Differential Islet and Incretin Hormone Responses in Morning vs. Afternoon after Standardized Meal in Healthy Men

Ola Lindgren, Andrea Mari, Carolyn F. Deacon, Richard D. Carr, Maria Sörhede Winzell, Jenny Vikman, Bo Ahrén

Department of Clinical Sciences, Lund University, Lund, Sweden (O.L., M.S.W., J.V., B.A.), National Research Council, Padua, Italy (A.M.), Dept of Biomedical Sciences, Copenhagen University, Copenhagen, Denmark (C.D.), Merck A/S, Glostrup, Denmark (R.D.C.)

Precis: Insulin secretion to equivalent meals is faster in the morning than in the afternoon and this corresponds to more pronounced incretin responses in the morning

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Corresponding author:

Dr Bo Ahrén

Department of Clinical Sciences, Lund

B11 BMC

SE-221 84 LUND, Sweden

Phone: +46462220758

Fax +46462220757

e-mail: bo.ahren@med.lu.se

Abstract

Context: The insulin response to meal ingestion is more rapid in the morning than in the afternoon. Whether this is explained by a corresponding variation in the incretin hormones is not known.

Objective: Assess islet and incretin hormones after meal ingestion in the morning versus afternoon.

Design, Settings and Participants: Ingestion at 8am and at 5pm of a standardized meal (524 kcal) in healthy lean males (n=12) at a University Clinical Research Unit.

Main Outcome Measures: 1)Early (30 min) area under the curve (AUC30) of plasma levels of insulin and intact (i) and total (t) glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) after meal ingestion. 2)Estimation of ß-cell function by model analysis of glucose and C-peptide.

Results: Peak glucose was lower in the morning than in the afternoon (6.1±0.2 vs. 7.4±0.3 mmol/l, P=0.001). $AUC30_{insulin}$ (4.9±0.6 vs 2.8±0.4 nmol/l*30 min; P=0.012), $AUC30_{tGLP\text{-}1}$ (300±40 vs. 160±30 pmol/l*30 min, P=0.002), $AUC30_{iGIP}$ (0.7±0.1 vs. 0.3±0.1 nmol/l* 30 min, P=0.002) and $AUC30_{tGIP}$ (1.1±0.1 vs. 0.6±0.1nmol/l*min, P=0.007) were all higher in the morning. $AUC30_{iGLP\text{-}1}$ (r=0.68, P=0.021) and $AUC39_{iGIP}$ (r=0.78, P=0.001) both correlated to $AUC30_{insulin}$. Model analysis of ß-cell function showed a higher first hour potentiation factor in the morning (P=0.009). This correlated negatively with the 60 min glucose level (r=-0.63, P<0.001).

Conclusions: The early release of GLP-1 and GIP are more pronounced in the morning than in the afternoon. This may contribute to the more rapid early insulin response, more pronounced potentiation of ß-cell function and lower glucose after the morning meal.

Introduction

It is known that the early (30 or 45 min) insulin secretion after oral glucose or meal ingestion is significantly higher in the morning than in the evening (1,2). This is a possible explanation for the more rapid glucose clearance from the circulation in the morning (1-4), because the early insulin response is of importance for the rapid reduction in hepatic glucose output after meal ingestion (5,6).

A potential explanation underlying the enhanced early insulin secretion in response to identical oral challenges in the morning versus the afternoon could be corresponding diurnal variations in the release of the incretin hormones, glucagon-like peptide 1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP). These hormones are important factors for insulin release after oral ingestion of glucose, amino acids, fat or mixed meals (7,8). However, no clear diurnal pattern of the incretin hormones has been observed (9-12), but these studies have not compared the incretin responses to identical meals given at different times during the day and only the total concentrations of the incretins have been assessed. Since both GLP-1 and GIP are rapidly degraded by the enzyme dipeptidyl peptidase-4 (DPP-4) after their release, concentrations of both the intact and degraded forms of the hormones need to be measured in order to draw full conclusions about their physiology (7).

To clarify whether incretin responses to identical meals are different in the morning versus in the afternoon, we compared the incretin response to a standardized meal in the morning (8am) and in the afternoon (5pm) in healthy men. We measured both the intact and total concentrations of GLP-1 and GIP by the use of N- and C-terminally directed antisera to allow conclusions both on the potential differences in incretin hormone secretion and on potential variability in DPP-4

activity in the morning versus the afternoon. To further assess the potential diurnal variability in incretin degradation, we also determined plasma activity of DPP-4. We also estimated ß-cell function by model analysis of glucose and C-peptide data after meal ingestion (12,13).

Methods

Subjects. Twelve lean male volunteers aged 20-30 (22.4±0.6) years and with a BMI of 20-25 kg/m^2 (22.7±0.4 kg/m^2) were were recruited through advertisements. They had no personal history of diabetes or gastrointestinal disease and they were not taking any medication. The study was approved by the ethics committee of Lund University, Sweden, and all subjects gave written informed consent before entry into the study.

Study protocol. After an overnight fast with no food after 10pm, subjects were provided with an antecubital vein catheter. After two baseline samples were collected, a standard breakfast was rapidly ingested at 8am. Further blood samples were taken throughout a 300 min study period. In the afternoon at 5pm, the same protocol was repeated. The subjects fasted between the two meals. The meal consisted of 524 kcal (19% protein, 18% fat and 63% carbohydrates) as rye and wheat bread (60g), margarine (10g), ham (15g), cheese, (15g), orange juice (150g), green pepper (40g), light sour milk, fat 0,5% (200g) and muesli with fruit (40g)

Analyses. Blood samples, collected in chilled tubes containing EDTA (7.4 mmol/l) and aprotinin (500 KIU/ml; Novo Nordisk, Bagsvaerd, Denmark), were immediately centrifuged at 4°C and plasma was frozen at –20°C. Glucose was measured using the glucose oxidase method. Insulin, C-peptide and glucagon were analysed with double antibody radioimmunoassay (Linco Research, St Charles, MO). Non-esterified free fatty acids (FFAs) were analyzed by colorimetric assays (Wako Chemicals GmbH, Neuss, Germany). Blood samples for determining incretins were collected into chilled tubes containing EDTA and aprotinin with addition of diprotin A (0.1 mmol/l; Bachem, Bubendorf, Switzerland). Intact and total GLP-1 and GIP were determined with radioimmunoassay. In the assay for intact GLP-1, N-terminal specific guinea-pig anti-GLP-1 was

used (Linco) and in the assay for total GLP-1, a C-terminally directed antiserum 89390 was used (14). In the assay for intact GIP, an antiserum (no 98171), which is specific for the intact N-terminus of GIP, was used, and in the assay for total GIP, the C-terminally directed antiserum R65 was used (15). DPP-4 activity was assessed kinetically using Gly-Pro-*p*-nitroaniline (1 mmol/l) as substrate (16).

Model analysis. Beta-cell function parameters were obtained by model analysis of glucose and C-peptide data, as previously described (12,13). Insulin secretory rate (ISR) was determined by C-peptide deconvolution (17). The model expresses ISR as the sum of two components. The first component describes the dose response between ISR and glucose concentrations. The mean slope of this dose-response function is denoted ß-cell glucose sensitivity. ISR at reference glucose levels of 4.5 mmol/l was calculated from the ß-cell dose response for estimating basal insulin secretion. The second component of ISR is the potentiation factor, which encompasses signals which modulate the dose response relation between ISR and glucose. The potentiation factor is set to have a mean value of 1 over the 300 min of the study; values above 1 means potentiation above average during the study. The ratio between the potentiation factor value at 60 min or 120 min, respectively, and that at time 0 is called the potentiation factor ratio. The model also quantifies a dynamic dependence of insulin secretion on the rate of change of glucose concentration, which is called ß-cell rate sensitivity. This dynamic factor is proportional to the time-derivative of glucose concentration when glucose rises and is zero otherwise and accounts for the enhancement of insulin secretion proportional to the rate of change of the plasma glucose.

Statistics. Means±SEM are shown. Areas under curves (AUC) were calculated by the trapezoid rule for suprabasal levels after meal ingestion. Separate AUCs were calculated for the early

(initial 30 min) and the total (entire 300 min) responses. This separation of responses in early and total was decided a priori based on previous findings that insulin secretion is progressively increased during these first 30 min after oral nutrient ingestion (18). Wilcoxon matched paired test was used for tests of significance between variables obtained during morning and afternoon meal ingestion. Spearman regression coefficients were obtained to estimate correlation.

Results

Glucose and FFA responses to the meal challenges

Fig. 1 shows that after the meal ingestion, the initial 30 min rise in plasma glucose was identical in the morning and afternoon. At 30 min, the glucose levels peaked in the morning, whereas in the afternoon, glucose continued to rise throughout a 60 min period. Peak glucose levels and total $AUC_{glucose}$ were lower in the morning than in the afternoon (6.1±0.2 vs. 7.4±0.3 mmol/l, P=0.001; 193±17 vs. 269 mmol/l*300 min, P=0.009). Baseline FFA was lower in the morning than in the afternoon (0.23±0.04 vs. 0.51±0.05 mmol/l, P=0.007). Meal ingestion reduced FFA levels similarly in the morning and in the afternoon (Fig. 1). The nadir FFA levels were lower in the morning than in the afternoon (0.03±0.01 vs. 0.12±0.05 mmol/l, P=0.009).

Insulin and C-peptide responses to the meal challenges

The insulin responses to the meal challenges were more rapid in the morning than in the afternoon (Fig. 2). Already after 5 minutes, insulin was significantly higher in the morning than in the afternoon (74±6 vs. 57±6 pmol/l, P=0.001). This difference persisted for 30 min; $AUC30_{insulin}$ was higher in the morning (4.9±0.6 vs 2.8±0.4 nmol/l*30 min, P=0.01). Following a peak after 30 min, insulin levels declined in the morning, whereas in the afternoon, they continued to rise and peaked after 60 minutes. Commensurate with the higher insulin levels after the initial 30 min in the afternoon, the total $AUC_{insulin}$ was lower in the morning than in the afternoon (24.8±3.3 vs. 34.4±4.1 nmol/l* 30 min, P=0.009). C-peptide levels after meal ingestion mirrored the insulin levels (Fig. 2) with a more rapid release in the morning with a higher value already after 5 minutes (0.62±0.04 vs. 0.47±0.04 nmol/l, P=0.005). $AUC30_{C\text{-}peptide}$ was higher in the morning than in the afternoon (21.1±1.2 vs. 12±1.1 nmol/l*30 min, P=0.005). C-peptide concentrations declined in the morning after the first 30 min, whereas in the afternoon,

they remained high until 120 min. Total $AUC_{C\text{-}peptide}$ was lower in the morning than in the afternoon (196±30 nmol/l*min vs. 334±20 nmol/l*30 min, P=0.007).

Model analysis

Table 2 shows the assessment of ß-cell function. The insulin secretory rate (ISR) at the glucose reference of 4.5 mmol/l and the ß-cell glucose sensitivity did not differ between morning and afternoon. The potentiation factor was higher in the morning than in the afternoon throughout the initial 90 min after meal ingestion, while the opposite was true after the 90 min (Fig. 2, Table 2). Moreover, the mean potentiation factor in the first hour correlated negatively with the 60 min glucose levels (r=-0.63, P<0.001; Fig. 2). There was also a clear trend for the rate sensitivity to be higher in the morning (1.3±0.5 nmol/m^2/mol/l) than in the afternoon (0.36±0.08 nmol/m^2/mol/l), although this did not reach significance (P=0.08). ISR was higher in the morning during the early 30 min phase (P=0.008; Fig. 2), whereas from min 45 to 180, ISR was lower in the morning; the 300 min total ISR was significantly lower in the morning than in the afternoon (P=0.03).

Glucagon response to the meal challenges

Fig. 2 also shows that in the morning, there was a rapid rise in glucagon levels after meal ingestion, where a significant difference compared to the afternoon was evident already after 5 min (81±7 vs. 71±6 ng/l; P=0.001). Furthermore, the elevated glucagon levels after meal ingestion were sustained for 180 min after meal ingestion. In contrast, in the afternoon, glucagon levels declined after an initial 15 min peak to reach a nadir after 90 min, whereafter they returned to baseline. Whereas $AUC30_{glucagon}$ did not differ between the two tests, total $AUC_{glucagon}$ was higher in the morning than in the afternoon (+2.4±0.9 versus. -0.7±0.6 ng/ml*30 min, P=0.02). $AUC30_{glucagon}$ did not correlate to AUC30 for the incretin hormones.

Incretin hormone responses to the meal challenges

GLP-1. Fig. 3 shows that both intact and total GLP-1 rose rapidly after meal ingestion. During the initial 30 min after meal ingestion, the increase was higher in the morning than in the afternoon. Thus, the peak levels of GLP-1 were significantly higher in the morning, both for iGLP-1 (12±3 vs. 9±2 pmol/l, P=0.03) and for tGLP-1 (35±4 vs. 28±2 pmol/l, P=0.03). After the initial 30 min, the levels of intact and total GLP-1 did not differ between the two tests. $AUC30_{iGLP-1}$ was not significantly different between the two tests, whereas $AUC30_{tGLP-1}$ was higher in the morning (300±40 vs. 160±30pmol/l*30 min, P=0.002). In univariate analysis of the whole data set, $AUC30_{iGLP-1}$ correlated significantly with $AUC30_{insulin}$ (r=0.68, P=0.021).

GIP. Fig. 3 shows that also iGIP and tGIP increased following meal ingestion. As for GLP-1, the rapid and initial 30 min increases in iGIP and tGIP were higher in the morning than in the afternoon. Thus, as early as 5 min after the meal, there were significantly higher iGIP levels in the morning than in the afternoon (14±3 vs. 9±2 pmol/l, P=0.003) and a similar difference was observed at 10 min (32±3 vs. 13±2 pmol/l, P<0.001). AUC30 for both iGIP and tGIP were significantly higher in the morning than in the afternoon (0.7±0.1 vs. 0.3±0.1nmol/l*30 min for $AUC30_{iGIP}$, P=0.002, and 1.1±0.1 vs. 0.6±0.1nmol/l*30 min for $AUC30_{tGIP}$, P=0.007). After the initial 30 min, there was no difference in iGIP or tGIP levels between morning and afternoon. In univariate analysis of the whole data set, $AUC30_{iGIP}$ correlated significantly with $AUC30_{insulin}$ (r=0.78, P=0.001).

DPP-4. Plasma DPP-4 activity before meal ingestion was 540±323 mmol/min/mg protein/ml in the morning and 520±36 mmol/min/mg protein/ml in the afternoon (NS). Plasma DPP-4 activity did not change after meal ingestion throughout the 5-h study period.

Discussion

In this study, we confirm previous reports showing that the insulin response to identical meals is higher in the morning than in the afternoon (1-3). Furthermore, we show that it is the early (30 min) insulin response which is higher in the morning. $AUC30_{insulin}$ was 1.75-fold higher and ISR during the first 30 min was 1.5-fold higher in the morning than in the afternoon. The main novel finding in our study is that this more rapid early insulin response to the meal in the morning versus in the afternoon is accompanied by a correspondingly faster incretin hormone response in the morning. We determined concentrations of both intact and total forms of the incretins and we found that levels of both intact and total forms of GLP-1 and GIP were higher in the morning than in the afternoon during the first 30 min after meal ingestion. These results would suggest that a mechanism underlying the more rapid early insulin response in the morning may be a more rapid incretin response. This conjecture is supported by the significant correlation between the AUC for the incretins and insulin. It should be emphasized that the conclusion is based on a close association between the incretin and insulin responses. However, the conclusion is strong since the association was consistent and also supported by the prediction that higher GLP-1 and GIP levels would result in a higher potentiation of ß-cell function. Future studies could be testing the hypothesis further by inhibiting the incretin hormone action with incretin hormone receptor antagonists.

In contrast to the difference between morning and afternoon in regard to 30 min incretin responses, the concentration curves after this time point were almost identical, indicating that the diurnal difference in incretin response to meal challenge is evident only for the rapid and early responses. This is similar for insulin; insulin levels being higher in the morning only during the

first 30 min after meal ingestion, although after this period, they were actually lower in the morning. This may be explained by the magnitude of the prandial glucose excursions, since glucose levels were higher after 30 min in the afternoon; the higher glucose levels thus enhancing insulin secretion.

The model analysis of glucose and C-peptide data for the estimation of ß-cell function showed that the time pattern of the potentiation factor differed. Thus, during the initial 90 min after meal ingestion, the potentiation factor was higher in the morning than in the afternoon. This factor represents the potentiation of the glucose-induced insulin response at a given time and can, for instance, be caused by neural effects, incretin hormones or amplifying changes within the ß-cell (12). For example, GLP-1 has previously been shown to augment the potentiation factor (19). The higher potentiation factor in the morning compared to the afternoon supports the idea that the higher incretin levels may cause higher insulin secretion in the morning, which is an effect assessed as the potentiation. Interestingly, the potentiation factor correlated negatively to the 60 min glucose level after mixed ingestion, which may suggest that the higher early insulin secretory rate in the morning versus in the afternoon contributes to the faster clearance of glucose in the morning. The time-related pattern of the responses of total versus intact incretin hormones did not seem to differ between morning and afternoon. Since the relation between intact and total incretin hormone concentrations reflects the degradation process of the hormones, this would suggest that degradation of incretin hormones does not show a diurnal variation. This is also supported by the analyses of DPP-4 activity levels in plasma, which did not differ in morning versus afternoon. The more rapid incretin hormone responses in the morning than in the afternoon, therefore, seems to reflect mainly enhanced secretion from the gut. The mechanism of the higher incretin hormone responses to breakfast in the morning versus the afternoon is not

known. A possibility would be diurnal variation in K and L cell activity, which in turn could be due to neural effects. A second possibility would be diurnal variation in gastric emptying rate, since this rate has been shown to correspond to changes in GLP-1 (but not GIP) secretion (7). These possibilities need now to be examined in more detail.

The difference in incretin hormone responses to breakfast between morning and afternoon was quantitatively small and may be insufficient to explain the entire difference in insulin release after a meal. This conclusion would also be supported by the time pattern of the potentiation factor, which was higher in the morning than in the afternoon well after the time of difference in incretin levels had passed. Other factors in addition to incretin levels may therefore also contribute. One possibility could be neural factors; it is well known that the initial insulin secretion after meal ingestion is neurally mediated during the so-called cephalic phase (23). Our results showing that insulin levels were higher in the morning already at 5 min after meal ingestions may therefore suggest a more rapid cephalic response in the morning. Besides a diurnal pattern of the cephalic phase, another explanation for such a difference would be differences from normal life since a second breakfast was served at dinner time. Although this unlikely, it remains to be tested in a separate study when also a lunch is served.

It is also possible that the ß-cell response to stimuli may show a diurnal pattern. In support of this, previous studies have shown a higher insulin response to intravenous glucose or intravenous sulphonylurea in the morning than in the afternoon/evening (1,21,22). Hypothetically, the ß-cell response to incretins may also display a similar diurnal variation, contributing to the higher insulin response in the morning. A potential diurnal pattern of the insulin response to incretins has, however, not been studied.

Insulin sensitivity is known to be inversely related to insulin secretion, such that reduction in insulin sensitivity upregulates insulin secretion (24). A potential explanation behind the higher insulin secretion in the morning would therefore be a lower insulin sensitivity than in the afternoon. Conversely, if insulin sensitivity is lowered during the afternoon, the difference in insulin secretion between morning and afternoon would be underestimated in this study. Furthermore, we have previously shown that incretin hormone secretion is reduced in subjects with insulin resistance (24). Hence, a different insulin sensitivity in the morning versus afternoon might therefore contribute to differences in islet and incretin hormone secretion. We did not directly measure insulin sensitivity in this study. Instead we have assessed insulin sensitivity indirectly using several different surrogates, such as HOMA-IR, Stumvoll´s method with and without BMI inclusion, Matsuda index (using 2- and 5-hour data) and OGIS indices (25; data not shown). When using these surrogates, no differences were observed between morning and afternoon. Although these indices are indirect, we conclude that there is no clear difference in insulin sensitivity which would have explained the differences in insulin or incretin hormone secretion between morning and afternoon in this study.

The diurnal variation for cortisol would be another possibility behind the higher insulin secretion in the morning, because cortisol levels are elevated during morning hours (26) and upregulated insulin secretion would be secondary to the insulin counterregulation by cortisol. However, such an effect would have resulted in lower insulin sensitivity using the surrogates, which was not observed, and cortisol, if anything, would have inhibited insulin secretion (27).

The second novel finding in this study was that the glucagon response to morning or afternoon meal ingestion also differed. Glucagon levels were sustained in the morning, whereas in the afternoon, they were reduced to reach a nadir after 90 min. This difference might be explained by the higher in insulin levels in the afternoon after the initial 30 min, since insulin is a powerful inhibitor of glucagon secretion (28). The difference might also be dependent on the differences in glucose levels, since these levels were lower in the morning which would have increased glucagon secretion. However, also the rapid glucagon response to meal ingestion showed a diurnal pattern and was higher in the morning, which was at a time when glucose levels did not differ. Instead, the higher early iGIP response to meal ingestion in the morning may have contributed to this higher glucagon, considering that GIP stimulates glucagon secretion during normoglycemia in humans (29). However, the potential diurnal variation of glucagon levels needs to be explored in more detail. Nevertheless, differences in glucagon can not explain the differences in glucose tolerance when comparing morning with afternoon, since glucagon levels were actually higher in the morning, at a time when glucose levels were lower.

In the study, we also measured FFA levels and found, as has been reported in previous studies, that both basal and nadir levels of FFA were lower in the morning than in the afternoon (1,2,30). This may contribute to the better glucose tolerance in the morning, because FFA reduces glucose uptake and phosphorylation in skeletal muscles (31) and impairs the insulin-induced suppression of glucose production by the liver (32), although overall insulin sensitivity does not seem to differ between morning and afternoon.

In conclusion, we have found a faster 30 min insulin secretion in response to an identical meal challenge in the morning than in the afternoon in healthy subjects due to a higher potentiation of

glucose-stimulated insulin secretion in the morning. This was associated with a higher 30 min GLP-1 and GIP response in the morning than in the afternoon. Therefore, we suggest that the rapid early insulin response which is evident in the morning may be explained, in part, by a more rapid incretin hormone response. However, also other factors may contribute, such as a more rapid cephalic response or a higher intrinsic ß-cell response to external stimuli in the morning.

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Figure legends

Fig 1. Plasma glucose and FFA levels after ingestion of a standardized mixed meal (524 kcal) in the morning (8 am) and afternoon (5 pm) in healthy men (n=12). Results are presented as mean±SEM with n=12 in each group.

Fig 2. Plasma insulin, C-peptide and glucagon levels, the potentiation factor of ß-cell function as assessed by model analysis of glucose and C-peptide data, and the insulin secretory rate after ingestion of a standardized mixed meal (524 kcal) in the morning (8 am) and afternoon (5 pm) in healthy men (n=12). Results are presented as mean±SEM. Lower left panel shows the relationship between the mean potentiation factor in the first hour and the 60 min glucose levels after ingestion of a standardized mixed meal (524 kcal) in the morning (8 am) and afternoon (5 pm) in the subjects.

Fig 3. Plasma levels of intact and total GLP-1 and intact and total GIP after ingestion of a standardized mixed meal (524 kcal) in the morning (8 am) and afternoon (5 pm) in healthy men (n=12). Results are presented as mean±SEM.

Table 1. Plasma levels of glucose, FFA, islet and incretin hormones in the morning (8am) and the afternoon (5pm) in healthy men (n=12) before meal was ingested. Results are presented as mean±SEM. P represents the probability level of random distribution between morning and afternoon, ns = not significantly different.

	Morning	Afternoon	P
Glucose (mmol/l)	4.6±0.1	4.3±0.1	0.002
FFA (mmol/l)	0.22±0.04	0.51±0.05	0.007
Insulin (pmol/l)	54±5	45±5	ns (0.11)
C-peptide (nmol/l)	0.52±0.04	0.42±0.03	0.03
Glucagon (pg/ml)	69±6	64±5	ns (0.08)
iGLP-1 (pmol/l)	7±3	5±2	0.03
tGLP-1 (pmol/l)	16±1	19±2	ns (0.16)
iGIP (pmol/l)	12±2	16±2	ns (0.11)
tGIP (pmol/l)	8±2	8±2	ns (0.38)

Table 2. Model analysis of glucose and C-peptide data after ingestion of a standardized mixed meal (524 kcal) in the morning (8 am) and afternoon (5 pm) in healthy men (n=12). Results are presented as mean±SEM. P represents the probability level of random distribution between morning and afternoon, ns = not significantly different. ISR = insulin secretory rate.

	Morning	Afternoon	P
ISR at glucose reference of 4.5 mmol/l (pmol/min/$m^{2)}$	140±17	163±17	ns (0.11)
ISR during time 0-30 min (nmol/m^2)	8.0±0.9	5.2±0.6	0.008
Total ISR (nmol/ m^2)	47±5	62±3	0.03
ß-cell glucose sensitivity (pmol/min x mmol/l/m^2)	123±18	142±10	ns (0.15)
ß-cell rate sensitivity (nmol/m^2/mol/l)	1.31±0.51	0.36±0.08	ns (0.08)
Potentiation factor ratio at 60 min	3.1±0.3	2.0±0.3	0.009
Potentiation factor ratio at 120 min	2.4±0.2	3.4±0.4	0.02

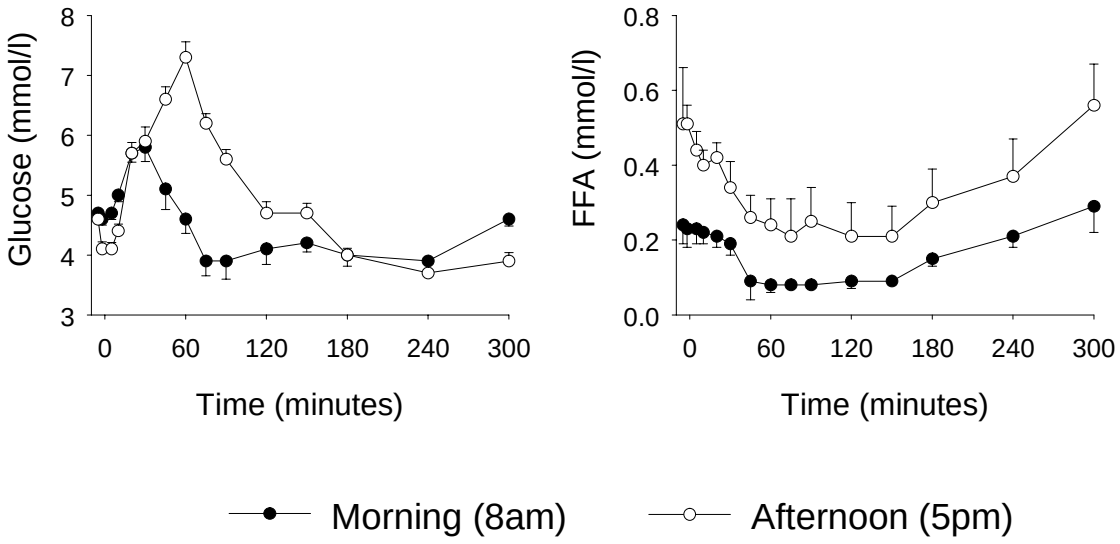
Glucose (mmol/l)
8
7
6
5
4
3
0
60
120
180
240
300
Time (minutes)
FFA (mmol/l)
0.8
0.6
0.4
0.2
0.0
0
60
120
180
240
300
Time (minutes)
Morning (8am)
Afternoon (5pm)

Fig. 1

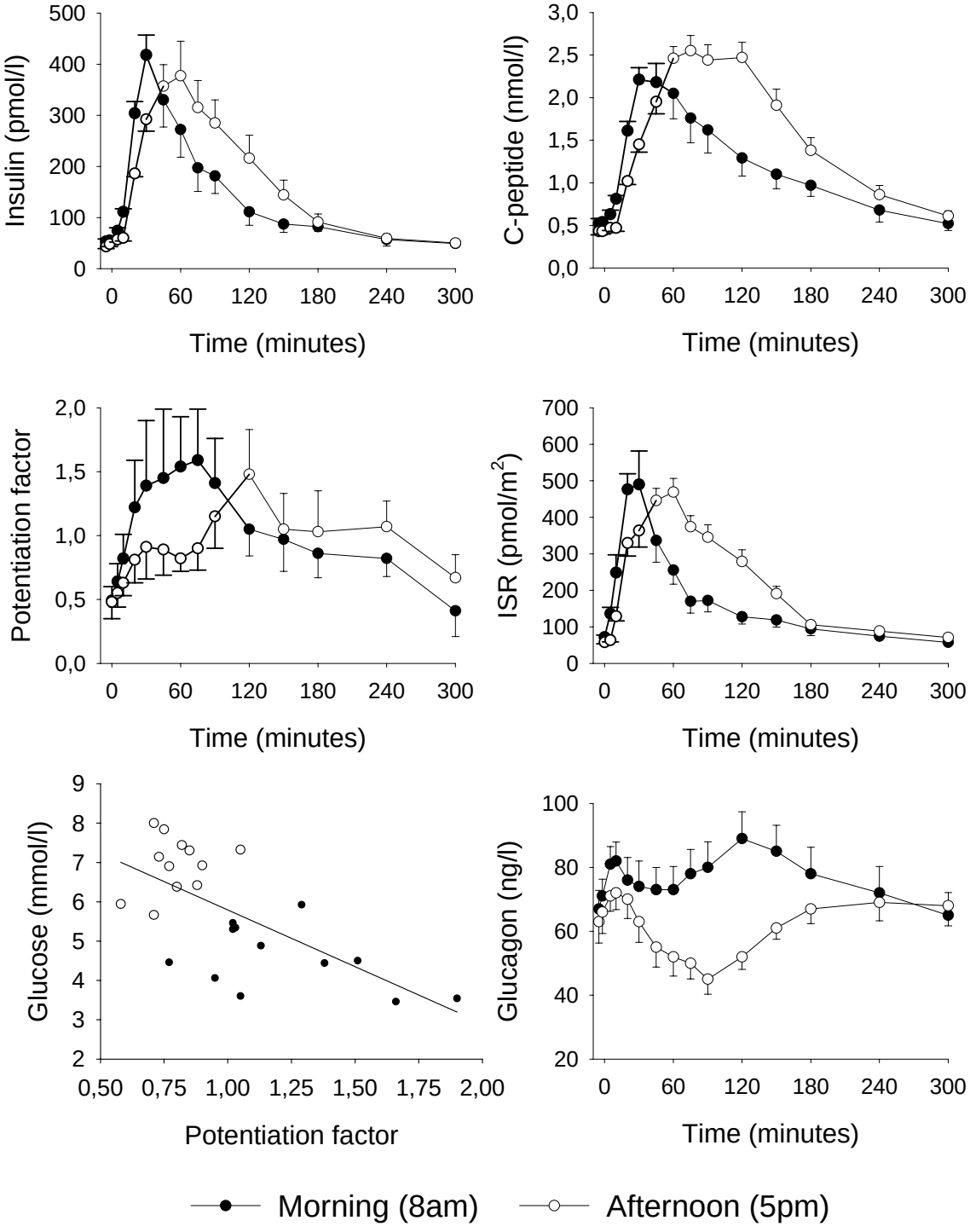
Insulin (pmol/l)
C-peptide (nmol/l)
Potentiation factor
ISR (pmol/m2)
Glucose (mmol/l)
Glucagon (ng/l)
Time (minutes)
Potentiation factor
Morning (8am)
Afternoon (5pm)

Fig. 2

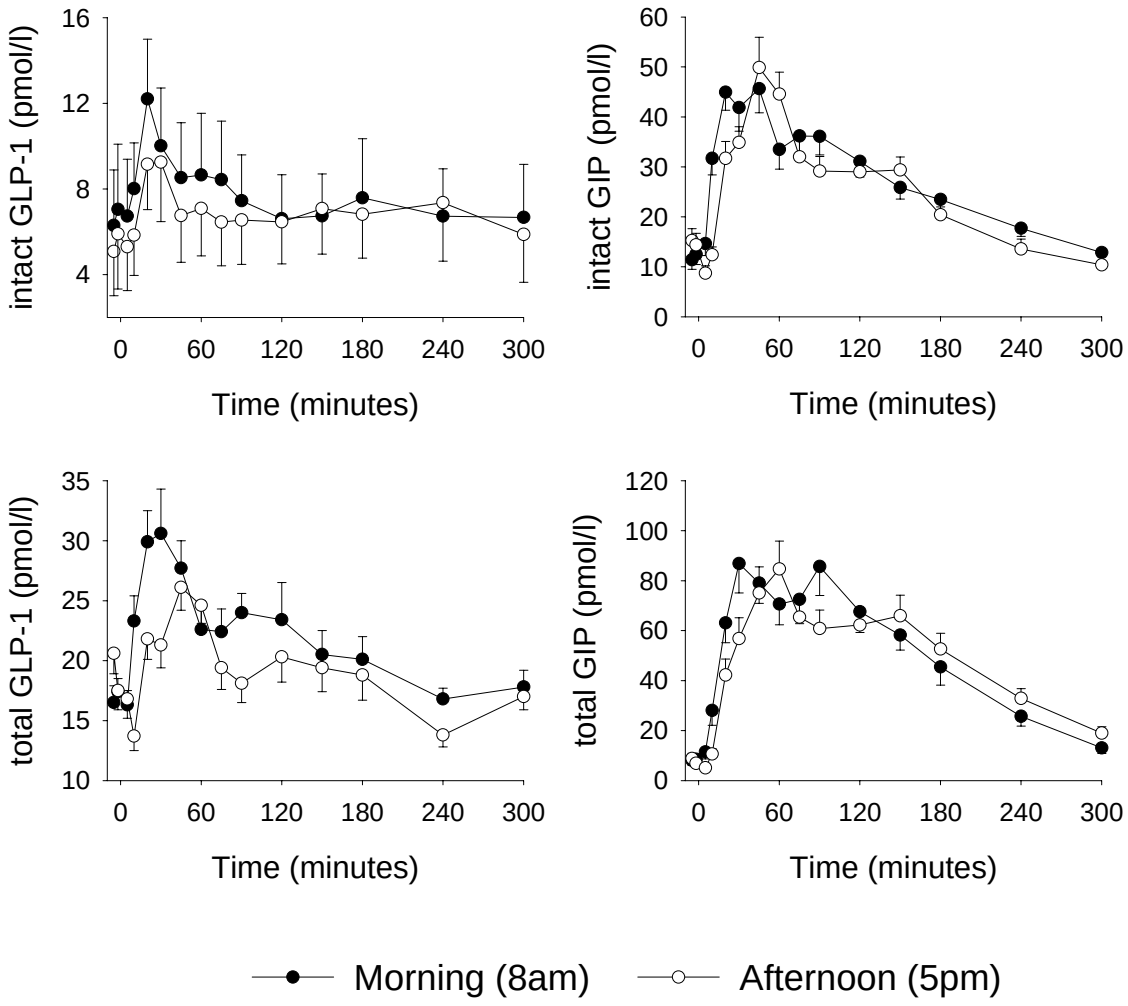
intact GLP-1 (pmol/l)
16
12
8
4
0
60
120
180
240
300
Time (minutes)
intact GIP (pmol/l)
60
50
40
30
20
10
0
Time (minutes)
total GLP-1 (pmol/l)
35
30
25
20
15
10
Time (minutes)
total GIP (pmol/l)
120
100
80
60
40
20
Time (minutes)
Morning (8am)
Afternoon (5pm)

Fig. 3