The impact of fasting and treatment omission on susceptibility to hypoglycaemia in children and adolescents with GH and cortisol insufficiency

Short title: Fasting in GH and cortisol deficient children

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Key words
Hypoglycaemia, GH, Cortisol, Children, Adolescents
Summary

Objective
Hypoglycaemia may be a frequent occurrence in young GH deficient patients and so we studied the response to fasting in children and adolescents with GH and/or cortisol deficiency.

Methods
20 patients (2-18y) fasted for 14h (2200-1200h) on 2 occasions as part of a randomised cross-over study. 14 had pituitary hormone deficiency (PHD) including GH deficiency (GHD). 7 of the 14 were ACTH sufficient (PHDC+) and 7 ACTH deficient (PHDC-). 6 had primary adrenal failure (PAF). Subjects administered or omitted their normal dose of evening GH and/or morning hydrocortisone. Glucose, insulin, GH, cortisol, ketones and catecholamines were measured at 04.00h and regularly from 0700h to 1200h. Insulin sensitivity was assessed by HOMA and hypoglycaemia defined as a blood glucose less than or equal to 3.3 mmol/l.

Results
BG was related to age and Body Mass Index on treatment but no subject became hypoglycaemic on or off therapy prior to 0700h. 5 children (aged 3,4,7,8 and 11y) were hypoglycaemic between 0700h and 1200h off treatment. There was a positive relationship between GH AUC and minimum BG in patients with PHD on treatment ($r^2=0.45$, $p=0.012$) with increased insulin sensitivity off treatment. Increased cortisol levels were seen in PHDC+ patients off GH ($p<0.001$). A negative relationship was observed between minimum BG and adrenaline ($r^2=-0.37$, $p=0.01$), ketone bodies ($r^2=-0.20$, $p=0.05$) and NEFA ($r^2=-0.35$, $p=0.02$). Noradrenaline levels were reduced in
patients with PHDC-. Low BMI (on treatment) and young age (off treatment) were determinants of low blood glucose levels in a multiple regression model.

Conclusions

Unrecognised overnight hypoglycaemia in children and adolescents on pituitary hormone replacement is uncommon but BG levels quickly become abnormal when treatment and meals are omitted. The insulin antagonistic actions of GH are important in preventing hypoglycaemia. Patients with PHD have altered sympathetic nerve activity.
Introduction

It has been known for many years that children with growth hormone deficiency (GHD) and ACTH deficiency are susceptible to hypoglycaemia before they are treated.\textsuperscript{1,2,3} Young children are particularly vulnerable with reduced gluconeogenesis, increased glucose utilisation, reduced fat mobilisation and ketone body generation potentially contributing to susceptibility. The normal child can fast for approximately 12 hours without any change in blood glucose (BG) concentrations but a more prolonged fast of 30 hours results in values around 3mmol/l \textsuperscript{4}. Lower BG and insulin concentrations are seen in the very young.\textsuperscript{4,5}

A surprising vulnerability of children and adolescents with GHD to hypoglycaemia was reported more recently by Houdjik and colleagues.\textsuperscript{6} In a study designed to determine the pharmacokinetics of GH administered using 2 different delivery devices, GH was omitted for 3 nights and then a single dose (2IU/m\textsuperscript{2}) was given at 1800h. BG was measured at intervals overnight and all 16 subjects were found to have a BG <2.6mmol/l on at least one of the two study nights.

As many as 50\% of children receiving GH treatment in the UK fail to comply fully with treatment.\textsuperscript{7} This can be improved with patients and family education\textsuperscript{8,9} but the fact remains that less than a quarter of adolescents with chronic disorders comply fully with treatment regimens.\textsuperscript{10}

We felt that the possibility of unrecognised overnight hypoglycaemia in our patients with GH and or primary / secondary adrenal insufficiency was an important issue that warranted further study. The findings of Houdjik and colleagues\textsuperscript{6} were intriguing in light of the relative infrequency with which neonates with isolated GHD become hypoglycaemic\textsuperscript{11} and we were keen to establish how quickly poor compliance and missed meals might impact on glucose levels.
The primary aims of this study were, firstly, to establish whether overnight hypoglycaemia was a common occurrence in young people with GH and/or cortisol deficiency on their usual treatment regimen and, secondly, to examine the impact of missing one dose of GH and/or hydrocortisone on BG concentrations during an overnight and morning fast.
Subjects and Methods

Patients

Local Ethical Committee approval was obtained for these studies. 14 children with pituitary hormone deficiency (PHD) and 6 with primary adrenal failure were recruited from the paediatric and adolescent endocrinology clinics at the Royal Victoria Infirmary in Newcastle-upon-Tyne. This was an observational study and we hoped to recruit a similar number of patients to that studied by Houdjik and colleagues. In this study hypoglycaemia had been a striking feature in all 16 patients and we anticipated that we would be able to recruit a similar number from our service, recognising that only some families would agree to participate. Of the patients with PHD, 7 had GH deficiency but were ACTH sufficient (PHDC+) whilst 7 had GH and ACTH deficiency (PHDC-). Patients were aged 3-18 years (median age 11.6 yrs) and all had a body mass index standard deviation score (BMISDS) between 2.5 SD above and below the mean. Patient details are shown in Table 1.

The diagnosis of PHD was made on the basis of the history, clinical examination, biochemical testing and, in the case of 2 children presenting with a craniopharyngioma, the pre-and post operative biochemistry as well as the operation record. Both of these patients had post-operative cranial diabetes insipidus. When interpreting biochemical testing, GH deficiency was defined as a peak GH to insulin-induced hypoglycaemia (ITT) or following glucagon stimulation that was less than 5mcg/l (13mU/l). GH production represents a continuum and we were keen to ensure that children were at the more severe end of this spectrum. ACTH deficiency was defined as a peak cortisol response to ITT or glucagon stimulation testing of less than 500nmol/l. All of the patients who had isolated GHD had undergone cranial magnetic
resonance imaging (MRI) and had characteristic features of PHD.\textsuperscript{14} The patients with primary adrenal failure had congenital adrenal hypoplasia due to a mutation in NROB-1 (n=2), antibody positive autoimmune Addison’s disease (n=2) and isolated glucocorticoid deficiency (n=2). Patients on GH received a dose (0.5 to 0.8 mg/m\textsuperscript{2}/day) that maintained an age appropriate growth rate. Patients on hydrocortisone replacement were treated with a dose (7.7 – 12.5mg m\textsuperscript{2}/day) that kept them free of symptoms attributable to glucocorticoid deficiency. GH was given by a single nightly subcutaneous injection and glucocorticoid replaced using a bd or tds hydrocortisone regimen.

Study Protocol

Each subject was admitted to hospital on 2 occasions as part of a randomised cross-over study involving a 14h overnight and morning fast. On one occasion subjects took their normal dose of GH and / or hydrocortisone and on the other occasion, 1 evening dose of GH was omitted and / or a single dose of hydrocortisone omitted on the morning of the study. We were therefore able to examine the impact of cortisol deficiency, GH deficiency and the combination of GH and cortisol deficiency on blood glucose levels. The two studies were conducted between 2 and 12 weeks apart and all studies were performed over a period of 11 months.

Patients were admitted in the afternoon and an intravenous cannula inserted for blood sampling. Height, weight, and pubertal status were recorded. Children were encouraged to undertake their normal physical activities and to take their medication consistently prior to each study with a similar diet on the evening of the two studies. The patients fasted from 2200h until 1200h the following day. Blood was taken at 0400h for the measurement of BG and then at intervals from 0700h until 1200h. BG,
GH and cortisol were measured at 20 minute intervals, insulin, adrenaline and noradrenaline at hourly intervals and intermediary metabolites (non-esterified free fatty acids or NEFA’s, alanine, aceto-acetate and beta-hydroxybutyrate) at 0700h and again at 1200h. Blood samples were spun and separated immediately after sampling with biochemical assays conducted the same day (glucose, GH, cortisol) or the samples frozen at -80°C for assays conducted at a later date.

Insulin sensitivity (IS) was calculated hourly using the computerised HOMA model.¹⁵

Hypoglycaemia

Blood glucose concentrations are a continuous variable but for the purpose of this study hypoglycaemia was defined as a blood glucose concentration less than or equal to 3.3mmol/l.

Laboratory methods

Glucose was measured using the hexokinase method (Olympus system – intra-assay CV 1.1%, inter-assay CV of 2.7% at 3.5mmol/l). GH was measured by two site chemiluminescence (Nichols Advantage HGH assay – intra-assay CV of 3%, inter-assay of 7%). Cortisol was measured using a competitive immunoassay and direct chemiluminescence (ADVIA Centaur assay - intra-assay CV of 3%, inter-assay CV 12%). Insulin was measured using an enzyme linked immunosorbent assay (DAKO – intra-assay CV of 5%, inter-assay CV 4.5 – 5.3%). Catecholamines were measured using in-house HPLC (inter-assay CV 10% at 0.48 nmol/l and 6.6% at 7.08 nmol/l). Intermediary metabolites were measured using a Cobas Centrifugal Fast Analyser (alanine intra-assay CV 2.3 % at 0.21 mmol/l and inter-assay CV 7.0% at 0.19 mmol/l; acetoacetate intra-assay CV 5.0 % at 0.26 mmol/l and inter-assay CV 6.0% at
0.30 mmol/l; betahydroxybutyrate intra-assay CV 2.0 % at 1.61 mmol/l and inter-assay CV 6.8% at 1.37 mmol/l) and NEFAs were measured enzymatically using a WAKO NEFA C Test kit (intra-assay CV 3.1% at 0.1mmol/l and 2.1 at 1.0 mmol/l and inter-assay CV 12% at 0.1mmol/l and 3.8% at 1.0mmol/l).

Statistics

Demographic data is expressed as mean +/- 1 standard deviation (SD) or median and range depending on whether it was normally distributed or not. Paired data for each subject were analysed using paired t-tests and area under the curve was used as a summary measure. Between group differences were analysed using ANOVA and Fisher’s pairwise comparisons. Spearman correlation co-efficients were calculated for linear relationships and multiple regression analysis was performed using a forward selection method with variables that could potentially affect blood glucose concentrations. Cortisol, GH and adrenaline production were calculated using the trapezoid rule for area under the curve (AUC). A summary of IS over the morning was also calculated in this way. IS, ketone body and NEFA concentrations were all normalised by log transformation prior to statistical analysis.
Results

Patient characteristics

There were no significant differences between the groups in terms of age or BMI.

Overnight BG concentrations

No child with PHD was found to be hypoglycaemic at 0400h on or off therapy and there was no difference between study nights (BG=5.4±1.0 mmol/l on treatment v 4.8±0.4 mmol/l off treatment; NS). None of the children with adrenal failure become hypoglycaemic overnight (BG=5.3±0.5 mmol/l). When treatment was omitted, children with PHDC- had lower BG concentrations at 0400h than those with PAF (4.7±0.4 mmol/l v 5.4±0.5 mmol/l; p=0.03).

Minimum BG concentrations between 0700h and 1200h

No child with PAF or PHD became hypoglycaemic on treatment. When treatment was omitted, 5 children had BG concentrations ≤3.3 mmol/l. 4 of the children had PHD (3 PHDC- and 1 PHDC+) and one child had PAF. Children with PHD had significantly lower BG concentrations on the morning when treatment had been omitted when assessed by either BG AUC (p=0.006) or by minimum BG between 0700h and 1200h (4.4 ±0.5 mmol/l v 3.9 ±0.5 mmol/l, p=0.008).

The difference in blood glucose concentrations between the 2 study nights in the 3 groups of patients is shown in figure 1.

Relationship between BG concentrations, age and BMISDS

There was a positive relationship between BG concentrations at 0400h and age off therapy ($r^2=0.22$; NS on treatment and $r^2=0.38$; $p=0.012$ off treatment). There was
also a relationship between minimum BG throughout the study and age on and off treatment ($r^2=0.20; p=0.05$ on treatment; $r^2=0.46; p=0.001$ off treatment) and glucose AUC and age ($r^2=0.57; p<0.001$ on treatment; $r^2=0.20; p=0.05$ off treatment). The minimum BG concentration between 0700h and 1200h was related to age in those with PHD both on and off treatment ($r^2=0.49, p=0.008$ and $r^2=0.38, p=0.018$) but not in those with PAF ($r^2=0.60, NS$). BG values at 0400h and at 0700h were positively related to BMISDS in all patients on treatment ($r^2=0.44, p=0.004$ and $r^2=0.46, p=0.001$) but not off treatment ($r^2=0.01, NS$ and $r^2=0.00, NS$). BMISDS was related to BG in patients with PHD (C+ and C-) at 0400h, 0700h ($r^2=0.69, p=0.001$), 1200h ($r^2=0.65, p=0.001$) and also to minimum BG concentrations ($r^2=0.61, p=0.002$) on replacement. There was no relationship between these variables when treatment was omitted.

GH levels and GH counter-regulation

a) GH

There was a positive relationship between the GH AUC and minimum BG in patients with PHD on treatment ($r^2=0.45, p=0.012$). There was no relationship between minimum glucose concentration and GH AUC in patients with PAF off treatment ($r^2=0.59, NS$).

b) Cortisol

When patients with PHDC+ omitted their evening GH, the cortisol AUC was higher than when treatment had been given ($2991 \pm 1073 \text{ nmol/l}$ versus $2374 \pm 756 \text{ nmol/l}; p=0.01$). There was no relationship between cortisol AUC and glucose concentrations, either on or off treatment.
c) Adrenaline

There was no difference in the adrenaline concentrations between the two
study nights and no difference in the adrenaline concentrations as assessed by AUC
between those with PHD and those with PAF (Table 2). When treatment was omitted
there was a negative relationship between minimum BG and adrenaline
concentrations in the group as a whole ($r^2=0.37$, $p=0.01$).

d) Noradrenaline

There was no difference in the noradrenaline concentrations as assessed by
AUC between those with PHD and adrenal failure. However when the groups were
analysed by ANOVA patients with PHDC- had significantly lower noradrenaline
concentrations than those with PHDC+ on and off treatment ($p<0.05$, Table 2).

e) Insulin sensitivity

Children with PHD (both PHDC+ and PHDC-) were more insulin sensitive on
the morning on which medication had been omitted ($p=0.05$ and $p=0.01$) respectively
(Figure 2). There was a negative relationship between IS and age in the group as a
whole (on treatment $r^2=0.16$, NS, off treatment $r^2=0.24$, $p=0.03$). There was a negative
relationship between IS and BMI SDS when treatment was given ($r^2=0.42$, $p=0.003$)
but not when treatment was omitted ($r^2=0.08$, NS).

Intermediary metabolites

a) Alanine

On treatment, children with PHDC- had higher concentrations of alanine than
those in the other 2 groups at 0700h (ANOVA $p=0.03$) There was no relationship
between alanine concentrations at the end of the fast and age when treatment was
given but when treatment was omitted, the younger children had lower alanine
concentrations ($r^2=0.79$, $p=0.011$). There was no relationship between alanine concentrations and minimum BG concentrations when treatment had been given in either those with PHD or PAF, but when treatment was omitted, there was a positive relationship between alanine and minimum BG in those with PHD ($r^2=0.56$, $p=0.002$).

b) NEFAs and ketones

There was no difference in the NEFA or total ketone bodies (TKB; acetoacetate + β-hydroxybutyrate) between the 3 groups on and off treatment, at the end of the fast. There was no relationship between age and TKB or NEFA concentrations either on or off treatment in the group as a whole (data not shown). On treatment TKB and NEFA concentrations were not related to minimum BG, but when treatment was omitted, there was a negative relationship ($TKB r^2=-0.20$, $p=0.05$; NEFA $r^2=-0.35$, $p=0.02$).

Multiple regression analysis

A forward selection multiple regression model was performed using the variables age, BMI SDS, GH AUC, cortisol AUC, adrenaline AUC, NEFA and TKBs, to establish which were significant determinants of minimum blood glucose concentrations. On treatment the only significant variable was BMISDS, $p=0.005$, accounting for 35% of the variability. Off treatment the significant determinants were age (positive relationship) and NEFA (negative relationship), $p<0.001$, accounting for 55% of the variability.
Discussion

We defined hypoglycaemia as a BG less than or equal to 3.3 mmol/l. This level was chosen for 3 reasons. Firstly, cognitive function becomes impaired at this blood glucose concentration. Secondly, neurophysiological changes can be observed in children when blood glucose concentrations are at this level and finally, a counter-regulatory hormone response was expected when BG concentrations fell to 3.3mmol/l. Jaquert and colleagues studied 10 children with either GH deficiency, GH and TSH deficiency or panhypopituitarism. The authors defined normal blood glucose values as being in the range 3.2 to 5.2 mmol/l but hypoglycaemia as a BG less than or equal to 2.6 mmol/l. 9 of the 10 patients became hypoglycaemic during a 24h fast (all patients if our definition of hypoglycaemia is used) but it is of note that these patients were generally younger than those studied by ourselves. They were also fasted for a longer and clinically less relevant period of time (24h).

In contrast to the work of Houdjik and colleagues we have not found any evidence of hypoglycaemia overnight in children or adolescents with GHD and / or cortisol deficiency on or off treatment. The patients in both studies received similar doses of GH at the same time in the evening but the fact that our patients were younger might have been expected to make them more susceptible to hypoglycaemia. Our definition of hypoglycaemia (less than or equal to 3.3 mmol/l) when compared to that of Houdjik and colleagues (less than 2.5mmol/l) serves to highlight the difference in behaviour of the two study groups. There are a number of possible explanations for the lower BG concentrations seen in Houdjik and colleague’s study. Firstly, the adolescents omitted three doses of GH in the nights prior to sampling in contrast to our study where children either took their GH as normal or were studied after the
omission of one dose. The cumulative effect of the three omitted doses may therefore have rendered patients more susceptible to low BG concentrations. Secondly, BG concentrations were measured more frequently in Houdijk and colleague’s study and we may have missed a short episode of overnight hypoglycaemia. However we believe this is unlikely since the 0400h sample was taken at the time at which the minimum BG concentrations had been observed. Finally, it is possible that the patients may have been quite different in terms of factors such as diet and preceding activity levels. However we are not aware of any selection criteria that are likely to lead to such profound differences in study findings.

As the fast continued into the morning there was evidence of decompensation when medication was omitted. 25% of our patients with PHDC+, PHDC- and PAF became hypoglycaemic during the morning, with a BG concentration less than or equal to 3.3mmol/l. Children with both GH and ACTH deficiency were, as expected, more susceptible with 3 from 7 becoming hypoglycaemic. Concentration is impaired in the well child if a breakfast containing complex carbohydrates is omitted. Children with GH or cortisol deficiency who miss breakfast could exacerbate differences in neurological performance that have been described between them and their peers. BG concentrations were related to age, in contrast to an earlier study in fasting children with hypopituitarism, but it was not just the youngest children who became hypoglycaemic and therefore any advice regarding hypoglycaemia should be given to all patients with PHD.

Studies with radioisotopes or with Magnetic Resonance Spectroscopy are the best way to assess the precise mechanisms that result in low blood glucose levels. However the positive relationship between GH AUC and minimum BG in patients with PHD underlines the pivotal role of GH in maintaining glucose concentrations in
the fasting child. We did not find a difference between the GH AUC on and off treatment in those with PAF which may reflect the small numbers studied or the fact that GH secretion in response to spontaneous hypoglycaemia is not a good measure of GH reserve. However, overnight BG levels were lower in patients making neither GH or cortisol (PHDC-) versus children unable to make just cortisol (PAF). The patients with PHDC+ produced more cortisol when treatment was omitted, despite the fact that only 1 child in this group became hypoglycaemic. This suggests that cortisol production is affected by absolute glucose levels and not just a particular glycaemic threshold.

When treatment was administered there was a negative relationship between IS and BMISDS, consistent with the findings of other authors who used the HOMA model. Multiple regression analysis identified a relationship between BMISDS and minimum BG and the fact that slim patients may be more susceptible to hypoglycaemia is a simple point that needs to be borne in mind by clinicians. The relationship between BMISDS and IS as well as minimum BG was lost when treatment was omitted which we suspect is in part a reflection of the impact of GH and cortisol on fuel mobilisation from fat. The fact that low BG was inversely related to NEFA levels in the multiple regression model may be linked to catecholamine production and associated fat mobilisation as part of the metabolic response to fasting and low BG levels.

The development of the adrenal medulla is dependent upon normal exposure to glucocorticoids antenatally. There is bi-directional signalling between the adrenal cortex and medulla and glucocorticoids regulate the expression of enzymes involved in catecholamine synthesis (tyrosine hydroxylase, dopamine-β-hydroxylase and phenylethanolamine-N-methyltransferase) and catecholamines enhance
adrenocortical steroidogenesis by stimulation of cytochrome P450 enzymes.\textsuperscript{31} Children with CAH have been observed to have lower morning adrenaline concentrations and lower adrenaline concentrations after exercise than normal children.\textsuperscript{32} The interpretation of circulating noradrenaline concentrations is more difficult because it is a neurotransmitter that is produced both from the adrenal glands and from the sympathetic chain. In terms of cortisol production these groups may be relatively heterogeneous since the ACTH deficiency in PHD may evolve over time\textsuperscript{33} and 3 of the patients had acquired PHD as a result of craniopharyngiomas and trauma. The patients with PAF also presented at different stages of childhood.

When treatment was omitted, those with PHDC- had lower noradrenaline concentrations. Voorhess et al.\textsuperscript{34} found that children with GHD but ACTH sufficient had no increase in noradrenaline in response to insulin induced hypoglycaemia whereas short normal children had a two fold increase, although others have observed a normal response to hypoglycaemia in those with GHD.\textsuperscript{35} These observations may reflect a developmental defect of the sympathetic chain as well as the hypothalamo-pituitary axis in some of these patients.

We conclude that children and adolescents with GH and ACTH/cortisol deficiency are susceptible to hypoglycaemia when a single dose of medication is omitted. The susceptibility is greatest in, but not confined to, the younger children who are both GH and ACTH deficient and in whom noradrenaline production is also compromised. We have demonstrated evidence of counterregulation with increased cortisol concentrations in those with PHD who were ACTH sufficient. We have also demonstrated failure to mobilise alternative fuels as a likely mechanism behind the low blood glucose concentrations that we observed. The importance of regular meals
and compliance with medication should be emphasised to this group of patients and their parents, particularly in those who are young and slim.
Acknowledgements

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References


syndromes and multiple pituitary hormone defects. *Pediatric Research*, 20, 1097-1101.
Table 1 Patient Characteristics

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PAF – Primary adrenal failure; PHDC+ - Pituitary Hormone Deficiency, cortisol sufficient; PHDC- - Pituitary Hormone Deficiency, cortisol insufficient
Table 2.
Adrenaline and Noradrenaline concentrations (area under the curve – AUC) during the morning fast.

<table>
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<th>Adrenaline AUC 0700h - 1200h</th>
<th>Mean On treatment (nmol/l/300mins)</th>
<th>SD</th>
<th>Mean Off treatment (nmol/l/300mins)</th>
<th>SD</th>
<th>‘p’ value</th>
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<td>0.6</td>
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<tr>
<td>PHDC+</td>
<td>2.0</td>
<td>2.2</td>
<td>0.5</td>
<td>0.7</td>
<td>NS</td>
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<tr>
<td>PHDC-</td>
<td>1.9</td>
<td>2.2</td>
<td>0.7</td>
<td>1.0</td>
<td>NS</td>
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<tr>
<td>Between groups</td>
<td>NS</td>
<td>NS</td>
<td></td>
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<table>
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<th>Noradrenaline AUC 0700h - 1200h</th>
<th>(nmol/l/300mins)</th>
<th>(nmol/l/300mins)</th>
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<td>4.0</td>
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<tr>
<td>PHDC-</td>
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<td>p=0.03</td>
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Figure 1

Difference in blood glucose concentrations (+ or − 1 SEM) between the 2 study nights (on and off treatment) in the 3 patient groups (O = PAF; ∇ = PHDC+; ∆ = PHDC−).
Figure 2
Difference in insulin sensitivity (with 1 SEM) between the study nights ‘on’ and ‘off’ treatment.