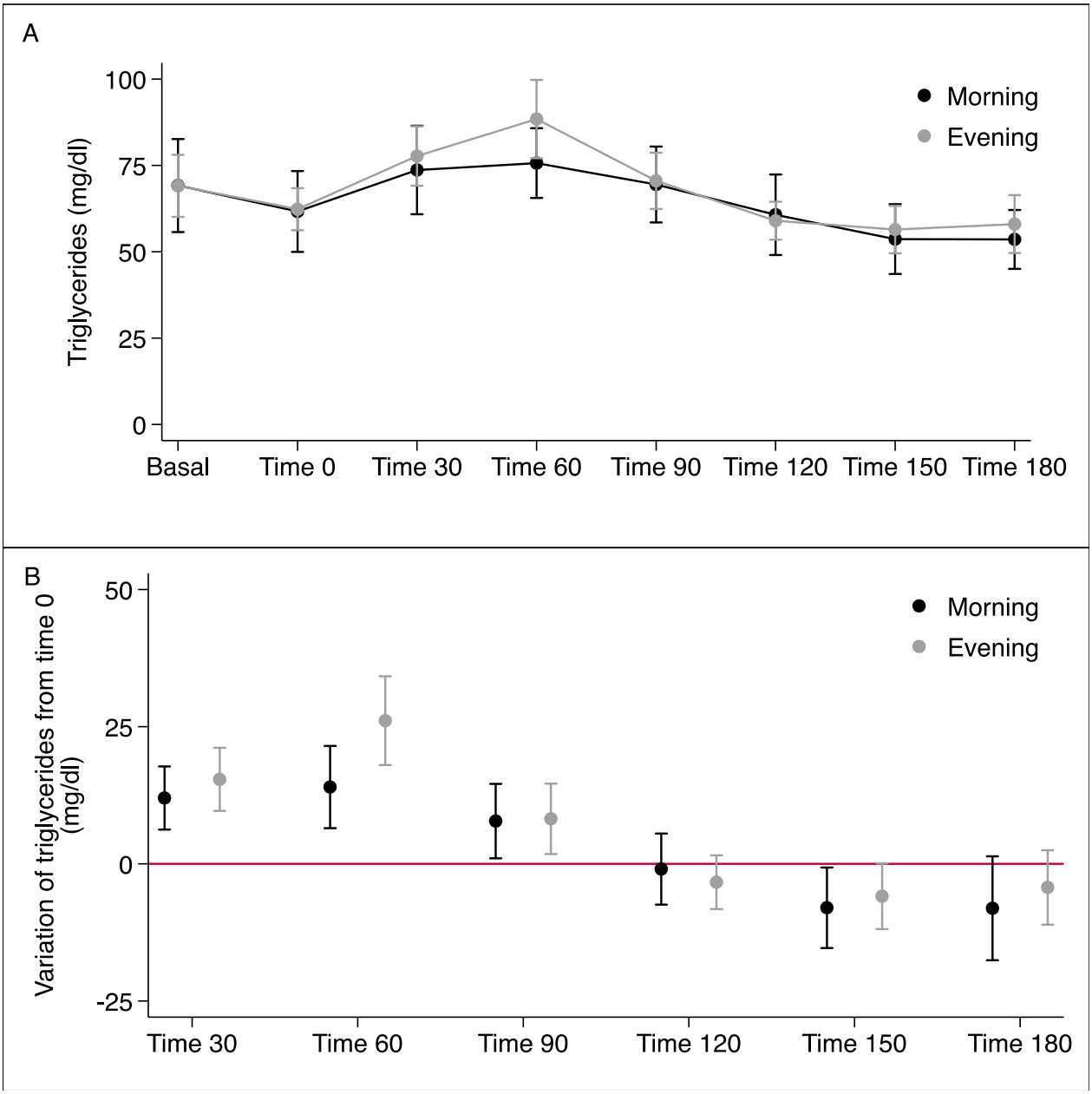
Mean insulin values at the different time points (panel A). Variation of insulin from time 0: changes from the value at time 0 (black dots), mean change from the value at time 0 (grey dots). The whiskers indicate the 95% CIs (panels A and B).

Figure 4. Mean and variation of FFA values at different time points (panel A) and variation of F



Mean FFA values at the different time points (panel A). Variation of FFA from time 0: morning and evening (black and grey, respectively) changes from the value at time 0 (panel B). The whiskers indicate the 95% CI (panel

Figure 5. Mean triglyceride values at different time points (panel A) and variation from time 0 (panel B)



Mean triglyceride values at the different time points (panel A). Variation of triglycerides from mean change from the value at time 0 (black dot) or the value at time 0 (grey dot) (panel B). The whiskers indicate the 95% CIs (panel A and B).

Table 2 Calorimetric variables before and after morning and evening mea

	Morning		Evening		p-value
Number	20		20		
Fasting RMR (kcal)	1588.	[1464.9;1	1518.	[1407.7;1	0.098
After meal RMR (kcal)	1916.	[1791.5;2	1755.	[1648.0;1	<0.00
DIT (kcal)	327.5	[279.0;37	237.0	[195.1;2	0.003
Fasting [1] RMR (kcal/kg FFI	30.2	[27.8;31	29.1	[27.0;31	0.180
After meal [1] RMR (kcal/kg F	36.7	[34.6;38	33.7	[31.4;36	<0.00
DIT [1] (kcal/kg FFM)	6.46	[5.16;7.	4.62	[3.55;5.	0.003
Fasting RQ	0.87	[0.85;0.	0.80	[0.78;0.	<0.00
After meal RQ	0.90	[0.89;0.	0.85	[0.82;0.	0.002
RQ Difference	0.03	[0.01;0.	0.05	[0.02;0.	0.055
Fasting CHO oxidat	0.13	[0.10;0.	0.05	[0.02;0.	<0.00
After meal CHO oxidat	0.20	[0.18;0.	0.12	[0.08;0.	<0.00
CHO oxidation diffe	0.07	[0.05;0.	0.08	[0.05;0.	0.856
Fasting fat oxidatio	0.01	[0.01;0.	0.04	[0.03;0.	<0.00
After meal fat oxidation (g	0.01	[0.00;0.	0.03	[0.02;0.	0.006
Fat oxidation differ	-0.01	[-0.01;0.	-0.01	[-0.03;0.	0.116

Mean [95% CI]; p calculated by t-tests for paired data

RMR = Resting Metabolic Rate; DIT = Diet-Induced Thermogenesis; RQ = Respiratory Quotient; CHO carbohydrates

[1] energy expenditure corrected in relation to fat free mass

Table 2. Estimates of morning effect adjusted for by Generalized Linear Mixed Models (GLMs)

	Effects	95% CI	p-value
RMR[1] (kcal)	90.5	[40.4, 140.6]	<0.001
RMR[1,2] (kcal/kg FFM)	1.84	[0.81, 2.87]	<0.001
RQ[3]	-0.02	[-0.04, 0.00]	0.035
CHO oxidation[3] (g/min)	0.00	[-0.02, 0.02]	0.848
Fat oxidation[3] (g/min)	0.01	[-0.00, 0.02]	0.089
Glucose[4] (AUC, mM*h)	-1800.1	[-2564, -1036.0]	<0.001
Log insulin[4,5] (AUC, mU/ml*h)	-0.19	[-0.30, -0.07]	0.001
Log insulin[4,6] AUC	0.83	[0.74, 0.93]	0.001
FFA[4] (AUC, mM*h)	-16.1	[-30.2, -2.09]	0.024
Log triglycerides (small AUC)[4,5]	-0.08	[-0.21, 0.05]	0.230
Log triglycerides AUC[4,6]	0.92	[0.81, 1.05]	0.230

RMR = Resting Metabolic Rate; RQ = Respiratory Quotient; CHO = carbohydrates; AUC = area under the curve; FFA = free fatty acids

[1] morning-delta Diet-Induced Thermogenesis (DIT); DIT

[2] RMR calculated in relation to fat free mass

[3] Morning delta minus evening delta

[4] Morning AUC vs. evening AUC

[5] Estimated effects expressed as mean difference in log

[6] Estimated effects expressed as ratio.