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Effects of low-dose oral hydrocortisone replacement versus short-term reproduction of physiological serum cortisol concentrations on insulin action in adult-onset hypopituitarism

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Summary

OBJECTIVE Hypercortisolism is associated with impaired glucose tolerance and insulin resistance. For many years hydrocortisone 30 mg was the standard total daily replacement dose in adult hypopituitarism. The use of this conventional dose has now been shown to result in mild biochemical hypercortisolism and might contribute to the increased cardiovascular risk reported in hypopituitarism. The use of lower doses of hydrocortisone replacement therapy might prevent some of the adverse metabolic effects seen with conventional doses.

PATIENTS In a randomized crossover study we assessed peripheral and hepatic insulin action in 15 ACTH-deficient patients with normal glucose tolerance on two occasions while receiving either a low-dose oral hydrocortisone replacement (LOR) therapy (15 mg at 0800, 5 mg at 1700) or a physiological hydrocortisone infusion (PHI), which achieved physiological serum cortisol concentrations.

RESULTS Exogenous glucose infusion rates required to maintain euglycaemia were similar for the LOR and the PHI protocols (26·2 ± 0·4 *vs.* 23·8 ± 0·6 μmol/kg/min, respectively). Endogenous glucose production was also similar (12·0 ± 2·5 *vs.* 11·6 ± 2·4 μmol/kg/min, respectively) and in the post-absorptive state suppressed to a similar extent following insulin (4·5 ± 2·0 *vs.* 5·1 ± 3·1 μmol/kg/min).

CONCLUSION Hydrocortisone replacement therapy at a dose of 15 mg with breakfast, 5 mg with evening meal does not increase peripheral or hepatic insulin resistance when compared to a hydrocortisone infusion designed to simulate physiological serum cortisol concentrations.

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Introduction

Recent work by Esteban *et al*. (1991) has indicated that daily cortisol production rates are considerably lower (at 5·7 mg/m^2) than the values that had previously influenced steroid replacement dose regimes (12–15 mg/m^2). Ideally, long-term therapy should mimic normal physiology as closely as possible. Further work based on cortisol day curves and 24-h urinary free cortisol estimates (Howlett, 1997), and also guided by markers of bone remodelling and bone mineral density (Peacey *et al.*, 1997), has suggested that the optimum hydrocortisone replacement dose is closer to 20 mg daily.

Circumstantial evidence suggests that conventional hydrocortisone replacement therapy for hypopituitarism (20 mg mane, 10 mg nocte) might partly explain the excess morbidity and mortality (Rosen & Bengtsson, 1990; Markussis *et al.*, 1992; Bulow *et al.*, 1997) described in this condition. It has been demonstrated that hypopituitary patients on 'conventional steroid replacement therapy' have an increased prevalence of diabetes and glucose intolerance compared to controls (Beshyah *et al.*, 1994). It has also been shown that an intravenous infusion of cortisol in normal volunteers induces both hepatic and peripheral insulin resistance (Rizza *et al.*, 1982; Dineen *et al.*, 1993; Rooney *et al.*, 1993).

Recent studies in growth-hormone deficient (GHD) adults have shown increased insulin resistance in comparison to controls (Johansson *et al.*, 1995; Hew *et al.*, 1996). Not all of these patients were receiving hydrocortisone replacement therapy and the timing of the administration varied from before to after the assessment of insulin sensitivity, confusing the issue further. Patients in these studies who were on hydrocortisone received the previous 'conventional' doses. Thus, even mild overtreatment with hydrocortisone could lead to adverse vascular risk factors and their consequences.

The effects on insulin action of the more recently advocated lower dose hydrocortisone replacement regimens are therefore of clinical relevance but at present no information is available regarding them. The present study compares insulin action during low-dose (15 mg with breakfast, 5 mg with evening meal) oral hydrocortisone replacement (LOR) therapy in adult-onset hypopituitary patients with that observed during an intravenous physiological hydrocortisone infusion (PHI) designed to simulate physiological serum cortisol concentrations (Al-Shoumer *et al.*, 1997). We have used the glucose clamp technique in conjunction with isotope dilution methodology to provide a detailed analysis of both peripheral and hepatic insulin action.

Subjects and methods

Subjects

Fifteen patients (mean age 45 ± 2·4 and BMI 27·5 ± 0·9 kg/m^2) with ACTH deficiency of at least 1 year's duration confirmed by insulin hypoglycaemia testing (peak serum cortisol < 550 nmol/l) were recruited from the Royal Victoria Hospital Endocrine Clinic (Table 1). ACTH deficiency was due to the effects or treatment of chromophobe adenoma in six patients, prolactinoma in three patients, craniopharyngioma in two patients and parasellar meningioma in one patient. Other causes included Sheehan's syndrome and isolated ACTH deficiency. Fourteen patients were also growth-hormone deficient (maximum GH < 5 mU/l on hypoglycaemia testing. Those with concurrent pituitary deficiencies were on stable replacement therapy unchanged for at least 1 year prior to entry (14 thyroxine, 13 sex hormone therapy and 1 desmopressin) but no patient had had growth hormone replacement before the study. Patients were excluded from the study if they exceeded 125% of ideal body weight (Metropolitan Life Insurance tables, 1959), had abnormal glucose tolerance (defined by a plasma glucose level > 7·8 mmol/l 2 h after a 75-g oral glucose tolerance test), had hypertension, or had a previous history of cardiac or cerebrovascular events or any significant hepatic or renal disease.

Approval for the studies was obtained from the Research Ethical Committee of the Queen's University of Belfast.

Study design

The hydrocortisone dose had been changed to 15 mg at 0800 and 5 mg at 1700 for at least 2 months prior to the study. A randomized crossover design was employed. Insulin action was studied twice, once after an overnight infusion of hydrocortisone [PHI], which then continued throughout the clamp, the rate varying to produce physiological serum cortisol concentrations, and once after 15 mg of hydrocortisone [LOR], taken at 0700. Studies were performed in random order 3–4 weeks apart.

Females were studied in the first 10 days of their menstrual cycle and males who were on androgen supplementation, between 7 and 14 days after the last injection of testosterone.

Assessment of insulin action

Insulin action was assessed using the euglycaemic glucose clamp technique (DeFronzo *et al.*, 1979; Neely *et al.*, 1992). In the PHI study, the patient was admitted to the Metabolic Unit, Royal

Table 1 Patient characteristics

Patient	Age	Sex	BMI (kg/m^2)	Cause of hypopituitarism	Duration (years)	Other treatments*
1	40	M	23·7	Chromophobe adenoma	3	1,2,3
2	52	M	27·4	Prolactinoma	16	1,2,3,5
3	37	M	23·5	Prolactinoma	4	1,2,3,5
4	43	F	29·4	Craniopharyngioma	9	1,2,3,4,5
5	34	M	26·1	Chromophobe adenoma	3	1,2,3
6	44	F	21·9	Parasellar meningioma	4	1,2,3
7	46	F	25·3	Sheehan's syndrome	23	1,2,3
8	45	M	26·6	Chromophobe adenoma	7	1,2,3
9	64	M	29·3	Chromophobe adenoma	1	1,2,3
10	39	M	32·1	Prolactinoma	2	1,2,3,5
11	44	F	30·9	Chromophobe adenoma	2	1,2,3
12	60	F	30·4	Chromophobe adenoma	1	1,2
13	39	F	31·1	Craniopharyngioma	20	1,2,3
14	56	M	31·9	Arachnoid cyst	2	1,2,3,4
15	32	M	22·2	Isolated ACTH deficiency	6	1
Mean	45	9M/6F	27·5		7	

BMI, body mass index.
*1, Hydrocortisone (all 15 mg with breakfast, 5 mg with evening meal); 2, thyroxine; 3, sex steroids; 4, desmopressin; 5, bromocriptine.

Victoria Hospital, at 1630 on the day preceding the clamp studies. A plastic cannula (18-gauge, Ventflon Viggo, Helsingborg, Sweden) was placed in a left forearm antecubital vein, and at 1700, 30 mg of hydrocortisone (Pharmacia and Upjohn, Crawley, Sussex, UK) in 250 ml of 0·9% NaCl was infused at a rate according to the patients weight (kg), pharmacokinetics and bioavailability, using an ambulatory Graseby 9400 infusion pump previously evaluated by Al-Shoumer *et al*. (1997). A second cannula was placed in the right forearm vein for the measurement of hourly serum cortisol levels. A pilot study had confirmed that the hourly rate, at which the hydrocortisone was infused, achieved physiological levels. Subjects were allowed to remain ambulatory before retiring to bed between 2200 and 2300, and fasted overnight. At 0745 a carrier infusion of 0·9% NaCl was connected at a rate of 50 ml per hour to the left antecubital vein which was already carrying the hydrocortisone infusion, which was continued throughout the glucose clamp. All subsequent infusions were connected to this line.

A dorsal hand vein on the opposite arm was cannulated retrogradely (21-gauge; Venflon Viggo) and the hand placed in a temperature-controlled plexiglass box (Northern Ireland Technology Centre, Automation Division, Queen's University of Belfast) maintained at 55°C to allow intermittent sampling of arterialized venous blood. On the morning of the LOR, patients were admitted to the Metabolic Unit at 0745, after a 12-h overnight fast and 15 mg of oral hydrocortisone taken at 0700. A left antecubital vein was cannulated (18-gauge; Venflon Viggo) and used for the carrier 0·9% NaCl and all subsequent infusions.

Glucose turnover was assessed using a primed continuous infusion of high-performance liquid chromatography purified [3-^{3}H]-glucose (New England Nuclear Research Products Division, Dupont Ltd, Stevenage, UK (NET100C) administered during a 2-h equilibration period (–120 min to zero time), and subsequent 2-h continuous (1 mU/kg/min) infusion of insulin (Humulin S; Eli Lilly and Co., Basingstoke, UK). Plasma glucose was maintained at a level of 5·1 mmol/l by an exogenous glucose infusion (20%). Exogenous glucose was prelabelled with [3-^{3}H]-glucose to match the predicted basal plasma glucose specific activity, with the primed continuous tracer infusion being reduced to 50% of the basal rate after 20 min and to 25% of basal after 40 min (to maintain tracer steady state), and was maintained at this rate throughout the remainder of the hyperinsulinaemic period.

Analytical techniques

Arterialized venous blood was used for all analyses. Blood samples for determination of plasma glucose specific activities were taken at 10-min intervals from –30 to 0, and 90 to 120 min, relative to the start of the insulin infusion. Plasma for measurement of glucose specific activity was deproteinized with barium dihydroxide and zinc sulphate by the method of Somogyi (1945). Samples were counted in a liquid scintillation spectrometer (Tri-Carb 2000 CA, Canberra Packard, Pangbourne). Aliquots of tracer infusate and labelled exogenous glucose infusion were spiked into nonradioactive plasma processed in parallel with plasma samples to allow calculation of [3-^{3}H]-glucose infusion rates.

Serum insulin concentration was measured by radioimmunoassay with insulin antibody precipitate (Hales & Randle, 1963), using reagents supplied by Abbott Laboratories (Maidenhead, Berkshire, UK) on an IMX analyser. The interassay coefficient of variation (CV) was 5·2% at a mean value of 7·3 mU/l, 3·8% at a mean value of 16·7 mU/l and 4·1% at a mean value of 58·4 mU/l. Serum cortisol was determined by radioimmunoassay, using reagents supplied by Diagnostic Products Corporation (Los Angeles, USA). The interassay CV was 3·5% at a mean value of 234 nmol/l, 3·8% at a mean value of 432 nmol/l and 3·6% at a mean value of 981 nmol/l. Commercially available reagent kits were used to measure serum free fatty acid (Wako Chemicals, Neuss, Germany), β-hydroxybutyrate (Randox Laboratories, Crumlin, UK) and serum glycerol (Randox Laboratories) concentrations. Blood samples for lactate and pyruvate were collected into glass tubes containing an equal volume of aqueous perchloric acid solution (8% w/v) and shaken immediately. After centrifugation, extracts were separated and analysed immediately or stored at –20°C until analysis (Sigma Chemical, Dorset, UK).

Calculations

Rates of glucose appearance (Ra) and disappearance (Rd) were determined during the periods –30 min to zero time and 90–120 min, using the nonsteady-state equations of Steele *et al*. (1956) as modified by De Bodo *et al*. (1963), assuming a pool fraction of 0·65 and an extracellular volume of 190 ml/kg. Infusion rates were calculated as the sum of the tracer infused continuously and the tracer in the labelled exogenous glucose infusion. Rates of endogenous (hepatic) glucose production were calculated by subtraction of the exogenous glucose infusion rates required to maintain euglycaemia from the isotopically determined rates of glucose appearance.

Statistical methods

The power of the study, calculated from previous clamp data (Rooney *et al.*, 1992; Harper *et al.*, 1994), gave a 90% chance of detecting a 10% change in insulin action at the 5% level of significance. Significance was assessed with Student's two-tailed *t*-test for paired data. Significance was taken as $P < 0{\cdot}05$. The values given in the text are means ± SEM.

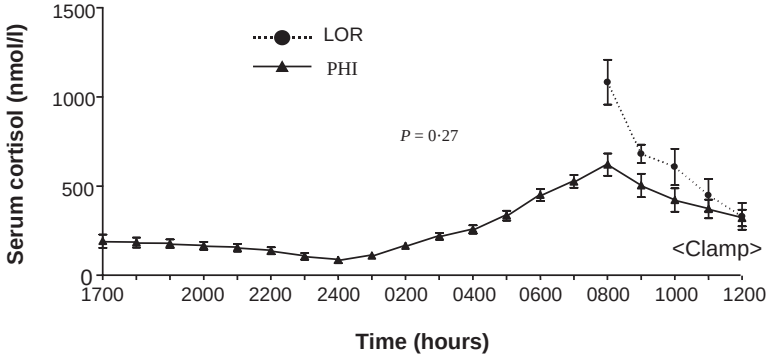

Fig. 1 Serum cortisol levels during low-dose oral hydrocortisone replacement (LOR) and physiological hydrocortisone infusion (PHI). *P*-value refers to comparison of area under curve during the clamp period (1000–1200).

Results

Serum cortisol concentrations during both studies are shown in Fig. 1. Hydrocortisone [LOR] 15 mg produced higher serum cortisol levels at 0800 (1083 ± 471 *vs.* 621 ± 225 nmol/l), reflecting the fact that the medication had been taken orally 1 h previously. During the clamp period 1000–1200, there was no statistically significant difference in the area under the curve for both treatments ($P = 0{\cdot}27$).

Fasting arterialized venous plasma glucose levels were similar in both groups (5·1 ± 0·0 *vs.* 5·1 ± 0.0 mmol/l) as was fasting serum insulin (6·1 ± 1·0 *vs*. 6·1 ± 0.9 mU/l). Plasma glucose levels remained constant in both studies (mean CV < 5·4% and 5·3%, respectively). The insulin infusion of 1 mU/kg/min led to comparable levels of steady-state insulin (80·1 ± 4·0 *vs*. 75·7 ± 3·5 mU/l). There was no statistically significant difference between LOR and PHI in exogenous glucose infusion rates (23·8 ± 0·4 *vs*. 26·2 ± 0·6 μmol/kg/min) required to maintain euglycaemia during the last 30 min of the glucose clamp, an index of peripheral insulin sensitivity. There was no difference in insulin-mediated glucose disposal determined isotopically between the two treatments (Fig. 2).

Post-absorptive endogenous glucose production (EGP), an index of hepatic insulin sensitivity, was similar in the fasting state (11·6 ± 0·6 *vs*. 12·0 ± 0·6 μmol/kg/min) and suppressed to the same degree during hyperinsulinaemia (5·1 ± 0·7 vs. 4·5 ± 0·5 μmol/kg/min) (Fig. 3). Post-absorptive levels of serum nonesterified fatty acids, β-hydroxybutyrate and glycerol were also similar in the fasting state and suppressed to the same degree during hyperinsulinaemia (Fig. 4).

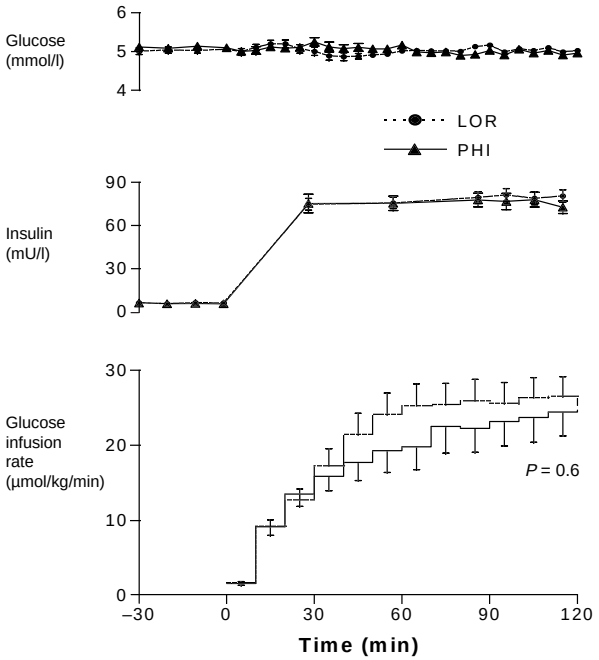

Fig. 2 Plasma glucose, insulin and glucose infusion rates on low-dose hydrocortisone replacement (LOR) compared to physiological hydrocortisone infusion (PHI) (mean ± SEM). *P*-value refers to the level of significance between exogenous glucose infusion rates (LOR and PHI).

Discussion

Our unit has previously demonstrated that a supraphysiological hydrocortisone infusion (2 μg/kg/min Solu-Cortef) over a 28-h period induces peripheral and hepatic insulin resistance (Rooney *et al.*, 1993) in normal volunteers when compared to a 0·9% saline infusion. Basal cortisol levels were similar but after 4 h, serum cortisol levels were higher (1008 ± 82 *vs*. 322 ± 28 nmol/l, $P < 0.001$). Glucose infusion rates, an index of peripheral insulin sensitivity, were significantly lower (34·5 ± 3·1 *vs*. 60·6 ± 3·9 μmol/kg/min, respectively, during 2 mU/kg/min glucose clamp) as was EGP (12·2 ± 0·5 *vs*. 13·3 ± 0·5 μmol/kg/min, respectively). Insulin resistance is associated with increased mortality (Howard *et al.*, 1996) and for this reason it is important to clarify whether the dose of hydrocortisone replacement therapy used in hypopituitarism could be contributing to the increased insulin resistance demonstrated in these patients (Johansson *et al.*, 1995; Hew *et al.*, 1996). This has become essential following Esteban's

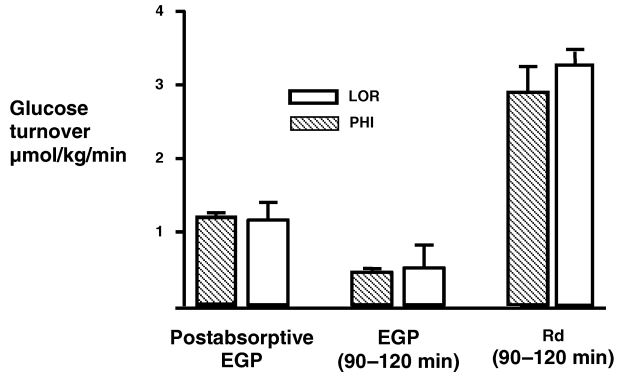

Fig. 3 Glucose turnover during hyperinsulinaemic euglycaemic clamps on low-dose oral hydrocortisone replacement (LOR) and physiological hydrocortisone infusion (PHI). No significant differences were observed. EGP refers to the rate of endogenous glucose production; Rd to the rate of glucose disposal.

more accurate assessment that the normal daily cortisol production rate in humans is considerably lower than previously estimated (Esteban *et al.*, 1991). The present study evaluated our currently used lower dose regimen in comparison with the more ideal physiological infusion.

Our results showed no statistically significant difference in either fasting plasma glucose or serum insulin during low-dose oral hydrocortisone (20 mg daily) replacement or physiological hydrocortisone infusion. Exogenous glucose infusion rates reflecting peripheral insulin resistance as well as endogenous glucose production rates in the fasting state and during hyperinsulinaemia (an index of hepatic insulin resistance) were similar with both treatments. The physiological hydrocortisone infusion was administered by an ambulatory Graseby pump and patients were encouraged to remain as mobile as they would otherwise have been at home. It is conceivable that there was a difference in energy expenditure which might have affected insulin action but this seems unlikely. The present study of well-characterized hypopituitary patients taking a total daily hydrocortisone dose of 20 mg therefore extends the observations that this replacement dose is closer to physiological concentrations and demonstrates no detrimental effect on peripheral and hepatic insulin action when compared to a hydrocortisone infusion designed to simulate physiological serum cortisol concentrations. Currently, there is no evidence that the higher dose of hydrocortisone (20 mg + 10 mg) increases insulin resistance compared to the low dose (15 mg + 5 mg). Other studies have suggested that the higher dose of hydrocortisone is supraphysiological (Peacey *et al.*, 1997) and we would expect this to induce a state of insulin resistance based on our previous study in which normal volunteers given supraphysiological doses of hydrocortisone had increased peripheral and hepatic insulin resistance (Rooney *et al.*, 1993).

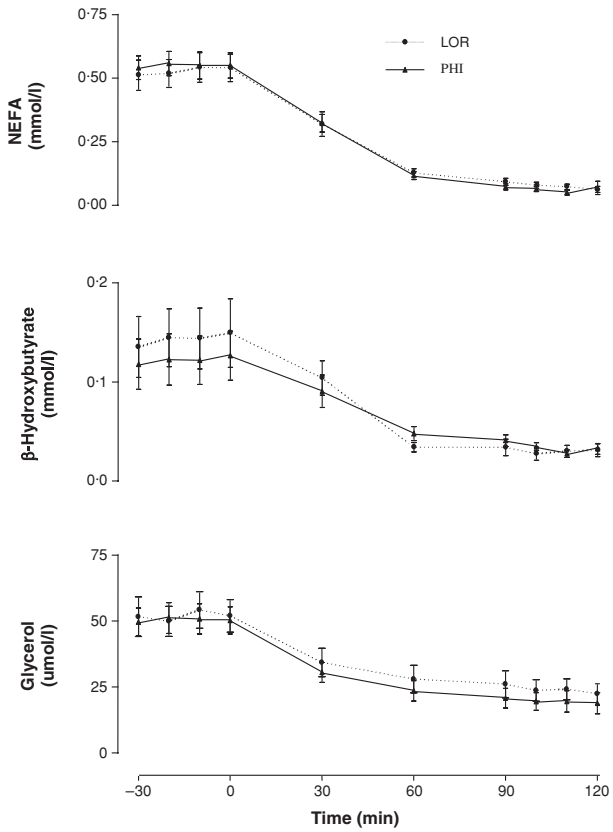

Fig. 4 Plasma nonesterified fatty acids (NEFA), 3-hydroxybutyrate and glycerol during low-dose oral hydrocortisone (LOR) and physiological hydrocortisone infusion (PHI). No significant differences were observed.

The temporal pattern of serum cortisol during the hydrocortisone infusion [PHI] is similar to that previously reported in normal unstressed controls with intact pituitary reserve (Weitzman *et al.*, 1970; Krieger *et al.*, 1971; Brandenberger *et al.*, 1984; Liu *et al.*, 1987). Our early morning 0800 peak serum cortisol of 621 ± 225 nmol/l equates to that of Weitzman *et al.* (1970) and Brandenberger *et al.* (1984), as does the basal nadir of 86 ± 63 nmol/l at 2400. Cortisol levels prior to 0800 on the day of the low-dose (15 mg) oral hydrocortisone were not measured. We therefore cannot exclude some cortisol deficiency during the night prior to assessment of insulin action, which has been demonstrated in hypopituitary patients on conventional doses (Al-Shoumer *et al.*, 1996). This might cause some reduction in insulin resistance as there is some evidence in hypophysectomized animal models that hypocortisolaemia induces a state of insulin sensitivity (Houssay & Biassoti, 1931). However, this would be the situation from day to day for these patients. In addition, serum cortisol levels were not measured for 1 h after the administration of the 15 mg hydrocortisone, and as absorption might be variable, it would have been worthwhile to sample more

frequently in the first few hours after administration. There was no significant difference in the area under the curve between the oral and intravenous serum cortisol concentrations, implying that the lower dose oral therapy achieves serum cortisol levels which simulate normal physiology. We accept that the metabolic observations might of course take several days to occur following a change to the hydrocortisone infusion (Van Cauter & Aschoff, 1989); however, our previous work in normal volunteers demonstrated a rapidly emerging difference over the same time interval (Rooney *et al.*, 1993). It would also have been interesting to examine insulin resistance in hypopituitary patients on a thrice daily cortisol replacement regimen more recently advocated by Howlett (1997), although the peak serum cortisol concentration after the 15 mg dose at 0800 equates with levels previously demonstrated in normal individuals.

In conclusion, the present study has demonstrated that low-dose oral hydrocortisone replacement therapy (15 mg with breakfast, 5 mg with evening meal) in the treatment of adult-onset hypopituitary patients, does not cause peripheral or hepatic insulin resistance when compared to a short-term infusion simulating physiological cortisol concentrations. The use of a total daily replacement dose of 20 mg might decrease some of the excess mortality and morbidity that has been identified with hypopituitarism.

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