GH stimulation tests: evaluation of GH responses to heat test versus insulin-tolerance test

Sanne Fisker¹, Jens Otto Jørgensen¹,², Hans Ørskov³ and Jens Sandahl Christiansen¹,²
¹Medical Department M (Endocrinology and Diabetes), ²Centre of Clinical Pharmacology and ³Institute of Experimental Clinical Research, Aarhus University Hospital, Aarhus, Denmark
(Correspondence should be addressed to S Fisker, Medical Department M, Aarhus Kommunehospital, DK-8000 Aarhus, Denmark)

Abstract
Objective: Heat exposure has been shown to stimulate GH release, but the specificity and the reproducibility have not been determined, and the test has not been compared with validated GH stimulation tests in adulthood. We therefore tested the specificity and the reproducibility of the heat exposure test in healthy subjects and compared the results with those obtained with the insulin-tolerance test (ITT).

Design: Ten healthy non-obese men, aged 31.3 ± 4.80 years, underwent four GH stimulation tests in random order: two ITTs and two heat exposure tests. In the heat test, subjects were placed in a hot bath with water temperature at 40.3 ± 0.11 °C for 45 min, resulting in an identical (P = 0.477) significant increase in tympanic temperature of 1.26 ± 0.05 and 1.41 ± 0.07 °C in the two tests.

Results: Peak GH response to the heat exposure test was less than the peak GH response to ITT (5.25 ± 1.72 vs 15.5 ± 3.17 µg/l, P = 0.006). Furthermore the specificity (arbitrary cut-off level = 3 µg/l) of the heat test was lower than of the ITT (8/17 vs 18/20, P = 0.006). The coefficient of variation did not differ between the two tests (heat test 0.31, ITT 0.36, P = 0.77). Peak GH values in the individual tests were highly correlated (heat, r = 0.908, P = 0.002; ITT, r = 0.815, P = 0.004).

Reproducible increments in the circulating levels of stress hormones were observed during ITT, but these hormones remained largely unchanged during heat exposure.

Conclusions: The heat exposure test is not a reliable GH stimulation test compared with the ITT in adults. This study documents that the ITT has a high specificity and reproducibility in the diagnosis of GH deficiency in adulthood. We propose that the heat exposure test is not used in the diagnosis of this condition in adulthood.

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Introduction
It is well known that heat exposure results in increased growth hormone (GH) secretion, even when only a modest increase in core temperature is achieved (1–4). Furthermore, it has been demonstrated that a temperature rise is essential for the GH secretion during exercise (2). GH stimulation tests have been shown to be superior to spontaneous GH secretion in the diagnosis of GH deficiency (GHD) in adults (5, 6), and since the heat test represents one of the few physiological stimulation GH tests it might provide an alternative to a pharmacological GH test. The specificity and the reproducibility of the heat test have, however, not been determined. The response to pharmacological tests, such as GH-releasing hormone (GHRH), L-arginine and the insulin-tolerance test (ITT) may not reflect the physiological GH secretory capacity, which theoretically is better measured in a physiological test such as heat exposure. In the present study we have evaluated the reproducibility and specificity of the heat exposure test compared with the ITT in healthy men and also evaluated the stress response in the two tests with respect to other hormones.

Subjects and methods
Ten healthy non-obese (mean body mass index (BMI) ± S.E.M. 22.7 ± 0.9 kg/m²) men (mean ± S.E.M. age 31.3 ± 4.80 years) participated in the study after written informed consent. The study was approved by the local ethics committee.

All participants underwent two ITTs and eight participants underwent two heat tests in random order after staying overnight at the hospital, performing minimal physical activity before the test, and having fasted for 10 h. Two participants left the study before performance of the heat tests: one was excluded before the second heat test because of commencement of antidepressive medication, and one left the study because of backache. The ITT was performed by i.v.
bolus injection of regular insulin (Novo Nordisk, Copenhagen, Denmark) in a dose of 0.075 IU/kg at 0 min. In all participants hypoglycaemia was achieved with blood glucose \( \leq 2.2 \) mmol/l. Blood samples were drawn from -30 min to 120 min every 15 min. From 0 to 30 min, blood samples were drawn every 10 min. In the heat test, subjects were placed in a hot bath with water temperature at 40.3 \( \pm \) 0.11 \(^\circ\)C for 45 min, resulting in a significant increase in tympanic temperature of 1.26 \( \pm \) 0.05 and 1.41 \( \pm \) 0.07 \(^\circ\)C (both \( P < 0.001 \)) in the two heat tests. The bath temperature was kept constant by adding hot water during the tests. Tympanic temperatures were measured with a thermocouple probe, type A115 (Ellab Instruments, Denmark) connected to an electronic direct reading thermometer (Ellab). The accuracy of the thermometer had previously been checked to be within \( \pm 0.1 \) \(^\circ\)C (2).

During the ITT, plasma glucose was measured immediately in duplicate on an autoanalyser (Beckman Instruments, Palo Alto, CA, USA) by the glucose oxidase method. All other blood, serum and plasma samples were stored at \(-20\) \(^\circ\)C until analyses were performed. All analyses from one individual were run in the same assay. Blood glucose was determined by the glucose oxidase method after stabilising the sample in NaF until analysis. Serum GH was determined in an immunofluorometric assay (TR-IFMA, Delfia, Wallac, Finland) with two monoclonal antibodies directed against the 22 kDa variant of human GH. Serum cortisol was determined by a competitive immunofluorometric assay (TR-IFMA, Delfia). Plasma glucagon was measured as previously described (7) after extraction with ethanol. Serum insulin-like growth factor-I (IGF-I) analyses were performed with an in-house TR-IFMA after acid–ethanol extraction of serum to remove binding proteins as previously described (8). Serum adrenaline and noradrenaline were determined by electrochemical detection after HPLC (9). Serum non-esterified fatty acids (NEFA) were measured by an enzymatic colorimetric method (Wako Chemicals, Neuss, Germany). Serum insulin was analysed by an ELISA (Dako A/S, Copenhagen, Denmark). BMI and waist/hip ratio (W/H) were determined. Whole body resistance was measured using the BIA 101 (RJL-Systems, Detroit, MI, USA). Percentage of body fat was calculated by the software supplied with the BIA 101.

Statistics

Differences between peak GH responses were analysed by paired \( t \)-test when data were normally distributed. Otherwise, the Mann–Whitney Rank sum test was used. GH, cortisol, catecholamine, and glucagon responses were analysed by two-way (time and condition) ANOVA. Variability was defined as mean coefficients of variation (CV) of individuals tested (standard deviation/mean peak). Specificity was defined as the number of subjects with a GH response above 3 \( \mu \)g/l divided by total number of tests. When comparing hormone responses in the two different GH tests, the responses of the first performed tests were used. For correlation analysis, Pearson’s correlation was performed. Data are expressed as means \( \pm \) S.E.M., \( P \) values \( \leq 0.05 \) were considered significant.

Results

GH

Serum GH concentrations increased significantly during heat exposure and ITT (ANOVA: both \( P < 0.001 \)), and there were no differences in peak responses between the first and second ITT and heat test (ANOVA: heat test \( P = 0.450 \), ITT \( P = 0.851 \)) (Fig. 1). The GH response during ITT was significantly higher compared with that of the heat test (ANOVA: \( P < 0.001 \)), and so were the peak GH values (15.5 \( \pm \) 3.2 and 5.2 \( \pm \) 1.7 \( \mu \)g/l, \( P = 0.006 \)). Furthermore the specificity of the ITT was markedly higher when using a cut-off level of 3 \( \mu \)g/l (9/10 and 9/10 (ITTs) vs 5/9 and 3/8 (heat tests) respectively, \( P = 0.006 \)). In 4/17 heat exposure tests GH peak response was less than 0.5 \( \mu \)g/l. The CV of the peak GH responses was equal with the two tests (ITT 0.36 vs heat exposure 0.31, \( P = 0.77 \)). Peak GH was obtained in the heat test after 45 min, at which time the heat exposure was terminated. Maximum temperature rise was attained 40 min after the start of heat exposure, but at least 1 \(^\circ\)C of temperature rise was attained after 20 min (data not shown). There was a high degree of correlation between both the two ITTs and the two heat exposure tests (ITT \( r = 0.815 \), ITT 0.004; heat exposure \( r = 0.908 \), \( P = 0.002 \)).

Peak GH in the ITTs was negatively correlated to body fat estimated by bioimpedance (test one, \( r = -0.774 \), \( P = 0.009 \); test two, \( r = -0.637 \), \( P = 0.048 \)), whereas peak GH did not correlate to BMI or W/H. In the heat test there was no correlation between peak GH and any measure of body composition (data not shown). Nor did the increase in body temperature correlate to peak GH (data not shown). IGF-I levels were 187 \( \pm \) 16.3 \( \mu \)g/l.

Blood glucose, NEFA and fasting insulin

Blood glucose decreased significantly during the ITT (ANOVA: \( P < 0.001 \)) (Fig. 2). In all subjects nadir blood glucose levels of less than 2.2 mmol/l were achieved. During heat exposure blood glucose levels did not change (ANOVA: \( P = 0.118 \)), but they were lower in test two (ANOVA: \( P < 0.001 \)). NEFA decreased during hypoglycaemia (\( P < 0.001 \)) but reached fasting levels after 120 min. During heat exposure there was a time-dependent increase in levels of NEFA to 60 min followed by a slight physiologically insignificant decrease (ANOVA: \( P = 0.003 \)). In both the ITTs and the heat test, levels of NEFA were lower in test two (ANOVA: \( P = 0.019 \) and \( P < 0.001 \)). Fasting insulin was identical in the two ITTs (8.00 \( \pm \) 1.13 vs 7.50 \( \pm \) 0.05 pmol/l,
P = 0.70) whereas fasting insulin in the second heat exposure test was higher than in the first (8.63 ± 1.38 vs 7.00 ± 0.96 pg/l, P = 0.045).

**Cortisol and glucagon**

Serum cortisol increased significantly and equally during the two ITTs (ANOVA: P < 0.001 and P = 0.272), whereas serum cortisol fell significantly (according to circadian rhythm) during heat exposure and was lower in test two (ANOVA: P < 0.001 and P = 0.011) (Fig. 3). Plasma glucagon also increased significantly and identically during both ITTs (ANOVA: P < 0.001 and P = 0.961), whereas there was no change during heat exposure.

**Catecholamines (adrenaline and noradrenaline)**

Plasma adrenaline increased identically and significantly during the two ITTs (ANOVA: P = 0.383 and P < 0.001), whereas there was an insignificant increase during heat exposure (ANOVA: P = 0.07), and no difference between responses in test one and two (Fig. 3). Noradrenaline increased during both the ITTs and the heat tests.

**Discussion**

This study suggests that the heat exposure test is less useful in the diagnosis of GHD in adulthood because of low specificity using the arbitrary cut-off level of 3 μg/l adopted from the ITT (10). The GH responses to heat were smaller compared with those attained in the ITT, whereas variability in peak GH responses was comparable in the two different test types.

In adults it has been demonstrated that spontaneous 24 h GH secretion does not separate GHD patients from healthy subjects (5, 6). Urinary excretion of GH has been proposed as another method of evaluation of physiological GH secretion. However, the specificity of this test is not acceptable for diagnostic use, at least not in patients above the age of 40 years (11). GH secretion is increased during exercise, which is, however, difficult to standardise, and furthermore there is evidence to suggest that the GH secretion during exercise is secondary to the concomitant increase in body temperature (2). We therefore found it relevant to investigate the diagnostic value of heat exposure. We found substantially lower peak GH values compared with those after ITT, despite a rise in core temperature of at least 1 °C.

In both series of ITTs only one normal subject responded with a peak GH below 3 μg/l. The low peak GH values were presented by two different subjects, who responded with low peak values during both ITTs, although peak GH just exceeded 3 μg/l in one of the tests for both concerned. This confirms the high specificity of the ITT (5). We found a low intra-individual variability of ITTs of 0.36, which is below that described by Hoeck et al. (12). The low variability was also illustrated by a high degree of correlation between peak GH in test one and two in both ITT and heat exposure test. The CV of the ITT is comparable to that found in the GHRH–pyridostigmine test (13), and the CVs between GH response to test one and two comparable to those found in the GHRH–arginine test in different age groups (14). We have previously demonstrated that external factors such as physical activity prior to testing may confound the test result (15), and therefore the subjects in our
study were admitted to hospital the night before testing. Pre-testing factors may thus explain some of the disagreement in peak GH variability in different studies. The intra-individual variability of heat tests was comparable to that of ITTs. Very low peak levels (below 0.5\( \mu g/l \)) were, however, found in almost 25% of the heat tests. This may reduce the applicability of the test to diagnose GHD in adults, even if the cut-off level was reduced. We have, however, not investigated GH response to heat exposure in GHD patients.

Adrenalin was significantly increased during ITT, but an insignificant increase was found during heat exposure. This is in agreement with the findings of Weeke & Gundersen (1), who found a significant increase in noradrenaline only during heat exposure for 60 min. In that study, body temperature increased to slightly higher levels than in our study, but a plateau was reached after 30-40 min at which time GH also reached a plateau. In a study by Møller et al. (16) it was demonstrated that a 3 h hot bath with an increase in core temperature of 1.2\(^\circ\)C (plateau reached after approximately 30 min), resulted in a significant increase in both adrenaline and noradrenaline. In that study GH peaked after approximately 45 min. Christensen et al. (2) found that 80 min of heat exposure applied by covering subjects in heated blankets (increase in core temperature approximately 1\(^\circ\)C) resulted in a significant increase in GH secretion with peak value after 80 min. The time of heat exposure and the kind of stress applied during the temperature rise may be of importance for the hormone responses and more prolonged heat exposure with continuous increase in core temperature may result in higher GH responses. Considering catecholamine responses, in the present study it appears that the stress caused by the heat test was not as extensive as in the ITT. We also measured other hormones during ITT and heat exposure. Generally the hormone levels during the tests were highly reproducible, although cortisol during heat exposure was lower in the second test. Glucose, NEFA, and fasting insulin levels also differed in the two heat tests, which is probably coincidental.

It has previously been demonstrated that body fat determines the GH response to arginine (17). Our data are in line with those findings with respect to the GH response to ITT, since peak GH was negatively correlated to body fat estimated by bioelectric impedance assessment (BIA). In contrast, peak GH in the heat test was
not correlated to body fat. This is, however, in contrast to the findings of Weeke & Gundersen (1). Nor was the increase in body temperature correlated to peak GH, which suggests that factors in addition to core temperature per se are important during heat stimulation, as also indicated by data from sauna studies, where very small increases in body temperature resulted in significant GH secretion (4). Whether these additional factors include skin temperature or duration of heat exposure has not been clarified. The GH stimulatory effect of arginine and ITT is probably mediated via a decrease in somatostatin tonus (18), which is increased in obesity. This might explain the negative relation between body fat and GH secretory capacity to arginine and ITT. Heat exposure has been suggested to act on GH secretion through GHRH rather than a decrease in somatostatin tone, as GHRH is elevated during heat exposure in younger men (4). On the other hand, Ovesen et al. (19) found that arginine infusion prior to heat exposure abolished GH secretion to subsequent

Figure 3 Mean noradrenaline (dashed lines), adrenaline (full lines), cortisol and glucagon responses during ITT (A), n = 10, and during heat exposure (B), n = 8. • test one, ○ test two. Significance level of time-dependent changes in: adrenaline, ITT P < 0.001, heat test P = 0.07; noradrenaline, ITT P < 0.001, heat test P = 0.002; cortisol, ITT P < 0.001, heat test P < 0.001; glucagon, ITT P < 0.001, heat test P = 0.318. Significance level of test difference in: adrenaline, ITT P = 0.383, heat test P = 0.851; noradrenaline, ITT P = 0.933, heat test P = 0.869; cortisol, ITT P = 0.272, heat test P = 0.011; glucagon, ITT P = 0.590, heat test P = 0.166. Arrow indicates time of insulin injection. Black bar indicates heat exposure.
heat stimulation, suggesting that arginine and heat exposure share common pathways of GH stimulation. So far the exact way in which heat exerts its influence on GH secretion has not been clarified.

In conclusion, heat exposure should be used with great attention to the low GH stimulatory potency if used to diagnose GHD or to characterise GH secretory capacity in healthy adults. Finally, this study confirms the high specificity of the ITT and provides new data to suggest acceptable reproducibility.

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