Clinical Endocrinology (2013) **79**, 14–19 doi: 10.1111/j.1365-2265.2012.04478.x

ORIGINAL ARTICLE

Determining the utility of the 60 min cortisol measurement in the short synacthen test

Aditi Chitale*, Patrick Musonda*, Alan M. McGregor† and Ketan K. Dhatariya*,‡

*Norwich Medical School, University of East Anglia, Norwich, †King's College London School of Medicine, Department of Medicine, London and ‡Elsie Bertram Diabetes Centre, Norfolk and Norwich University Hospital NHS Foundation Trust, Norwich, UK

Summary

Objective Despite the widespread use of the short synacthen test (SST), there remains no clear consensus on sampling times for the measurement of serum cortisol that best determines adrenal reserve. We set out to establish whether there is any value in measuring serum cortisol at 60 min following administration of synacthen.

Design Retrospective data analysis of 500 SST results measuring 0, 30 and 60 min cortisol levels after administration of 250 μg of synacthen at 2 large urban National Health Teaching Hospitals in the UK.

Patients and measurements Individuals thought to have primary or secondary adrenal insufficiency given 250 μg of synacthen.

Measurements Serum cortisol levels measured at 0, 30 and 60 min, looking to see how many people who had adrenal insufficiency at the 30 min sample but in whom the 60 min sample showed adequate adrenal reserve.

Results The results from 384 people were analysed**.** A total of 276 had normal responses at 30 min and also at 60 min. A sum of 33 individuals had 'insufficient' (i.e., <550 nmol/l) 30 min cortisol levels, rising to $\geq$ 550 nmol/l at the 60 min test. All 75 individuals who were insufficient at 60 min were also insufficient at 30 min. No individuals passed ($\geq$550 nmol/l) at 30 min and then failed (<550 nmol/l) at 60 min.

Conclusions These results suggest that a significant proportion of people undergoing a SST may be inappropriately diagnosed as having adrenal insufficiency if the 60 min sample is not measured. We suggest that the 60 min sample is measured in all individuals having a SST to prevent unnecessary over-diagnosis of adrenal insufficiency.

(Received 28 December 2012; returned for revision 31 May 2012; finally revised 12 June 2012; accepted 18 June 2012)

Correspondence: Ketan K Dhatariya, Elsie Bertram Diabetes Centre, Norfolk and Norwich University Hospitals NHS Foundation Trust, Colney Lane, Norwich, NR4 7UY, UK. Tel.: +441603 288170; Fax: +441603287320; E-mail: ketan.dhatariya@nnuh.nhs.uk

Introduction

The short synacthen test (SST) was introduced over 45 years ago as a faster and safer alternative to the gold standard insulin tolerance test (ITT) for the assessment of primary adrenal insufficiency.[1] In recent years the test (variously referred to in the literature as the co-syntropin or short tetracosactide test) has also been employed to assess secondary adrenal insufficiency based on the assumption that chronic adrenocorticotrophic hormone (ACTH) deficiency leads to adrenal atrophy and therefore hyporesponsiveness to exogenous, synthetic ACTH (synacthen).[2] For this reason, the test has grown in popularity among UK endocrinologists, with a rise in use from 24% in 1988 to 50% in 1994.[3,4]

Given the widespread use of this test, it is surprising that there remains no consensus on sampling times for the measurement of serum total cortisol.[5] Samples for cortisol have historically been taken at 0 and 30 min, with some units also taking samples at 40, 45 or 60 min postsynacthen administration.[5] Two UK surveys conducted 15 years apart show an increasing trend in clinicians discarding the 60 min sampling time and relying more heavily on the 30 min sample,[5,6] the only time point that has been validated against the ITT.[2,7]

Adrenocorticotrophic hormone concentrations have been shown to be high immediately following its administration,[8,9] and its half life is between 10 and 20 min.[10,11] This would suggest that maximal adrenal stimulation would last for several hours. Despite this, however, some authors have stated that, based on the results of their studies, the 60 min sample has no benefit over the 30 min sample.[4,12] Other authors believe that, because the 60 min value has not been validated against the ITT, it may be a less reliable index of hypothalamo-pituitary-adrenal (HPA) axis function.[13] However, Mansoor and colleagues produced evidence to support the use of the 60 min sample as a sole measurement postsynacthen, stating that it is equally effective in identifying abnormal cases as values at both 30 and 60 min.[14]

Based on these data, our study set out to establish whether there is any value in measuring cortisol at 60 min. In particular, the aim of our study was to determine how often a patient would be misdiagnosed as having adrenal insufficiency if the 60 min sample was not taken.

Subjects and methods

The study was conducted at King's College Hospital NHS Foundation Trust (KCH), London, and the Norfolk and Norwich University Hospitals NHS Foundation Trust (NNUH) in Norwich. At both institutions ethical approval was sought and deemed not to be required due to the retrospective nature of the data collection and analysis. In addition, consent was deemed not to be required from participants for a similar reason, and also because this was deemed to be 'a service improvement exercise'.

Consistent with UK practice, both centres measured serum total cortisol (nmol/l) at time 0 min, then administered the supra-physiological dose of 250 μg synacthen intravenously or intramuscularly.[5] The serum cortisol response was measured at 30 and 60 min.

At KCH, serum total cortisol was determined using the ADVIA Centaur chemiluminescence immunoassay (Bayer Diagnostics, Berkshire, UK). At NNUH, serum total cortisol is measured using the Roche Elecsys 2010 electrochemiluminescence immunoassay (Roche Diagnostics, West Sussex, UK). Both methods are assessed by the National External Quality Assurance Scheme (NEQAS) ensuring they maintain low coefficients of variation and remain relatively comparable.

Whilst there are significant differences between immunobased assays and less so in mass spectrometry based assays, the UK NEQAS regularly sends out a single sample equally divided to a number of laboratories using different analysers to access the precision and accuracy of their results, and comparing them to others. The NEQAS data for the duration of the study showed that the coefficient of variation for the Bayer ADVIA: Centaur analyser (Bayer Diagnostics) used at KCH was a mean of 5·92% (SD 0·94) using 5 samples, with that for the Roche Elecsys analyser (Roche Diagnostics) used at NNUH was 5·36% (SD 0·55). The national average for all 5 analysers was 12·26% (SD 6·55). A snapshot from September 2011 from the UK NEQAS Steroid Annual Review 2011 would indicate that the average bias (B score) of the Centaur was + 7·8% and the average bias (B score) of the Elecsys was + 0·2% (Finlay MacKenzie, NEQAS, personal communication)

In all, 250 consecutive SSTs were viewed in each centre. At KCH, these SSTs were conducted between January and October 2010. At NNUH, the SSTs were conducted between March 2009 and August 2010.

A 'pass' response to synacthen was defined as a serum total cortisol concentration of $\geq$ 550 nmol/l at 30 and 60 min. Both centres use this widely adopted cut-off value at 30 min when interpreting SST results.[6]

In each centre, the 250 consecutive SSTs were accessed using respective electronic pathology systems. All subjects who had the SST conducted to assess adrenal reserve were included in the study. The following groups were excluded: those taking glucocorticoids which had not been omitted prior to the test; those taking oestrogen-containing preparations, i.e., the combined oral contraceptive pill or hormone replacement therapy; those within 4 weeks of a pituitary insult, i.e., surgery or apoplexy; pregnant women; those who had the test performed for congenital adrenal hyperplasia and those who had an incomplete test, i.e., where cortisol was not measured at all three time points (0, 30 and 60 min).

Each subject's SST result was then categorized according to the outcome. There were four possible outcomes. A 'pass' for the SST was a cortisol response $\geq$ 550 nmol/l at 30 and 60 min whilst a 'fail' was a cortisol response < 550 nmol/l at 30 and 60 min. A 'pass at 30 min only' was a cortisol response $\geq$ 550 nmol/l at 30 min but < 550 nmol/l at 60 min. A 'pass at 60 min only' or a 'delayed response' was a cortisol response < 550 nmol/l at 30 min but $\geq$ 550 nmol/l at 60 min. The focus of the present study was those subjects falling in the latter category. They would have a false negative test if the 60 min sample was not taken, i.e., they would be misdiagnosed as having adrenal insufficiency. Therefore, we wanted to determine how many subjects fell into this category in both centres to determine whether the 60 min sample is required in the SST.

Results

All 250 subjects from NNUH were included in the data analysis; however, there were 116 exclusions from KCH. Of these, 114 were patients with chronic liver disease, and 2 were incomplete tests – i.e., samples were not taken at all three time points. Of the 384 subjects included in the study from both centres, the median age was 51 years (IQR 37, 65), 61% were female (95% CI, 56% to 66%) and median serum cortisol concentrations was 323 nmol/l (IQR 208, 442) at 0 min. Following synacthen administration, median cortisol concentration rose to 685 nmol/l (IQR 502, 819) at 30 min and 780 nmol/l (IQR 595, 941) at 60 min.

Looking at those individuals with results that were not a clear pass, at NNUH 6 patients had marginal results at 30 min (i.e., between 500 and 549 nmol/l) and 12 were clear fails at 30 min (<500 nmol/l at 30 min), and at KCH 5 patients were marginal and 10 were clear fails. The combined mean (i.e., NNUH together with KCH, $n = 22$) 0 min cortisol for these clear fails at 30 min was 219 nmol/l (±SD 93), the 30 min value was 471 nmol/l (±24) and the 60 min value was 581 nmol/l (±22). For those that were marginal (i.e., $\geq$500 nmol/l at 30 min), the combined mean (i.e., NNUH together with KCH, $n = 11$) of the 0 min cortisol at 30 min was 258 nmol/l (±124), the 30 min value was 515 nmol/l (±11) and the 60 min value was 602 nmol/l (±32). The numbers of patients were too small to do any statistical analyses.

Table 1 shows pair-wise comparisons of raw cortisol responses, taking account of the paired nature of the data. The differences shown were all strongly statistically significant (P-value < 0·0001).

Table 2 compares the cortisol responses in each centre at 30 min and 60 min using the McNemar's test. This test assesses the significance of the difference between two correlated proportions. It is the discordant pairs that were of interest to our study. We noted significant differences in the responses at 30 min compared to those at 60 min, and this is mainly influenced by the fact that if a subject passes at 30 min, they are unlikely to fail at 60 min. The main findings of the current

Table 1. Inferential statistics, taking account of paired nature of the data. The data show the results of the administration of synacthen on all possible time points in the test at both centres. After the administration of synacthen, mean cortisol values rise at 30 min and continue to rise at 60 min at both centres

Pair-wise comparison	NNUH, $n = 250$	NNUH, *P*-value	KCL, $n = 134$	KCL, *P*-value	Overall, *P*-value
Cortisol 0 min *vs* 30 min	330 (221, 432) *vs* 723 (550, 841)	<0·0001*	306 (182, 458) *vs* 625 (489, 716)	<0·0001*	<0·0001*
Cortisol 0 min *vs* 60 min	330 (221, 432) *vs* 843 (612, 965)	<0·0001*	306 (182, 458) *vs* 713 (571, 840)	<0·0001*	<0·0001*
Cortisol 30 min *vs* 60 min	723 (550, 841) *vs* 843 (612, 965)	<0·0001*	625 (489, 716) *vs* 713 (571, 840)	<0·0001*	<0·0001*

NNUH, Norfolk and Norwich University Hospital; KCH, King's College Hospital.
*Wilcoxon matched pairs signed-rank test was used. Values are median in nmol/l (Inter-quartile range).

study was that for the 33 people with delayed responses (responses < 550 nmol/l at 30 min, but ≥ 550 nmol/l at 60 min – 18 (7%) from NNUH and 15 (11%) from KCH), the mean 0 min cortisol level was 232 nmol/l (±104), the 30 min response was 486 nmol/l (±29) and the 60 min response was 588 nmol/l (±27).

We also wanted to check the sensitivity, specificity, negative predictive value and positive predictive value of the 60 min sample assuming that the 30 min value was gold standard. This is shown in Table 3 and the corresponding ROC curve given in Fig. 1. We then repeated the calculations assuming 60 min as the gold standard. This is shown in Table 4 and the corresponding ROC curve given in Fig. 2. Tables 3 and 4 and Figs 1 and 2 confirm that when the 60 min were taken as the gold standard, higher specificity, positive predictive value (PPV) and ROC area were achieved, indicating that using the 60 min as the gold standard was better than using the 30 min value as the gold standard.

Discussion

Our study shows that up to 11% of patients having an SST would be inappropriately diagnosed with adrenal insufficiency if the 60 min sample was not used. Recent data have shown that in healthy humans, cortisol levels continue to rise for up to 60 min after the administration of ACTH before reaching a plateau.[15] However, there has been previous controversy surrounding sampling times in the SST. Previous work has supported the use of the 30 min sample due to excellent correlation being documented between the 30 min sample and the peak cortisol response to hypoglycaemia in the gold standard ITT,[2,3,7,12,16,17] although this is not always the case.[18] Some clinicians still include the 60 min sample when conducting the SST, but its use is declining due to lack of validation against the ITT. Inconsistency in sampling times for the SST in centres across the UK could lead to erroneous diagnoses of hypoadrenalism.[6]

Subjects passing the SST only at 60 min tend to exhibit a 'delayed response' to exogenous ACTH but in essence have normally functioning adrenal glands. If their management was to be based solely on the 30 min sample, they would be commenced on unnecessary, long-term steroid replacement therapy. The adverse effects of long-term steroids such as osteoporosis, diabetes mellitus and hypertension have long been known to significantly increase morbidity and mortality in patients.

To justify removing the 60 min sample, the percentage of subjects with a 'delayed response' to synacthen would need to be as close to zero as possible. However, because a significant percentage of subjects in both centres are at risk of misdiagnosis without the 60 min sample, its value in improving the accuracy of diagnosis in the SST has been brought to light.

Our study had some limitations. These include the fact that it was a retrospective data analysis taking samples from only two centres in the UK. We acknowledge that there are some underlining biases inherent in a retrospective study compared to a prospective one. However, we suggest that our results indicate a particular direction and that confirmation of our findings using a larger dataset of subjects prospectively and from multiple centres would strengthen the value of our results. Previous work surveying endocrine function testing in the UK showed that very few laboratories were using the guidelines for interpreting cortisol responses that were published 10 years prior to their survey.[6,19] The guidelines recommended testing cortisol at 30 and 60 min, but

Table 2. Comparisons of responses at 30 min and 60 min. These data show that a proportion of people at each site who fail at 30 min go on to pass at 60 min. However, those who failed at 60 min also failed at 30 min

Cortisol response at 30 or 60 min	NNUH, $n = 250$ 60 min # (%)			KCL, $n = 134$ 60 min # (%)			ALL, $n = 384$ 60 min # (%)		
	Pass	Fail	*P*-value	Pass	Fail	*P*-value	Pass	Fail	*P*-value
30 min									
Pass	188 (75%)	0 (0%)	<0·00001*	88 (67%)	0 (0%)	0·0001*	276 (72%)	0 (0%)	<0·00001*
Fail	18 (7%)	44 (18%)		15 (11%)	31 (23%)		33 (9%)	75 (20%)	

NNUH, Norfolk and Norwich University Hospital; KCH, King's College Hospital; Pass = cortisol response greater or equal to 550 nmol/l or else it is fail.
*McNemar's test for matched pairs.

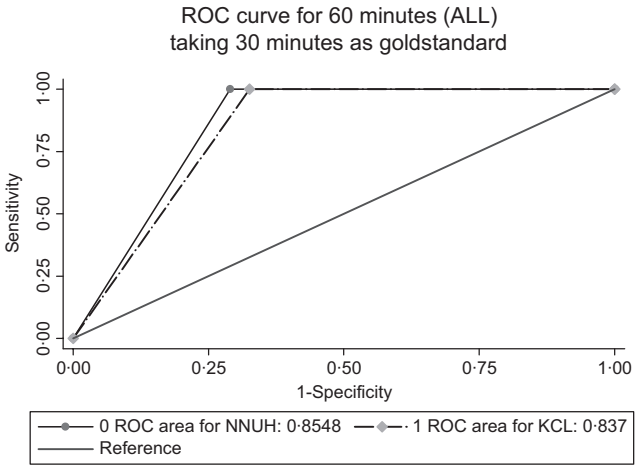

Fig. 1 Receiver operating characteristic curve for 60 min taking 30 min as the gold standard.

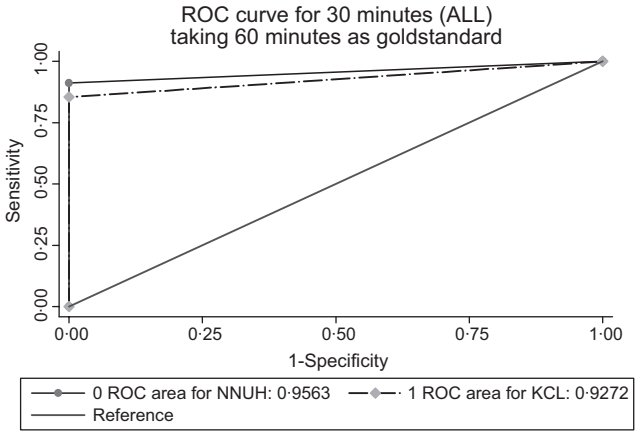

Fig. 2 Receiver operating characteristic curve for 30 min taking 60 min as the gold standard.

Table 3. Sensitivity, specificity, receiver operating characteristic (ROC) area, positive predictive value (PPV), negative predictive value (NPV) of responses at 60 min taking 30 min as the gold standard. These data suggest that a value of < 550 nmol/l at 60 min is always indicative of a failed response at 30 min, whist 11% of individuals with a value of > 550 nmol/l at 60 min may have had a suboptimal response at 30 min

	NNUH, n = 250 Estimate (95% CI)	KCL, n = 134 Estimate (95% CI)	ALL, n = 384 Estimate (95% CI)
Sensitivity	100% (98–100%)	100% (96–100%)	100% (99–100%)
Specificity	71% (58–82%)	67% (52–81%)	69% (60–78%)
ROC area	0·85 (0·80–0·91)	0·84 (0·77–0·91)	0·85 (0·80–0·89)
PPV	91% (87–95%)	85% (77–92%)	89% (85–93%)
NPV	100% (92–100%)	100% (89–100%)	100% (95–100%)

with a cut-off for a 'normal' value of 650 nmol/l.[19] These findings are discrepant to those of Klose *et al.* who suggested that the cut-off of 550 nmol/l was too high.[20] These authors also discussed the high false positive rates associated with different forms of assay. The general use of a cut-off of 550 nmol/l varies between centres, mainly due to the use of different cortisol assays. The discussion of which assays to use or whether the 30 min value is incorrect remain beyond the remit of this discussion, and the focus of our study was to assess the relevance of widespread current UK practice. Neither was it the purpose of the current study to assess the validity of the 60 min value against the results seen with an ITT – this has been carried out previously.[4] Furthermore, there is no consensus about whether the dose of synacthen should be given intramuscularly or intravenously – with both methods being quoted regularly in the literature.[20,21]

Table 4. Sensitivity, specificity, receiver operating characteristic (ROC) area, positive predictive value (PPV), negative predictive value (NPV) of responses at 30 min taking 60 min as the gold standard. These data suggest that a value of >550 nmol/l at 30 min is always indicative of a sufficient response at 60 min, whist 31% of individuals who were identified as having a suboptimal response at 30 min went on to have a sufficient response at 60 min

	NNUH, n = 250 Estimate (95% CI)	KCL, n = 134 Estimate (95% CI)	ALL, n = 384 Estimate (95% CI)
Sensitivity	91% (87–95%)	85% (77–92%)	89% (85–93%)
Specificity	100% (92–100%)	100% (89–100%)	100% (95–100%)
ROC area	0·96 (0·94–0·98)	0·93 (0·89–0·96)	0·95 (0·93–0·96)
PPV	100% (98–100%)	100% (96–100%)	100% (99–100%)
NPV	71% (58–82%)	67% (52–81%)	69% (60–78%)

We believe, however, that whilst absolute values are important, it is the principle that some people who have suboptimal results at 30 min (whatever the assay used and the local reference range) go on to have results that fall into the reference range at 60 min, and thus do not have adrenal insufficiency. Thus individual units should assess their own practice to determine the utility of their results at different times.

Results from patients with liver disease were excluded because it was felt that their hormone binding was likely to be variable due to changes in the levels of free cortisol, serum binding proteins, albumin and corticosteroid binding globulin or sex hormone metabolism thus making interpretation of the results difficult. As discussed by Keenan et al., these parameters need to be stable to allow accurate interpretation of the rapid changes in the equilibrium of the complex interaction between these variables.[22] In patients with ongoing liver disease this is unlikely to be the case.

Another limitation was that due to the small numbers of individuals who had delayed responses [<550 nmol/l at 30 min, but $\geq$ 550 nmol/l at 60 min – 18 (7%) from NNUH and 15 (11%) from KCH], our data did not have sufficient power to assess if there were any differences in outcomes according to age, gender or type of adrenal failure – primary or secondary. This work will need larger data sets.

In many units a 9 am cortisol may be carried out prior to deciding on the need for a SST. If this is low, then a zero, 30 and 60 min value are carried out. The results of the current

study suggest there seems little need for the zero and 30 min samples to be taken if the results of the 60 min samples are sufficient to make a diagnosis. Thus it may initially seem that the test may be confined to a single cortisol sample, taken 60 min after the administration of synacthen, affording cost, efficiency and comfort advantages. However, we feel that this would be an incorrect course of action for three reasons. First, given that it is the 30 min sample that has been shown to have the best correlation with the ITT, it is unlikely that without comparative data that the 30 min test will be abandoned. Secondly, the zero time basal sample is useful because it allows clinicians to know whether stressed patients 'passed the test' before being given Synacthen. Finally it also allows the clinician to get some feel for the severity of any cortisol deficiency, having the opportunity to assess the extent of the response to synacthen and, perhaps more importantly, being able to interpret the subsequent samples if the basal sample itself is elevated because of stress, etc. One hesitates to suggest that the comparison of the 0 min sample in relation to the other values is part of the *art* of interpreting the SST.

Thus, we would continue to recommend the use of the 30 min sample due to its repeated validation against the peak cortisol response to hypoglycaemia in the ITT, but would suggest that a 60 min sample also be taken due to the data we have presented, and the data presented by Dorin *et al.*[15]

In summary, to our knowledge, this clinical study is the first of its kind to support the use of the 60 min sample alongside the 0 and 30 min samples in the conventional SST.

Acknowledgements

The authors would like to acknowledge Sondra Gorick, Christine Jermy and Dr Haris Marath of the Department of Diabetes and Endocrinology at the Norfolk and Norwich University Hospital and Dr Hagosa Abraha of the Department of Clinical Biochemistry at King's College Hospital for their invaluable assistance in accessing SST results. We are also extremely grateful to Dr Jamshid Alaghband-Zadeh of the Department of Chemical Pathology at King's College Hospital, for their ongoing advice throughout the duration of the study. We would also like to acknowledge Dr Finlay MacKenzie at NEQAS in Birmingham for providing the data on assay bias. Finally, we are grateful to the reviewers for their invaluable comments and suggestions.

Conflict of interest

No grants or fellowships. The authors have nothing to disclose.

References:

1 Wood, J.B., James, V.H., Frankland, A.W. *et al.* (1965) A rapid test of adrenocortical function. *Lancet* **285**, 243–245.
2 Stewart, P.M., Seckl, J.R., Corrie, J. *et al.* (1988) A rational approach for assessing the hypothalamo-pituitary-adrenal axis. *Lancet* **331**, 1208–1210.
3 Davies, M.J. & Howlett, T.A. (1996) A survey of the current methods used in the UK to assess pituitary function. *Journal of the Royal Society of Medicine* **89**, 159–164.
4 Gleeson, H.K., Walker, B.R., Seckl, J.R. *et al.* (2003) Ten years on: Safety of short synacthen tests in assessing adrenocorticotropin deficiency in clinical practice. *Journal of Clinical Endocrinology & Metabolism* **88**, 2106–2111.
5 Chatha, K.K., Middle, J.G. & Kilpatrick, E.S. (2010) National UK audit of the Short Synacthen Test. *Annals of Clinical Biochemistry* **47**, 158–164.
6 Barth, J.H., Seth, J., Howlett, T.A. *et al.* (1995) A survey of endocrine function testing by clinical biochemistry laboratories in the UK. *Annals of Clinical Biochemistry* **35**, 442–449.
7 Lindholm, J. & Kehlet, H. (1987) Re-evaluation of the clinical value of the 30 min ACTH test in assessing the hypothalamic-pituitary-adrenocortical function. *Clinical Endocrinology* **26**, 53–59.
8 Mayenknecht, J., Diederich, S., Bahr, V. *et al.* (1998) Comparison of low and high dose corticotropin stimulation tests in patients with pituitary disease. *Journal of Clinical Endocrinology & Metabolism* **83**, 1558–1562.
9 Darmon, P., Dadoun, F., Frachebois, C. *et al.* (1999) On the meaning of low-dose ACTH(1-24) tests to assess functionality of the hypothalamic-pituitary-adrenal axis. *European Journal of Endocrinology* **140**, 51–55.
10 Dorin, R.I., Ferries, L.M., Roberts, B. *et al.* (1996) Assessment of stimulated and spontaneous adrenocorticotropin secretory dynamics identifies distinct components of cortisol feedback inhibition in healthy humans. *Journal of Clinical Endocrinology & Metabolism* **81**, 3883–3891.
11 Veldhuis, J.D., Carlson, M.L. & Johnson, M.L. (1987) The pituitary gland secretes in bursts: appraising the nature of glandular secretory impulses by simultaneous multiple-parameter deconvolution of plasma hormone concentrations. *Proceedings of the National Academy of Sciences* **84**, 7686–7690.
12 Hurel, S.J., Thompson, C.J., Watson, M.J. *et al.* (1996) The short Synacthen and insulin stress tests in the assessment of the hypothalamic-pituitary-adrenal axis. *Clinical Endocrinology* **44**, 141–146.
13 Wang, T.M., Wong, M.S., Smith, J.F. *et al.* (1996) The use of the short tetracosactrin test for the investigation of suspected pituitary hypofunction. *Annals of Clinical Biochemistry* **33**, 112–118.
14 Mansoor, S., Islam, N., Siddiqui, I. *et al.* (2007) Sixty-minute post-Synacthen serum cortisol level: a reliable and cost-effective screening test for excluding adrenal insufficiency compared to the conventional short Synacthen test. *Singapore Medical Journal* **48**, 519–523.
15 Dorin, R.I., Qiao, Z., Qualls, C.R. *et al.* (2012) Estimation of maximal cortisol secretion rate in healthy humans. *Journal of Clinical Endocrinology & Metabolism* **97**, 1285–1293.
16 Bangar, V. & Clayton, R.N. (1998) How reliable is the short synacthen test for the investigation of the hypothalamic-pituitary-adrenal axis? *European Journal of Endocrinology* **139**, 580–583.
17 Kehlet, H., Blichert-Toft, M., Lindholm, J. *et al.* (1976) Short ACTH test in assessing hypothalamic-pituitary-adrenocortical function. *British Medical Journal* **1**, 249–251.
18 Borst, G.C., Michenfelder, H.J. & O'Brian, J.T. (1982) Discordant cortisol response to exogenous ACTH and insulin-induced hypoglycemia in patients with pituitary disease. *New England Journal of Medicine* **306**, 1462–1464.
19 Moore, A., Aitken, R., Burke, C. *et al.* (1985) Cortisol assays: guidelines for the provision of a clinical biochemistry service. *Annals of Clinical Biochemistry* **22**, 435–454.

20 Klose, M., Lange, M., Rasmussen, A.K. *et al.* (2007) Factors influencing the adrenocorticotropin test: role of contemporary cortisol assays, body composition, and oral contraceptive agents. *Journal of Clinical Endocrinology & Metabolism* **92**, 1326–1333.

21 Clark, P.M., Neylon, I., Raggatt, P.R. *et al.* (1998) Defining the normal cortisol response to the short Synacthen test: implications for the investigation of hypothalamic-pituitary disorders. *Clinical Endocrinology* **49**, 287–292.

22 Keenan, D.M., Roelfsema, F. & Veldhuis, J.D. (2004) Endogenous ACTH concentration-dependent drive of pulsatile cortisol secretion in the human. *American Journal of Physiology - Endocrinology And Metabolism* **287**, E652–E661.