LETTERS TO THE EDITOR

Different Effects of GH Treatment on Cognitive Function in Girls with Turner’s Syndrome and in Adults with GH Deficiencya

To the editor:

We read with great interest the paper by Ross et al. (1), concerning the absence of GH effects on cognitive function in girls with Turner’s syndrome. In our opinion, the statement that these results are in agreement with most of the previous studies that found no apparent GH treatment effects on cognitive function in patients with GH deficiency (GHD) needs a fair degree of caution.

In fact, although GH treatment is recommended worldwide for ameliorating the final height of girls with Turner’s syndrome, these patients are generally not classically GH-deficient. For this reason, we believe that the absence of GH effects on cognitive function, as clearly demonstrated by Ross et al. (1) in girls with Turner’s syndrome (without estrogen replacement treatment), is not easily comparable with the data obtained in GHD patients, in whom GH represents the “substitution” therapy.

Furthermore, the concern by Ross et al. (1) that the presence of multiple pituitary hormonal defects in adults with GHD could have a negative impact on brain development and could potentially interfere with the results of the psychological tests is not completely justified, as the majority of clinical studies [including the only mentioned (2)] have been performed in patients receiving stable and adequate hormonal replacement therapies.

As far as the effects of GH therapy on cognitive functions in adults with GHD are concerned, Degerblad et al. (3) actually found no significant effects of GH treatment; however, they suggested that the negative results could be tentatively explained by the difficulty in optimizing the measurement of subtle changes of mood and cognitive functions, rather than by a real lack of effects exerted by GH. In a short-term study (1 month) using GH treatment, Almqvist et al. (4) demonstrated that recombinant GH was able to improve cognitive psychometric testing, in particular the face recognition test, a test primarily for evaluating memory function.

Our experience in adults with childhood-onset GHD (5, 6) showed that 6 months of GH treatment caused an overall improvement in relation to intellectual tasks, accompanied by a lower level of stress during their performance. In particular, the scores of the tasks in the nonverbal Wechsler Adult Intelligence Scale (WAIS) and in the mental arithmetic test increased significantly, while those of “sensitivity,” “thought,” “impulsiveness,” and “anxiety” scales (evaluated using the “Experiential-World Inventory”-EWI) reduced. The finding that the psychological characteristics of patients reverted to those before treatment after stopping recombinant GH supports the GH dependence of the effects observed during treatment.

In conclusion, as also stated by Ross et al. in their interesting paper (1), we agree that cognition in girls with Turner’s syndrome is more probably estrogen-dependent rather than GH-dependent, as the former actually represents the main feature of the syndrome. Further additional studies, aimed at correcting the real hormonal defects, are required to understand the potential reversibility (or not) of the neurocognitive deficits observed in Turner’s syndrome.

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References

Psychological Stress and Skydivingb

To the editor:

Recently, two interesting papers dealing with psychological stress produced by skydiving appeared in The Journal of Clinical Endocrinology and Metabolism (1, 2). Because in 1992 and 1993 we described the response of subjects about to make their first jump with a parachute (3, 4), we would like to offer some comments.

The plasma concentrations of a number of hormones were found not to increase significantly during the period preceding the jump (1, 2). However, in our hands, the skydiving test—before a novice enters the plane—induced a significant rise in plasma antidiuretic hormone and a similar tendency in serum cortisol (3, 4). Also, there was a significant and positive correlation between the changes in cortisol and C-reactive protein.

One explanation for the negative findings could be that the responses of some hormones were blunted, as each stressor might induce its specific hormone pattern (5). More likely, the discrepancy between our results is due to the different design of the experiments, the selection of controls, and the influence of preanalytical factors (factors acting before a specimen is collected and analyzed). Stress responses are known to exhibit considerable interindividual variation. To increase the detectability of the response, we used each individual as his or her own control (during a stress-free situation some days before or after the jump) instead of different control individuals. In the articles quoted there is no mention of the possibility of preanalytical influences (e.g. time of day, posture, food intake, exercise, experiencing cold, and changes in altitude and speed, etc. during the flight and the fall). Because we have previously demonstrated that physical activity and changes in posture have strong effects (e.g. refs. 6–10), the investigators (1, 2) may have been unable to keep the volunteers absolutely free of physical activity—parachutists need to change clothes, take on a harness, etc. Actually, we considered it important, as recommended (6), to collect the specimens after 15 min of sitting to achieve hemodynamic equilibrium and to eliminate the influence of many other preanalytical factors. Therefore, it would have been interesting to receive information on analytes that reflect physical activity (e.g. serum creatine kinase and lactate dehydrogenase) and hemoconcentration.

Because of ethical and legal considerations it is difficult to induce pure psychological stress experimentally. Therefore, imminent skydiving—before a novice enters the plane—seems to be an excellent model for such studies. However, later phases in the skydiving sport appear to involve so much somatic stress that one should be cautious in using them as models of pure psychological stress.

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b Received December 5, 1997. Address correspondence to: Dr. Benoît Dugué, Minerva Foundation Institute for Medical Research, Tukholmankatu 2, Helsinki 00250, Finland.
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References

Psychological Stress and Sky Diving—Authors’ Response

To the editor:

We appreciate the comments of Dr. Dugué and colleagues, in the preceding letter, concerning a first-time parachute jump as a model for acute psychological stress. This model has been employed in a number of studies and has been shown to induce a pronounced neuroendocrine stress response (1–5). The authors argue however, that physical activity before jumping and in particular during the jump itself, influence the psycho-neuroendocrine response. Furthermore, it is argued that anticipatory stress of the novice jumper, incurred immediately before the first jump is a superior model for analyzing the effects of purely psychological stress on neuroendocrine parameters. However, in the studies cited as examples, the prejump psychological arousal did not significantly increase cortisol plasma concentrations (6, 7). This is in accordance with previously published observations in first-time tandem parachutists where the prejump stress did not increase cortisol, prolactin, GH, or TSH levels (3, 5). Both studies however, observed increased sympathetic activity before the jump as indicated by elevated salivary catecholamine (5) and plasma noradrenaline levels (3). The blunted neuroendocrine response before the jump itself was most probably due to stress coping mechanisms of novice parachutists (5). These subjects appear to cope well with the forthcoming jump. However, after boarding the aircraft and during ascent of the plane, heart rate values and endocrine parameters start to increase, and they peak during the jump itself as this potentially life threatening situation elicits an emergency (fight-flight) response with a weakening of psychological coping mechanisms (2, 3, 8).

We agree that there is some physical activity involved in performing a tandem-parachute jump. However, physical exercise, is mainly associated with the release of noradrenaline. In contrast, psychological stress, primarily elicits an increase in adrenaline plasma concentrations. The much greater jump-induced increase in adrenaline (700%) plasma concentrations when compared with a 100% increase in noradrenaline levels, demonstrates that psychological stress is predominantly responsible for the neuroendocrine changes during parachute jumping (3).

Whereas for ethical reasons it remains difficult to induce psycholog-

cal stress in an experimental setting with human subjects, a first-time (tandem) parachute jump is a very good, though not perfect, model to investigate the effects of intense acute psychological stress on neuroendocrine parameters.

LETTERS TO THE EDITOR

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Psychological Stress and Skydiving—Author’s Response

To the editor:

Dugué, in the letter above, implies that there is a significant difference between the data of his group and that published by us (1). However, the only analyte measured by both of us was cortisol in men before a parachute jump. His group found a mean increase in serum cortisol of from 504 to 582 mmol/L, P < 0.15 (2). In females the difference was even less significant. There did appear to be a hemoconcentration effect in his subjects, but it was only 2.0% (3). We also found no significant increase before the actual experience of parachuting. Assuming their initial value was actually 504 mmol/L rather than 504 nmol/L as listed, it would appear that their subjects were quite stressed at the time of the initial blood sample. This may, in part, be because blood was drawn by venipuncture, a stressful procedure for many persons.

In our case an indwelling catheter was inserted early in the day; the initial mean value at 0800 h for the parachutists on the day of the skydive was 250 mmol/L and at 1230 h was 225 mmol/L. We did include control day data on the parachutists, but only at one time during the day. The mean cortisol concentration in the parachutists themselves at 1400 h, 3–5 days before the skydive, was 226 mmol/L. Our subjects were also seated for at least 15 min before the preboarding sample was obtained. Hemoglobin measurements were not taken but, if similar to those of Dugué et al. (2), little correction for hemoconcentration would have been necessary.

Thus, we see no evidence that cortisol was increased in first-time skydivers before the actual event in either study and, to quote the conclusion of Dugué et al. (2), “cortisol, commonly used to indicate the level of stress, did not react much and is therefore not a good index of psychological stress.” In fact, we found that the concentration of plasma cortisol increased before the jump itself was most probably due to stress coping mechanisms of novice parachutists (5). These subjects appear to cope well with the forthcoming jump. However, after boarding the aircraft and during ascent of the plane, heart rate values and endocrine parameters start to increase, and they peak during the jump itself as this potentially life threatening situation elicits an emergency (fight-flight) response with a weakening of psychological coping mechanisms (2, 3, 8).

We agree that there is some physical activity involved in performing a tandem-parachute jump. However, physical exercise, is mainly associated with the release of noradrenaline. In contrast, psychological stress, primarily elicits an increase in adrenaline plasma concentrations. The much greater jump-induced increase in adrenaline (700%) plasma concentrations when compared with a 100% increase in noradrenaline levels, demonstrates that psychological stress is predominantly responsible for the neuroendocrine changes during parachute jumping (3).

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References
cortisol progressively decreased significantly until mid-morning before skydiving, and we comment on this in relation to other studies in which a similar phenomenon was observed.

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References


Childhood Graves’ Disease—Remission Rate and Risk Factors

To the editor:

The management of Graves’ disease in childhood is often difficult for both patients and their parents, and places a great deal of responsibility on physicians for appropriate treatment guidelines. Glaser and Styne (1) recently presented a retrospective investigation of 191 patients aged 1–19 yr treated for Graves’ disease at 5 pediatric hospitals in California from 1976–1996. They found that among patients with BMI scores above 0.5 sd and small goiters, 86% responded favorably to pharmacological treatment (remission, defined as normal T4 or fT4 concentrations when off medication for 6 months within 2 yr after initiation of therapy). In a group with lower BMI and larger goiters, remission was seen in only 31%. Despite the large number of patients the authors were unable to identify other predictive factors, possibly because several parameters were missing in the records reviewed. A total of 85/191 patients were excluded from the analyses. These patients had been subjected to radioactive or surgical therapy, failed to achieve remission or were lost to follow-up. This seems to imply that the true number of patients who failed to achieve remission was larger than that of the final comparison between 27 remitters and 79 nonremitters. Moreover, the 6 month follow-up period was remarkably short, contributing additional bias to the study.

In their report, Glaser and Styne subscribe to previous claims that 25% of children with Graves’ disease enter remission every 2 yr on pharmacological treatment (2). We disagree, and we object to this frequently cited and oversimplified figure. In our experience, comprising 31 carefully monitored cases subjected to a long-term thyrostatic-thyroxine regimen, only 19% entered remission after a median of 6.5 yr (range 4.5–8 yr) (3). The mean age at diagnosis was 11 yr, and the majority of the patients were girls. Although our material was smaller, it was population-based, and all the patients were followed into adulthood. We argue strongly that a proper evaluation of therapeutic response must be based on long-term follow-up.

In our view, the majority of children with Graves’ disease are notoriously difficult to cure with pharmacotherapy, and even during long-term therapy only a small fraction will enter permanent remission. We therefore recommend a limited period of thyrostatic drug treatment. If the disease activity is not readily reduced (as reflected by the dose of thyrostatics required and the level of TSReceptor antibodies), extensive thyroid surgery is advised. We believe that this active strategy reduces the risk of exposing children with Graves’ disease (and their families) to complications of medical therapy, 12 (14%) opted to discontinue medical therapy because of dissatisfaction with frequent office visits and blood testing, and 3 (4%) chose surgical therapy for cosmetic reasons. Only 9 (11%) continued to have elevated T4 and/or T3 concentrations at the time of surgery or radioablation and thus could possibly be said to have failed medical therapy. Of these 9 patients, however, noncompliance was suspected in 5. Thus, there is no clear indication that the exclusion of these 5 patients would have been likely to bias the results of the study.

Despite the limitations of our study as described above, we do not feel that the stated remission rate of 25% with every 2 yr of treatment is markedly inaccurate. The remission rate observed in our study agrees with that observed by Lippe et al. (2) and Collen et al. (3) in their previous prospective studies. In the initial study by Collen et al., the authors followed a cohort of 65 pediatric patients with hyperthyroidism for periods ranging from 3 months to 16 yr. They used life-table analysis methods to determine the distribution of remission times in the population. In that study, the authors defined remission as maintenance of clinical and biochemical euthyroidism for at least 1 yr without antithyroid medication. They determined that approximately 25% of patients achieve remission with every 2 yr of treatment. In a second study by Lippe et al., the same group followed the cohort of patients from the first study for an additional 5 yr. Even with this additional follow-up period, the remission rate remained the same. The authors note that only one of 36 patients who achieved remission experienced a relapse after remaining euthyroid for greater than 1 yr without antithyroid medication. The remaining 35 patients had been followed for a mean of 3.3 ± 2.9 yr (range 1–11.7 yr) without experiencing a relapse. Both the relatively long follow-up period in these studies as well as the concordance of the

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References

remission rate observed in these studies with that observed in our study argues strongly that the figure is likely an accurate one.

Karlsson et al. (4) state that, in their study of 31 pediatric patients with hyperthyroidism, only 19% entered remission after a median of 6.5 yr of medical treatment. The authors, however, do not report data regarding predictive variables such as goiter size and body mass, index which were determined in our study to influence the likelihood of early remission. In addition, the sample size for their study was small. Finally, 21% of the patients in their study were treated with antithyroid medication for 2 yr or less before surgery and 67% were treated for 4 yr or less. We feel that determination of accurate remission rates from this small study group, many of whom were treated for relatively brief periods, is difficult.

Karlsson et al. favor the use of surgery after a brief attempt at medical management. While the optimal treatment for pediatric hyperthyroidism continues to be controversial, we feel that their strategy would subject many children to unnecessary surgical procedures and the risk of surgical complications. We continue to advocate medical therapy as the first line of treatment, particularly in patients who present with small goiters and body mass index sd scores greater than –0.5 sd, indicative of a high likelihood of achieving remission within 2 yr.

As concerns the issue of increased risk of autoimmune thyroid disease in children, the presence of lipoid CAH was formerly misdiagnosed as primary hyperthyroidism. This is a well-established fact. Children with Down’s syndrome constituted 3% of our study population. This information (along with information regarding other concurrent medical disorders in the study population) was not included in the manuscript merely because of the desire to keep the manuscript concise. Children with Down’s syndrome were not excluded from the analysis, and the care of children with Down’s syndrome in our clinics certainly is no different from that of other children.

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Sacramento, California 95817

References


Why Nobody Has P450scc (20,22 Desmolase) Deficiency*

To the editor:

Okuyama et al. (1) recently reported a case of congenital lipoid adrenal hyperplasia (lipoid CAH) caused by a splicing mutation in the gene for the steroidogenic acute regulatory protein (STAR). In citing the relevant literature, they pointed out that nearly all patients with the lipoid CAH phenotype have been found to have STAR mutations. In two large series, Bose et al. (2) and Nakae et al. (3) found STAR mutations in 39 of 40 patients with lipoid CAH. Thus, lipoid CAH was formerly misdiagnosed as primary hyperthyroidism. This is a well-established fact. Children with Down’s syndrome constituted 3% of our study population. This information (along with information regarding other concurrent medical disorders in the study population) was not included in the manuscript merely because of the desire to keep the manuscript concise. Children with Down’s syndrome were not excluded from the analysis, and the care of children with Down’s syndrome in our clinics certainly is no different from that of other children.

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Serum Leptin Levels in a Patient with Pheochromocytoma

To the editor:

We read with great interest the report of Masuzaki et al. (1), which showed that serum leptin levels were increased in patients with Cush- ing’s syndrome. However, serum leptin levels in a patient with pheochromocytoma, another adrenal tumor, were not described.

A 72-year-old man complaining of episodic headaches, nausea, palpitations, and perspiration was referred to our department in October 1997. Physical examination showed that the patient was agitated and had striking peripheral vasoconstriction. Blood pressure varied between 240/130 and 80/40 mm Hg; the patient did not have orthostatic hypo-
tension. Plasma noradrenaline and adrenaline levels were increased (2.20 ng/mL and 0.86 ng/mL, respectively), although serum cortisol and aldosterone levels were normal. Twenty-four-hour urinary noradrenaline and adrenaline were also increased (882 µg/day and 138 µg/day, respectively). A tentative diagnosis of pheochromocytoma was made. Computed tomography of the abdomen showed a mass measuring approximately 4 cm in diameter in the left adrenal gland. Pathological examination after left-sided adrenalectomy were compatible with pheochromocytoma. After surgery, the patient had no symptoms and was normotensive. Although serum leptin levels were low before surgery, serum leptin levels were increased with diurnal rhythms 1 month after surgery (Table 1).

It was recently reported that norepinephrine decreases ob gene ex-
pression in vitro (2) and in vivo (3), and also decreases circulating leptin levels in animals (4, 5). Therefore, high serum norepinephrine levels, which pheochromocytoma produces, might decrease serum leptin levels.

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References

Differential Expression of HGF and Met in Human Placenta

To the editor:

We read the paper by Kauma et al. (1) with interest. Their technique for separating and culturing trophoblast from villous core tissue showed that only the villous core tissue produces hepatocyte growth factor (HGF). This confirms evidence from previous in situ hybridization studies by ourselves and others, where HGF mRNA was localized to the villous core (2–4). They attribute the HGF production to villous core villous core fibroblasts. However, hybridization studies, which clearly show that by far the strongest signal comes from the syncytiotrophoblast cells and isolated villous core fibroblasts failed to increase their technique for isolating villous core tissue resulted in damage or loss of perivascular tissue, then it would not be surprising to see a dramatic reduction in HGF production. One possible explanation for this lies in our in situ hybridization studies, which clearly show that by far the strongest signal for HGF came from the perivascular smooth muscle of the stem villi. If their technique for isolating villous core tissue resulted in damage or loss of perivascular tissue, then it would not be surprising to see a dramatic reduction in HGF production. Vascular smooth muscle has previously been shown to be a potent source of HGF (5). Furthermore, in Fig. 3 of the Kauma paper, it appears that the whole tissue culture supernatant contained around 240 ng HGF per µg total protein. This implies that

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## Table 1. Cathecholamine and serum leptin levels in a patient with pheochromocytoma

<table>
<thead>
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<th>Before surgery</th>
<th>After surgery</th>
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<td>1.9</td>
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* Serum leptin levels were determined in duplicate by a radioimmunnoassay kit (Linco Research, Inc., St. Charles, MO).
nearly 25% of total secreted protein was HGF, which is implausibly high and raises questions about the measurements reported.

The failure by Kauma et al. to immunolocalize HGF to the villous trophoblast is at odds with the previously published work of ourselves and others, showing co-localization of HGF and c-met protein to the vasculo-syncytial membrane in the third trimester (3, 6). One study in human placenta from the first trimester also failed to localize HGF to the trophoblast, but did localize it to the basal membrane area, where the cytotrophoblasts come into contact with the villous core (4). A fourth study failed to immunolocalize HGF to trophoblast through-out gestation (2). The reasons for this discrepancy may well lie in the specificity of the antibodies involved as, while the latter study (Clark et al., ref. 2) and Kauma et al. used the same monoclonal antibody, the other three studies all used different polyclonal antibodies. It is possible that the polyclonal antibodies may bind to forms of HGF not detected by the monoclonal antibody, which is likely to recognize a single epitope. Alternative forms of HGF likely to exist in the trophoblast layer include the inactive monomer, the active dimer, and the receptor bound or internalized HGF. A further difference identified between these studies lies in the tissue fixation used: both Wolf et al. (6) and ourselves (3) used Formalin-fixed wax embedded sections, whilst the other three studies used frozen sections, post-fixed in acetone. This may also affect antigen recognition, especially by monoclonal antibodies. It would be interesting to know if the authors were able to perform successful HGF immunolocalization using the Formalin-fixed, wax embedded sections that they used for the c-met immunohistochemistry.

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References

Differential Expression of HGF and Met in Human Placenta—Authors’ Response

To the editor:

We greatly appreciate the interest of Dr. Somerset and his colleagues (see preceding letter) in our paper concerning the production of hepatocyte growth factor (HGF) in placental villous core mesenchymal cells and the presence of the HGF receptor, Met, in placental trophoblast cells. They note that, in our study, intact placental villous explants produced 24 times more HGF than did isolated placental villous core tissues. Furthermore, they speculate that possible damage to the perivasculary smooth muscle in the villous core may result in decreased HGF production from these cells. We acknowledge that tissue damage to cells in the villous core during isolation may be one possible explanation for decreased HGF production in our studies.

That this effect would be selective for the perivascular smooth muscle cells seems unlikely, as the short Disperse digestion isolation procedure used is gentle and rapid, leaving the villous core tissue completely intact, including the perivascular tissues within the villous core (1).

Dr. Somerset raises question our reported values of HGF production by intact placental villi stating in the letter that “25% of total secreted protein was HGF.” This interpretation would be correct if the denominator were μg total protein secreted. However, as stated in the Materials and Methods section, HGF production in this figure was standardized between samples by measuring the extractable protein from the tissue and cell samples and is not a value for total secreted protein. These procedures for standardizing in vitro and tissue secretion of proteins have previously validated (1, 2). Standardizing HGF secretion by total wet weight of cultured placental villous explants demonstrates production rates of approximately 1 ng HGF/mg tissue/24 h.

Our study did not show immunolocalization of HGF to placental trophoblast. We initially attempted immunostaining with both polyclonal and monoclonal antibodies to human HGF on formalin-fixed paraffin embedded tissue sections, but could not demonstrate any specific staining using these methods of fixation and processing. We would agree with Dr. Somerset et al. that they may have detected receptor-bound or internalized HGF on trophoblast cells, not detected by our HGF immunolocalization procedure, in their studies using their immunolocalization procedure. This explanation would be consistent with our findings that trophoblast do not produce HGF but do express Met.

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Low BMD in Calcium Stone Formers with Hypercalciuria

To the editor:

The article by Ghazali et al. (1) entitled “Low Bone Mineral Density and Peripheral Blood Monocyte Activation Profile in Calcium Stone Formers with Idiopathic Hypercalciuria” evokes substantial interest in relation to mechanisms that induce osteoporosis. Secondary hyperparathyroidism has been described in patients with idiopathic hypercalciuria of renal origin (2). The authors have stated that they have excluded patients with hyperparathyroidism from their study. It would be of interest to know if those patients with renal hypercalciuria had bone elevations in serum PTH or had HGF mRNA levels in the upper limits of the reference range as compared to those patients with dietary dependent hypercalciuria or the normal controls.

PTH acts on cells other than those of bone and kidney, and mitosis of lymphocytes has been described (3, 4). It may be interesting to note if there is a positive correlation between the PTH levels and the cytokines of patients with renal hypercalciuria, and whether this was responsible for the reduction in bone density.

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References

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