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

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19 Is the Timing of Caloric Intake Associated with Energy Expenditure and the
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43 Abstract

44 Background/Objective: The energy expenditure is generally reported to be higher in the morning
 45 although contrasting results to differences in energy expenditure have been reported. The purpose of this study was to compare the calorimetric
 46 energy expenditure, diet-induced thermogenesis, and resting energy expenditure in a group of healthy
 47 metabolic responses to a standardized meal and a standardized evening meal in volunteers, after
 48 standard diet, physical activity of fast subjects (age 20-30 years, BMI 18-25 kg/m²) were
 49 35 year, BMI 18-25 kg/m²) were randomized over 2 days in a laboratory setting. The only difference
 50 standard meal and evening meal was the timing of the meal. Basal calorimetry was
 51 performed; a 60-min indirect calorimetry was done at the beginning of the meal. Blood
 52 were drawn every 15 min for 180 min. General lines (GLM) and adjusted of rapid recovery were
 53 used to evaluate the difference of meal-induced thermogenesis (MIR) (adjusted value
 54 minus evening meal) (fasting) of the variables. Resting metabolic rate
 55 (RMR) did not change from morning to evening. MIR was significantly higher in the
 56 morning (meal; 95% CI 175, 240-68, cal; $p < 0.001$). RMR was significantly increased
 57 after the morning meal (95% CI 40, 41-106, cal; $p < 0.001$) while differences in the
 58 glucose (-1.60; -2.56-4.03 mg/dL, $p < 0.001$), insulin (-0.19; -0.07 U/dL; $p = 0.001$) and fatty free
 59 acid concentration (-0.2, 0.0 mmol/l; $p = 0.001$) were significantly larger
 60 increases in glucose and insulin were found for the evening meals.

61 Conclusion: The same meal consumed in the evening, BMI increased
 62 glycaemic and insulinemic responses, suggesting higher energy expenditure in
 63 pattern in healthy individuals. Timing of meals probably be considered when nutritional
 64 recommendations are given.

65 ClinicalTrials.gov NCT02314380

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67 Introduction

68 An increasing number of studies have shown that the timing of food intake influences energy
69 risk of weight gain and obesity [1]. Total daily caloric intake, dietary composition and energy
70 expenditure [2].

71 Early insulin secretion after a meal is significantly higher in the morning than in the evening
72 to a more rapid glucose clearance [3], whereas insulin resistance is higher in the evening [4].

73 Circadian variation in concentrations of hormones such as leptin and ghrelin, appetite
74 as generated by the circadian clock, have been implicated in the specific modification of
75 metabolic pathways. Gastric emptying seems to be more rapid in the evening [5], and an
76 increased efficacy of dietary carbohydrate absorption has been reported in obese individuals
77 under conditions [6].

78 A few studies evaluated the circadian variation of the thermic effect of food (TEF), the
79 thermic effect of food (TEF), induced thermogenesis (DIT), is defined as the increase in
80 metabolic rate (RMR) after the ingestion of a meal. This component accounts for approximately
81 energy expenditure (about 10%) and is reported to be higher in the morning than in the evening
82 of obesity [7].

83 This is significantly higher after the consumption of a meal than after a snack at night [8].
84 A reduced evening response may contribute to the increased risk of obesity [9].

85 Furthermore, habitual nighttime eating or snacking have been associated with
86 potentially promoting weight gain [14].

87 However, data in the literature show contrasting findings in the relationship between the duration of
88 hours of sleeping, and the duration of the sleep period and the intensity of the activity.
89 A low number of the subjects are included in the studies, and the ideal setting to
90 diurnal variation in energy expenditure and absorptive capacity (RMR) under the same conditions at
91 different times. This has not been our study was planned to evaluate the metabolic

91 responses to identical protein and carbohydrate meals in the morning (8:00
 92 in the evening) by healthy volunteers after standardizing diet, physical activity
 93 and resting.

94

95 **Subject Methods**

96 **Recruitment of participants**

97 Twenty healthy (volunteers) were enrolled among students and graduates
 98 the Department of Medicine at the University of

99 Inclusion criteria: age 20-35 years, body mass index (BMI) moderate exercise level
 100 smoking <10 cigarettes per day. Exclusion criteria: acute or chronic disease, drugs or

101 supplementary dietary restrictions or specific diets, were asked to give a
 102 written informed consent

103 **Design**

104 This was a randomized controlled

105 **Outcomes**

106 The primary outcome was changes in RMR after meals compared to
 107 changes in RMR after the evening meal. Secondary outcomes were changes in circulating
 108 concentrations of glucose, free fatty acids (FFA) and triglycerides and evening
 109 consumption.

110 **Intervention**

111 Participants were randomly allocated to either a standard meal and the week after the
 112 standard meal or vice versa. They ate the meal (respectively 12:00-2am),
 113 participants ate a standard meal (with protein supplement), and then
 114 asked to abstain from drinking coffee, alcohol and tobacco for 7 days

115 permitted the preceding day participants were instructed not to eat and to
 116 refrain from heavy physical activity. Urinary collection was collected the day before
 117 determined urinary nitrogen excretion.
 118 The standard meal consisted of 100g white bread, 100g ham, 50g cheese, 125g yogurt,
 119 25g protein supplement (Research Protein, Nestlé, Small Company) and 100g fruit
 120 meal was 30% protein, 31% carbohydrate, 39% fat and 1.6g sodium. Participants consume each
 121 meal in 30 min. Participants take a taxi to the laboratory (or pm, according to
 122 randomization), the participants provide anthropometric measurements and to the insertion of
 123 indwelling catheter into an antecubital vein of the forearm, subsequently
 124 500 ml of saline solution until the end of the testing period. In the second day
 125 the blood samples have been withdrawn from an extension line tubing.
 126 A 30 min basal calorimetric examination was performed in a supine position
 127 awake and motionless until the whole procedure, during the meal, when they could
 128 eat and were allowed. At 8:00 am (or pm), the participants and then returned to the
 129 supine position. In the following second calorimetric evaluation to obtain a
 130 compliance to the experiment, the second calorimetric trial was at 18:00 min after the
 131 beginning of the meal, respectively previously performed in order to evaluate the
 132 tolerance to the carbohydrate load. It was found that maintaining immobility while
 133 more than 1 hour consecutive.
 134 Blood samples were drawn every 30 min in the basal period (at the end of the second
 135 (post-prandial) period) and after the first calorimetry and before the meal. The
 136 and 0.5 l referred to the time interval beginning of the meal time
 137 schedule was adopted in the case of the morning meal (at 8:00 am) and
 138 second test was carried out after 7 days from the first.

139 Sample size

140 A sample size of 20 subjects in the intervention group was
 141 to test a 0.66 effect size with a 0.20% and a α value = 0.05.

142 Randomization

143 The random sequence (generated by a computer) was used, using blocks
 144 different lengths in random order.

145 Measurements

146 The Minnesota Leisure Time Physical Activity Log was used in a cohort
 147 [17], was completed by all the participants with, which consisted of
 148 written food diaries. Subjects were instructed to record every 2 hours the type of
 149 week days and weekend day food record data were loaded on the Win Food
 150 (Medimatica, Colonnella, Teramo, Italy), and the mean nutritional values
 151 Body weight and height were measured to the nearest 0.1 kg and 0.1 cm,
 152 (SECA Hamburg, Germany) respectively. Circumference by was
 153 measured by a plastic tape meter at the top of the iliac crest, as normal
 154 were determined by frequency electrical impedance analysis (BIA) (Akern
 155 Italy) indirect calorimetry by a DEXA (Dixtal of Instruments Corp. Helsinki,
 156 measure rate of energy expenditure, by measuring the inspired and expired
 157 and carbon dioxide (V_O2 and V_ECO₂) by a metabolic cart (one of the reference
 158 tools for reliable measurements in vivo, and accuracy has been validated in several
 159 studies). Before measurements, the instrument was placed on
 160 the subject and carefully checked to prevent any leaks in the system. Gas sample
 161 were continuously analyzed by a paramagnetic and infrared gas analyzer (Oxycon
 162 During the calorimetric exams, participants had to be exams were performed in

163 room with a temperature of 23°C. The energy expenditure was calculated from the
 164 beginning of the meal, because it has been reported that the DIT response is not
 165 sufficient precise and it prevents the movements of the limbs and periods of
 166 immobility [

167 Blood samples were immediately centrifuged and aliquots of plasma were
 168 analyzed. The following determinations were performed: glucose was
 169 measured by enzymatic assay (Merck Diagnostica, Florence, Italy); serum
 170 determined by immunochemical assay (Coulter, Immunotech, Prague, Czech
 171 coefficients of variation of 9.8% and 10.1% for glucose and FA concentrations were
 172 measured by a fluorometric assay (Sigma, St. Louis, MO, USA) glycerides were assayed
 173 enzymatic colorimetric method (Hitachi, Mannheim, Germany). nitrogen
 174 excretion was determined during the 24 h of the day before each test. Total
 175 was assessed by a kinetic assay (Hitachi, Roche Diagnostics, Mannheim, Germany).

176 Definitions

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

180 Both basal and anteprandial RMR were calculated according to the following formulae
 181 in relation to fat free mass (FFM) and expressed as: RMR/FFM and expressed as
 182 DI was considered as the difference between anteprandial RMR and basal RMR
 183 basal RMR).

184 The Respiratory Quotient (RQ) was calculated as the ratio between VCO₂ and VEO₂.

185 Glucose and fat oxidation were calculated according to the following formulae:

186 Carbohydrate oxidation (g/min) = $\frac{VCO_2 - 3.21 \cdot VEO_2}{2.87}$ N (g/min)

187 Fat oxidation ($\text{g/m}^2(\text{h})\text{min}^{-1}$) = $0.67 \frac{V_{O_2} - V_{CO_2}}{V_{O_2} + V_{CO_2}} \times N$ (g/min)

188 V_{O_2} = oxygen consumption; V_{CO_2} = carbon dioxide production; N = urinary nitrogen excretion

189 Rate of N, an index of protein metabolism, was determined during the calorimetry.

190 Area under the curve (AUC) for glucose, insulin, FFA and triglycerides were calculated

191 using a trapezoidal model [12].

192 We defined as Delta the difference between the value at fasting and the value at the end of the intervention.

193 $\Delta = \text{variable value after intervention} - \text{variable value at fasting}$

194 In the case of the calorimetric variables, the value at fasting was the value at the start of the calorimetry; delta RMR therefore corresponded to the values at time 0.

195 In the case of the laboratory variables, the value at fasting corresponded to the values at time 0.

196 Blinding

197 Blinding

198 Due to the nature of the intervention, blinding participants and health

199 laboratory personnel was not possible. The investigators were blinded to the group assignment.

200 Ethics

201 The study was approved by the Comitato Etico della Società della Salute e della Ricerca of the University of Turin.

202 All procedures conformed to the principles of the Helsinki Declaration and all participants gave their informed

203 consent to take part to the study.

204 Statistical methods

205 Clinical and laboratory variables were presented as mean and standard deviation. For normally distributed

206 values were logarithmically transformed in order to obtain a normal distribution. For non-normally distributed

207 paired data was applied the Wilcoxon test. The significance level was set at 0.05. All analyses were performed

208 with subject characteristics in the morning and the evening for the consumption of energy and macronutrients.

209 In order to estimate the effect of the intervention on the variables, we calculated the delta of each variable

210 delta

211 Morning effect Δ AUCs were calculated as the difference between
 212 In the case of AUCs, the morning effect was the difference between morning
 213 evening AUC for the variable.
 214 General linear models (GLM) with patients as random effects were performed for
 215 possible periods of effects and to estimate model coefficients (95%
 216 confidence intervals). In order to make easier the interpretation of the
 217 adjusted estimates of triglycerides and insulin AUCs were expressed by
 218 In an explorative analysis, GLM were analyzed on glucose, insulin, FFA and
 219 values at 0 minutes.
 220 The repeated measures from 0 to 180 minutes of glucose, insulin, FFA
 221 reported as means and 95% CI from time 0.
 222 Statistical analyses were performed using GraphPad Prism 8.0 (San Diego, CA, USA).

223

224 Results

225 Mean age, weight, height, body mass index (BMI), and waist circumference
 226 years, 67.3 ± 12.5 kg, 1.70 ± 0.07 m, 28.4 ± 3.0 m², respectively. Fat mass and
 227 determined by bioelectrical impedance analyses were 14.5 ± 6.0 kg and
 228 Participants were moderately active: their median METs h/wk were 4
 229 low fiber diet (total kcal 1989.9 ± 523.0 ; fat 39.9 ± 15.7 % total kcal; saturated
 230 monounsaturated fatty acids 14.9 ± 4.2 % total kcal; carbohydrates 46.6
 231 There were no meaningful differences between groups in anthropometric variables
 232 first for anthropometric variables (Table 1SI, Supplementary Information
 233 In Table 1, the morning and evening calorimetric variables that 20 participants
 234 reported fasting were significantly lower in AMR DIT values significantly higher

235 after the meal fasting and after meals at morning were significantly higher than
 236 corresponding RQ. Both the resting and after HO oxidations were significantly higher after
 237 fasting and after the oxidation significantly lower in the morning with the evening
 238 Period and over days were the testing GLM and results are significant
 239 variables. The crude adjusted effects of morning effects differ; therefore, only the
 240 adjusted effects were reported in the RMR, values are indicated higher
 241 DIT. The results are shown in the evening meal (Table 2) to be different, differences in
 242 RQ values and differences between morning and evening meals and FFA concentrations
 243 were negative, indicating a negative effect of the evening meal on these variables
 244 meal.

245 Adjusted estimates of the effects of meal by GLM were significant for glucose at
 246 time 60, 90, 120, 150, values of insulin at time 90, 120, 150, 180,
 247 and values of triglycerides (Table 2, Supplemental material).
 248 Basal values of glucose, insulin, FFA and triglycerides (Figure 5) differ
 249 (panels A) and according to the different time points of glucose, insulin
 250 reported. In the panels -B, the error bars represent 25% CI variations from time 0
 251 were presented.

252

253 Discussion

254 The results of the present study indicate that the metabolic response to both the thermogenic
 255 the metabolic response to meals can have practical implications for diet and body
 256 be considered when planning a healthy diet.

257

258 Energy expenditure and metabolic responses to meals

259 Our data show the immediate DIT after the morning meal is higher than that observed
 260 during the evening meal, while basal MR were only slightly lower. Sensitivity analysis of the
 261 [71]. Furthermore, we found FFA values were significantly higher at the evening meal
 262 rather than at morning.

263 A circadian pattern in metabolic responses to nutrients has been hypothesized
 264 DIT consists of two components: an obligatory component, linked to the energy
 265 absorption and metabolism of nutrients, and a facultative component, likely
 266 sympathetic nervous system [23, 24]. A circadian rhythm in circulating noradrenaline
 267 has been found with increased values in the evening. This increase in metabolic rate,
 268 oxidation [25]. Other explanations for the evening reduction in thermic and metabolic rate may
 269 be the slower gastric emptying, with a decrease in dietary carbohydrate absorption
 270 the reported decrease in sensitivity to glucose [26]. Possible contributors to the
 271 variations in thermic effect of food include the circadian
 272 fluctuations in concentrations of ACTH, glucagon-like peptide 1, glucocorticoids, insulinotropic
 273 polypeptide and in the reduced glucagon response to evening meals
 274 [23, 27]. Insulin resistance determined by increased thermic effect of food is diminished
 275 insulin-mediated glucose uptake and metabolism by skeletal muscle that results in
 276 thermogenesis [28]. Furthermore, sympathetically mediated thermogenesis is decreased in
 277 hyperinsulinemic states [29]. Therefore, both circadian variations in catecholamine
 278 concentrations and sensitivity to these hormones contribute to the evening reduction in
 279 reported reduced insulin sensitivity at evening, we found lower glucose
 280 delayed and larger increases in the concentration of glucose (Fig. 1, Table 2, insulin
 281 Supplementary Information).

282 A circadian control of FFA metabolism in the skeletal muscle is reported to be [31
 283 afternoon [2], and an increased activity of lipolysis and lipase activity
 284 clock target genes in adipocytes may impact on lipid breakdown and ad
 285 mobilization from adipose tissue, thus acting on energy homeostasis a
 286 difficult to distinguish with our data if the higher increase in FFA AUC after the
 287 consequence of insulin resistance or rather the cause of the impaired
 288 effects of FFA on the reduction in lipolysis in skeletal muscle and o
 289 impairment in insulin signaling and action [34, 35]
 290
 291 Not all authors found a lower DIT after the evening meal. In a study by
 292 difference between morning and evening DIT during the fasting period w
 293 shorter in the afternoon than in the morning and the metabolic rate of
 294 were not the same [8]. However, DIT is slightly higher during the
 295 between energy intake and expenditure. Values of RQs and CHO oxidat
 296 significantly increased with our results [9]. A study found a higher energy exp
 297 during enteral nutrition in hospitalized neurologic unit on artificial
 298 therefore these results are difficult to compare with those of healthy individuals and no
 299 difference in energy expenditure is reported between normal (7:00 pm) o
 300 (10:30 pm) evening meal. In Japan, this measure of DIT was not a variable, the
 301 experimental meals were (approximately) consistent with our postprandial
 302 glucose AUCs significantly increased [10]. Similarly, delay of the time
 303 identical meal-offer about 8 h did not change postprandial energy expendi
 304 oxidation and glucose tolerance [12]

305 In our study we used extreme conditions (a meal at high intensity which is
 306 known to exert a greater energy expenditure than a maximal meal we could
 307 have had lower values and smaller differences between the morning and evening
 308 Nevertheless circadian variation in DIT might explain at least in part the
 309 individual variability in DIT and CHO and fat oxidation not by a circadian
 310 antecedent or in the method of measurement, as we found in the morning
 311 evening DITs were predicted well by energy expenditure as the FFM in relation
 312 (Table 2)

313

314 Respiratory Quotients

315 RQ values generally range from 0.70 (100% lipid oxidation) to 1.00 (100%
 316 carbohydrate oxidation). We found reduced CHO oxidation and RQ values and increased
 317 lipid oxidation in the evening, suggesting of metabolic pathways toward a higher utilization
 318 in the evening supported by the decrease of FFA levels after the evening meal (Figure 3,
 319 although starting from higher FFA values (Figure 3, panel A).

320 After the experimental infusion of glucose and insulin at the meal increase in CHO
 321 oxidation and a decrease in lipid oxidation, as reported with other studies have found
 322 higher RQ in the morning (17, 21, 59). A significantly higher lipid oxidation and
 323 CHO oxidation were described with meal two between 6:00 and 8:00 am in these
 324 subjects after a short (3 days) adaptation (18 days) in the morning considered
 325 the time of the day in which anabolic metabolism is at the place of the circadian rhythm
 326 glycogen synthesis if delta RQs were relatively higher in the evening (Table 2), increased
 327 evening fat oxidation and FFA AI consumption suggested a higher reliance on lipid
 328 substrates, a preferential use of lipid substrates in our sample

329

330 Clinical perspectives

331 Human studies have shown that adolescents consume 10-20% of the daily energy
 332 at evening associated with an increased risk of obesity, hyperglycemia, lipid
 333 syndrome, alcoholic fatty liver and cardiovascular disease [14, 20, 42, 44, 45]. Circadian
 334 misalignment has adverse metabolic and cardiovascular health consequences [46]. Examples
 335 of this phenomenon are shift work and sleep deprivation, both having increased
 336 risk of obesity, metabolic syndrome and cardiovascular disease [17, 47].
 337 The timing of meals influences the success of weight loss. Studies suggest that
 338 early eating is associated with weight/obesity significantly more weight reduction in low weight
 339 loss program than after a high-calorie dinner program [48]. Dietary recommenda-
 340 tions should ideally include indications of daily energy expenditure, besides advice
 341 and quantity.

342

343 Limitations

344 First of all, it is needed when trying to link results to lifestyle interventions.
 345 We did not evaluate the energy expenditure in the beginning of this meal,
 346 [7], but other authors who recommended a 30 min interval [10]. However, our experiment was
 347 consistent with studies showing energy expenditure after a meal that DIT response to meals
 348 be assessed with sufficient accuracy in a comparison across subjects [7, 49].
 349 Furthermore, most of the differences in the metabolic patterns we found were
 350 meal-related. We used the glucose equation to calculate the rate of CO₂ oxidation [21],
 351 plasma glucose turnover is dependent of glycogenolysis, liver and other comparisons were performed

352 individuals under the same conditions including exclusive glucose oxid

353 not introduce a major error

354

355 Conclusions

356 Consuming a high-carbohydrate evening meal seems energetically

357 unfavorable with respect to the consumption of the same meal at morning. En

358 metabolism may be tightly linked to circadian rhythms; gaining further

359 useful to curb the current increasing rate of metabolic disorders.

360

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365

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

367

368 Supplementary information is available at journal's website.

369

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Figure Legends

Figure 1

The arrows indicate the time when blood samples were drawn. During the visit, participants submitted to anthropometric measures and blood was periodically sampled from the time before the first calorimetric examination was first performed and before the meal. The times 30, 60, 90, 120, 150 and 180 were referred to the beginning of the meal.

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

Figure 2

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

Figure 3

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

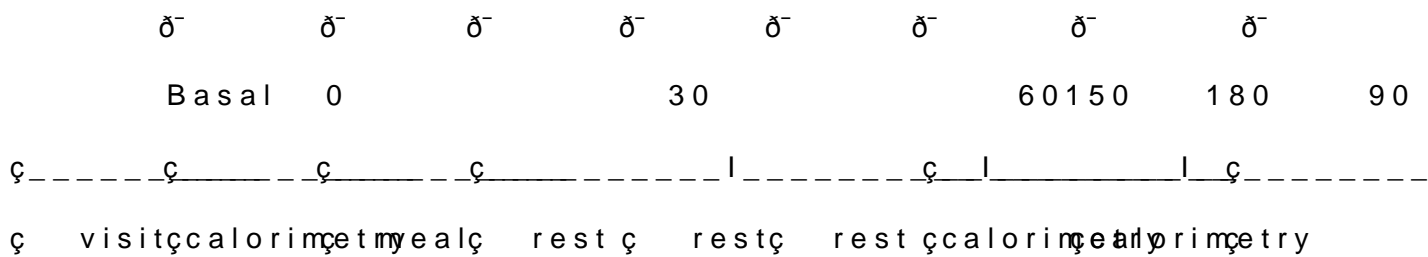
Figure 4

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

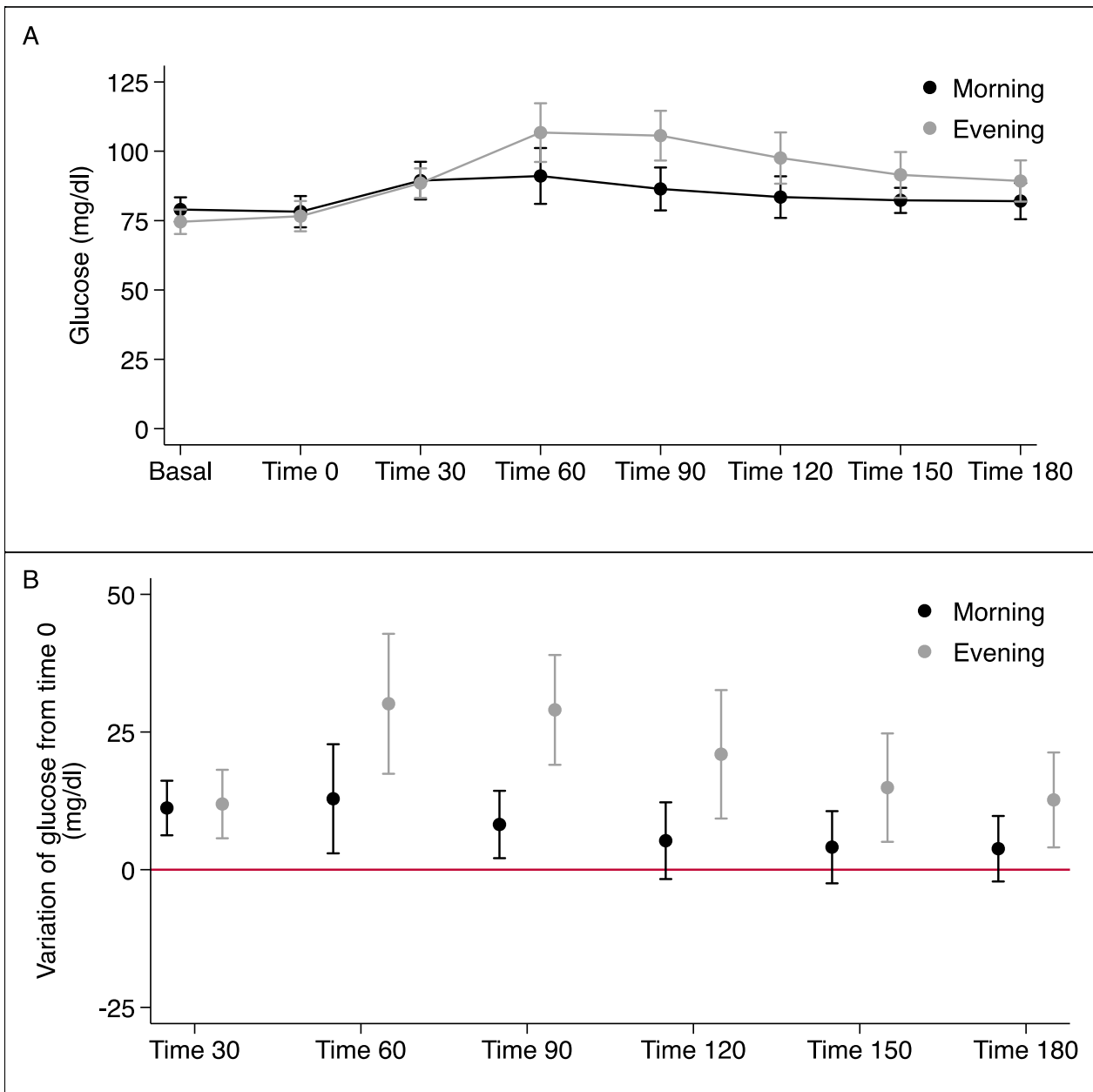
Fig 1. Time schedule of the study



The arrows indicate the time when the blood samples were drawn. During the study, the subjects were submitted to anthropometric measurements and blood samples were drawn at the following times: Basal, 0, 30, 60, 90, 120, 150, 180, and 90 min after the beginning of the meal.

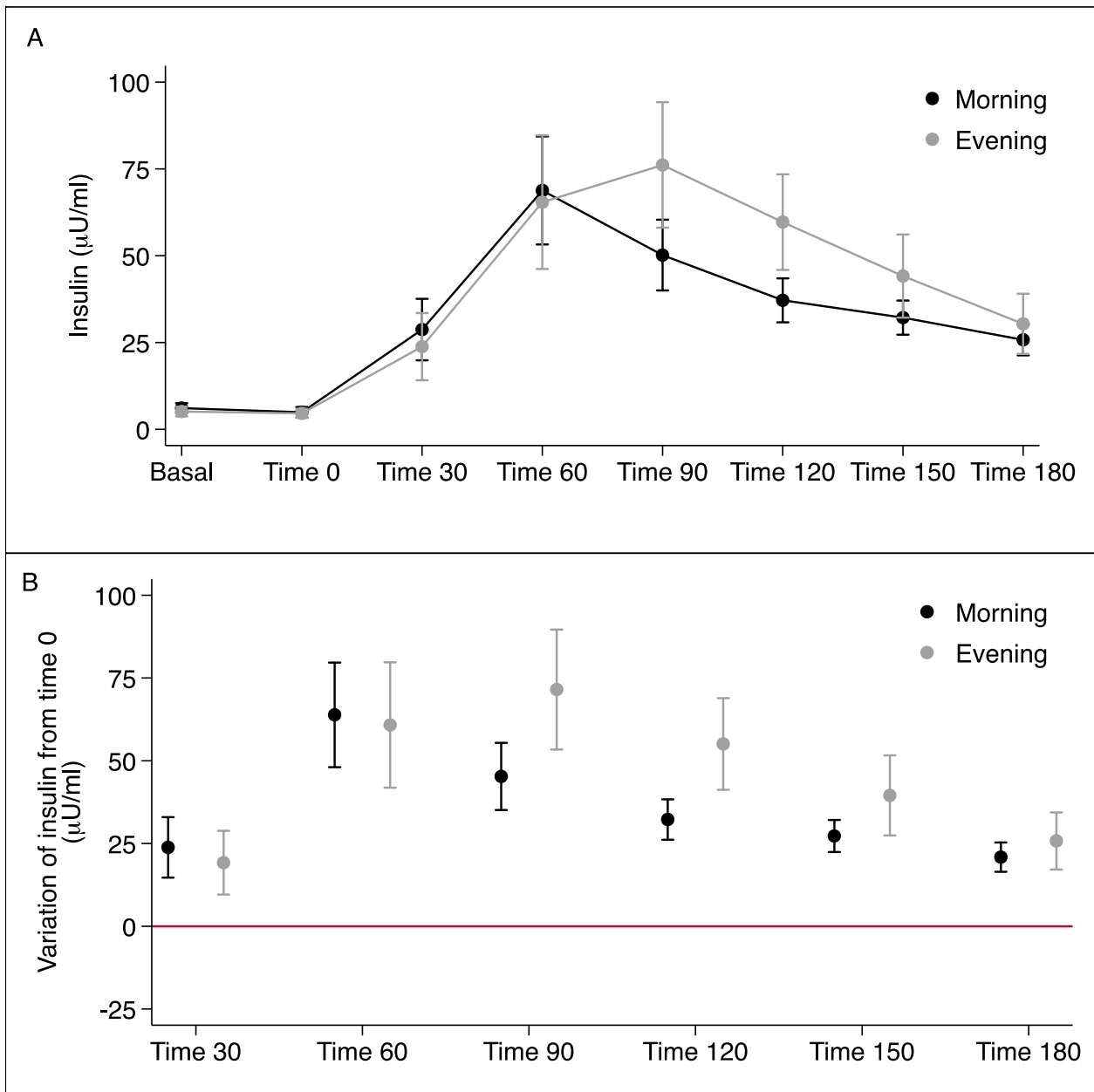
The same time schedule was adopted in the case of the morning meal (at 8:00 pm).

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



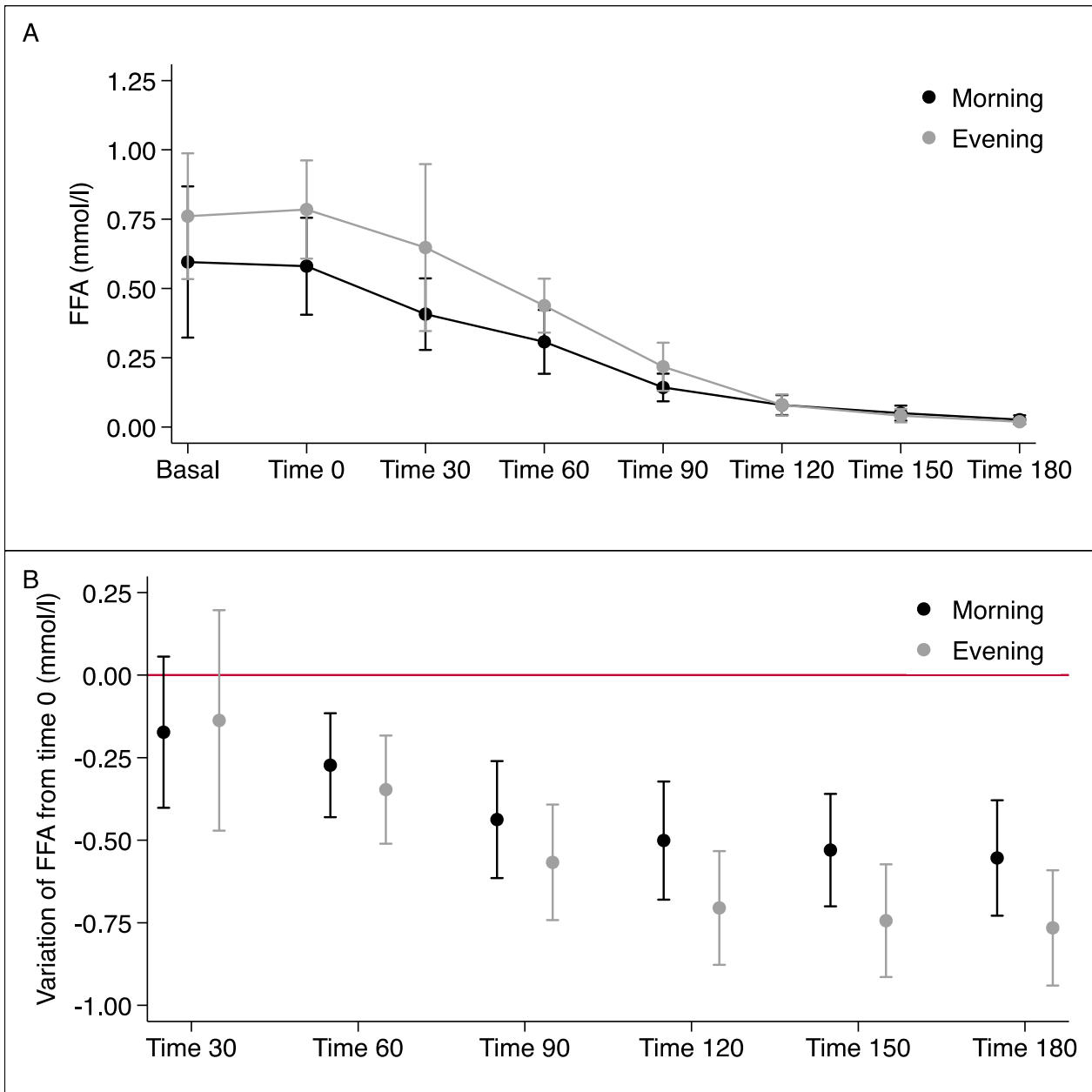
Mean glucose values at the different time points (panel A) and variation of glucose from time 0 (black circles) and evening group (grey circles) (panel B). Whiskers indicate the 95% CIs (panel B).

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



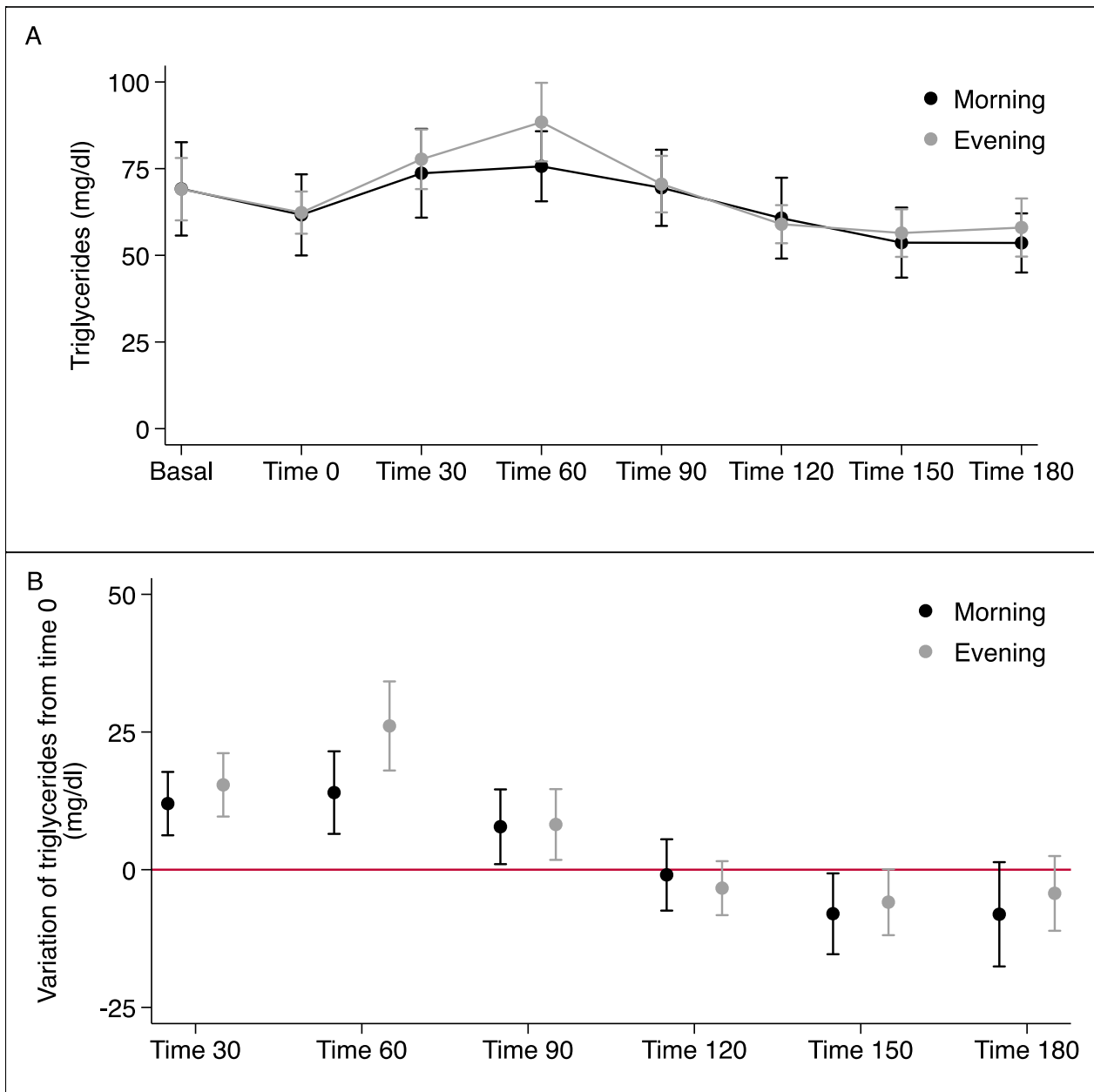
Mean insulin values at the different time points of insulin from time 0: changes from the value at time 0 (black dot) are indicated. Change dot whiskers indicate the 95% CIs (panel

Figure 4. Mean FFA at different time points (panel A) and variation of FFA from time 0 (panel B).



Mean FFA values at the different time points (panel A) and variation of FFA from time 0: morning (black) and evening (grey) groups. Error bars indicate the 95% ACaB (panel B).

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 dots) for the morning group and the 0 mg (grey dots) for the evening group. The whiskers indicate the 95% CIs (panel B).

Table 1. Calorimetric variables before and after morning and evening meals

	Morning	Evening	p-value
Number	20	20	
Fasting RMR (kcal)	1588. [1464.9;1711.7]	1518. [1407.7;1628.3]	0.098
Aftermeal RMR (kcal)	1916. [1791.5;2040.5]	1755. [1648.0;1862.0]	<0.001
DIT (kcal)	327.4 [279.0;375.8]	237.0 [195.1;278.9]	0.003
Fasting ¹ RMR/kg FFM	30.2 [27.8;32.6]	29.1 [27.0;31.2]	0.180
Aftermeal RMR/kg FFM	36.7 [34.6;38.8]	33.7 [31.4;36.0]	<0.001
DIT ¹ (kcal/kg FFM)	6.46 [5.16;7.76]	4.62 [3.55;5.69]	0.003
Fasting RQ	0.87 [0.85;0.89]	0.80 [0.78;0.82]	<0.001
Aftermeal RQ	0.90 [0.89;0.91]	0.85 [0.82;0.88]	0.002
RQ Difference	0.03 [0.01;0.05]	0.05 [0.02;0.08]	0.055
Fasting CHO oxidation	0.13 [0.10;0.16]	0.05 [0.02;0.08]	<0.001
Aftermeal CHO oxidation	0.20 [0.18;0.22]	0.12 [0.08;0.16]	<0.001
CHO oxidation difference	0.07 [0.05;0.09]	0.08 [0.05;0.11]	0.856
Fasting fat oxidation	0.01 [0.01;0.02]	0.04 [0.03;0.05]	<0.001
Aftermeal fat oxidation	0.01 [0.00;0.02]	0.03 [0.02;0.04]	0.006
Fat oxidation difference	-0.01 [-0.01;0.00]	-0.01 [-0.03;0.01]	0.116

Mean [95% CI]; p calculated by paired data

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

¹energy expenditure related in relation to fat free mass

Table 2 Estimates of morning effect adjusted for general linear model (GLMs)

	Effects	95% CI	p-value
RMR ¹ (kcal)	90.5	[40.4, 140.6]	<0.00
RMR ² (kcal/kg FFM)	1.84	[0.81, 2.87]	<0.00
RQ	-0.02	[-0.04, 0.01]	0.035
CHO oxid ³ (g/min)	0.00	[-0.02, 0.02]	0.848
Fat oxid ³ (g/min)	0.01	[-0.00, 0.02]	0.089
Glucose ⁴ (mmol)	-1800.1	[-2564.1, -1036.0]	<0.00
Log insulin ⁴ AUC (h)	-0.19	[-0.30, 0.07]	0.001
Log insulin ⁴ AUC	0.83	[0.74, 0.93]	0.001
FFA AUC (h)	-16.1	[-30.2, 0.09]	0.024
Log triglyceride ⁵ (mmol)	-0.08	[-0.21, 0.05]	0.230
Log triglyceride ⁶ AUC	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

¹morning-DIT - Thermogenesis vs evening-DIT

²RMR calculated in relation to fat free mass

³Morning delta minus evening delta

⁴Morning AUC - Evening AUC

⁵Estimated effects expressed as difference in log

⁶Estimated effects expressed as ratio.