Skin morphological changes in growth hormone deficiency and acromegaly

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Abstract

Objective: To evaluate the histomorphology of skin and its appendages, especially eccrine sweat glands, in patients with GH disorders, because reduced sweating ability in patients with growth hormone deficiency (GHD) is associated with increased risk of hyperthermia under stressed conditions.

Design and methods: A skin biopsy was obtained from 17 patients with GHD treated with GH, five patients with untreated GHD, 10 patients with active acromegaly and 13 healthy controls.

Results: The sweat secretion rate (SSR) was significantly decreased in both the untreated (median 41 mg/30 min, range 9–79 mg/30 min) and the GH-treated (median 98 mg/30 min, range 28–147 mg/30 min) patients with GHD compared with that in controls (median 119 mg/30 min, range 90–189 mg/30 min; \(P < 0.001\) and \(0.01\) respectively). Epidermal thickness was significantly decreased in both untreated (median 39 μm, range 28–55 μm) and GH-treated patients with GHD (median 53 μm, range 37–100 μm), compared with that in controls (median 66 μm, range 40–111 μm; \(P < 0.02\)). A statistically non-significant tendency towards thinner epidermis (median 59 μm, range 33–83 μm) was recorded in acromegalic patients \(P = 0.08\) compared with controls. There was no significant difference in the area of the sebaceous glands in the biopsies between the three groups and the controls. The area of eccrine sweat gland glomeruli was significantly decreased in the untreated patients with GHD (median 16407 μm², range 12758–43976 μm²) compared with that in controls (median 29446 μm², range 13511–128661 μm²; \(P = 0.03\)), but there was no significant difference between the GH-treated patients with GHD and controls.

Conclusions: We conclude that GH, either directly or via IGF-I, may have both a structural and a functional effect on human skin and its appendages, and that patients with GHD have histomorphological changes in skin compared with controls. Importantly, these changes are not fully reversed despite long-term and adequate GH treatment in patients with childhood onset GHD.

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Introduction

The ability to perspire is of minor importance in the maintenance of body temperature during rest. However, in certain conditions such as exercise, infections and exposure to high environmental temperature, sweating becomes imperative for thermoregulation. Sweating ability seems to be influenced by sex, age (1) and degree of physical training (2). Patients with childhood onset (CO) growth hormone deficiency (GHD) have been shown to have impaired sweating ability (1, 3) as have men, but not women, with adult onset (AO) GHD (4). The sweating ability has been shown to improve, but not normalise, in patients with CO-GHD when treated with growth hormone (GH) (3). A similar improvement has not been demonstrated in those with AO-GHD (4). The impaired sweating ability is also found in patients with GH insensitivity (Laron-type dwarfism) (5). Furthermore, most clinicians agree that patients with GHD have thin and dry skin. The decreased sweating ability in GHD imposes a risk of hyperthermia (6, 7). In contrast, patients with active acromegaly have thick and greasy skin and the prevalence of excessive sweating in such patients has been reported to be 65–88% (8). Furthermore, the sweat secretion rate (SSR) has been found to be abnormal in both active and inactive acromegaly (4). Previous studies of skin morphology in GHD have shown decreased skin thickness with decreased collagen content (9), whereas the opposite findings are present in acromegaly (8). Little, if any, attention has been paid to changes in the eccrine or sebaceous glands. It has been
suggested that the effect on the skin and its appendages is in part a direct effect of GH (9). This suggestion is supported by identification of the GH receptor in skin tissue, including the eccrine sweat glands (10, 11).

The aim of this study was to determine skin morphological changes present in GHD and acromegaly, with special emphasis on identifying factors that might explain the difference in sweating capacity between the two entities.

**Participants and methods**

Under local anaesthesia (2% lidocaine), a skin biopsy from the posterior of the right shoulder was obtained from a total of 45 individuals. Five were male patients with CO-GHD (median age 31 years). One of them had not received GH treatment at all and four had been without GH treatment for a period of 7–23 years. All five patients had one or more additional pituitary deficiencies, and were receiving adequate substitution therapy for the respective deficiencies. A further 17 (12 men, five women) were patients with GH-treated CO-GHD (median age 20 years). Of these, 13 had one or more additional pituitary deficiencies, and were receiving adequate substitution therapy for the respective deficiencies. These patients had been on discontinuous GH substitution since diagnosis, for a median duration of 10 years (range 4–21 years). Ten of the 45 patients (six men, four women; median age 39.5 years) had active acromegaly; five of them had one or more additional pituitary deficiencies, and were receiving adequate substitution therapy for the respective deficiencies. The remaining 13 participants (nine men, four women) were healthy controls (median age 23 years).

The characteristics of the study participants are summarized in Table 1.

The biopsy specimens (4 mm punch biopsies) were fixed in Lillies fixative, dehydrated and embedded in paraffin. They were then cut into 4 μm sections using a microtome and stained with haematoxylin and eosin (H&E). The specimens were examined using a Zeiss Axiophot microscope (Carl Zeiss, Oberkocken, Germany) (H&E). The specimens were examined using a Zeiss Axiophot microscope (Carl Zeiss, Oberkocken, Germany) connected to a high-resolution camera (Hamamatsu Photonics, Hamamatsu City, Japan). The area of the total biopsy, and of the eccrine sweat gland glomeruli (defined as adjacent eccrine sweat gland tubuli) and the total area of the sebaceous glands were measured by means of NIH Image 1.60 (public domain image processing and analysis program provided by National Institute of Health, Bethesda, Maryland, USA) as described previously (12). Eccrine sweat glands and sebaceous glands were not present in all biopsies. When they were present, one to three sebaceous glands were identified in each specimen and the sum of their areas was taken to comprise the total area. After calibration, the border of the biopsy and the boundaries between gland tissue and surrounding tissues were outlined by the computer cursor, allowing calculation of the area. The mean epidermal thickness (calculated by 10 randomly selected measurements in each biopsy) was determined by NIH image 1.60.

Serum concentrations of insulin-like growth factor I (IGF-I) and insulin-like growth factor binding protein 3 (IGFBP-3) were determined in all participants, by radioimmunoassays.

A pharmacological sweat test was performed using previously published methods: two filter papers (Whatmann Ashless Roundfilters, diameter: 2.5 cm) soaked in 0.2% pilocarpine were positioned on the flexor side of the distal forearm underneath two quadrangular negative electrodes (size: 3 × 3 cm). Iontophoresis was performed, using a current of 2 mA for 5 min. After 5 min, the area of iontophoresis was rinsed with deionised water and 61% ethanol and thoroughly dried. Sweat was collected during a 30-min period using three filter papers sealed by a plastic roundel and tape to prevent evaporation. Sweat mass was measured by weighing the filter paper (Mettler scales, precision: ±0.1 mg) before and after the sweat collection (1).

The local Ethics Committee approved the study, and signed informed consent was obtained from all patients.

**Statistical methods**

The results were analysed by Mann–Whitney’s U-test. Correlations analyses were performed using Spearman’s test.

**Table 1** Patient characteristics expressed as medians and ranges. Additional hormone deficiencies are stated. Patients with additional hormone deficiencies were in adequate substitution therapy for the respective deficiency. Values are absolute numbers or median (range).

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Sex (M/F)</th>
<th>Age (years)</th>
<th>Height (cm)</th>
<th>Weight (kg)</th>
<th>BMI (kg/m²)</th>
<th>ACTH</th>
<th>TSH</th>
<th>ADH</th>
<th>FSH/LH</th>
</tr>
</thead>
<tbody>
<tr>
<td>GHD untreated</td>
<td>5</td>
<td>5/0</td>
<td>31 (25–64)</td>
<td>167 (157–182)</td>
<td>75 (37–102)</td>
<td>28 (15–31)</td>
<td>4</td>
<td>5</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>Acromegaly</td>
<td>10</td>
<td>6/4</td>
<td>39.5 (26–64)</td>
<td>174 (165–190)</td>
<td>78 (64–100)</td>
<td>26 (21–31)</td>
<td>4</td>
<td>1</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Controls</td>
<td>13</td>
<td>9/4</td>
<td>23 (20–28)</td>
<td>184 (160–190)</td>
<td>76 (56–93)</td>
<td>23 (21–26)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

BMI, body mass index; ACTH, adrenocorticotrophic hormone; TSH, thyroid-stimulating hormone; ADH, antidiuretic hormone; FSH, follicle-stimulating hormone; LH, luteinizing hormone.
Results

Skin biopsies

The biopsies were very inhomogeneous between the groups. Those from the patients with GHD, in particular the untreated patients, were characterized by a greater content of fat compared with controls. There was a tendency, though not significant, towards a greater area of sebaceous glands in the acromegalic patients compared with the other groups. In some of the biopsies in the acromegalic patients and in some controls, the dermis was so thick that the subcutis was not included in the biopsies and could therefore not be measured accurately. The dermis in the untreated patients with GHD, however, appeared thinner compared with that in the other groups. The biopsies from the acromegalic patients were furthermore characterised by the presence of a dense and thick, almost membrane-like, border of connective tissue under the epidermis that was not as pronounced in the other groups (Fig. 1A–C).

Eccrine sweat glands

Six eccrine sweat gland glomeruli were present in five biopsies of the untreated patients with GHD. 14 were present in 17 biopsies of the GH-treated patients with GHD. 13 were present in 10 biopsies of the acromegalic patients and 18 glomeruli were present in 13 biopsies of the controls. The area of a glomerulus was significantly smaller in the untreated patients with GHD (median 16407 μm², range 12758–43976 μm²) compared with that in controls (median 29446 μm², range 13511–128661 μm²; P = 0.03). There was no significant difference between the untreated patients with GHD and the GH-treated patients with GHD (median 33936 μm², range 3315–105497 μm²), and no significant difference between the GH-treated patients with GHD or the acromegalic patients (median 40421 μm², range 9978–167971 μm²²) compared with the controls. No difference was found between the untreated patients with GHD and the acromegalic patients (Figs. 1G–H and 2A).

Sebaceous glands

Sebaceous glands were present in the biopsies of two of the five untreated patients with GHD, seven of the 17 GH-treated patients with GHD, six of the 10 acromegalic patients and 11 of the 13 controls. There was no difference in the area of sebaceous glands in the three groups of patients compared with the controls.

Epidermal thickness

Epidermal thickness was significantly reduced in both patients with untreated GHD (median 39.0 μm, range 28–55 μm) and GH-treated GHD (median 53 μm, range 37–100 μm) compared with that in controls (median 66.0 μm, range 40.0–111.0 μm; P = 0.005 and 0.02 respectively; Figs. 1D–F and 2B). There was a tendency, though not significant, towards reduced thickness in patients with acromegaly (median 59 μm, range 33–83 μm) compared with controls (P = 0.08). The epidermal thickness was, however, increased in the acromegalic patients compared with that in untreated patients with GHD (P = 0.01).

Pilocarpine iontophoresis sweat test

Pilocarpine iontophoresis tests showed a lower SSR both in patients with untreated GHD (median 41 mg/30 min, range 9–79 mg/30 min) and in those with GH-treated GHD (median 98 mg/30 min, range 28–147 mg/30 min) compared with the controls (median 119 mg/30 min, range 90–189 mg/30 min; P = 0.001 and 0.01 respectively). SSR was, however, significantly lower in patients with untreated GHD than in patients with GH-treated GHD (P = 0.03). SSR was greater in patients with acromegaly (median 168 mg/30 min, range 61–260 mg/30 min) than in the controls, but the difference did not reach statistical significance (P = 0.26). The SSR was, however, significantly greater in the acromegalic patients than in the untreated patients with GHD (P = 0.01) (Fig. 2C).

IGF-I

IGF-I concentrations were decreased in untreated patients with GHD (median 43 ng/ml, range 20–60 ng/ml) compared with GH-treated patients with GHD (median 302 ng/ml, range 89–886 ng/ml) and the controls (median 244 ng/ml, range 169–636 ng/ml; P = 0.0009 and 0.001 respectively). IGF-I concentrations were increased in acromegaly (median 745 ng/ml, range 523–1037 ng/ml) compared with those in controls (P = 0.0002). Patients with GH-treated GHD had similar IGF-I values as the controls (Fig. 2D).

IGFBP-3

IGFBP-3 concentrations were significantly lower in untreated patients with GHD (median 1300 ng/ml, range 562–1593 ng/ml) compared with GH-treated patients with GHD (median 3983 ng/ml, range 1638–7078 ng/ml) and the controls (median 3376 ng/ml, range 2593–4274 ng/ml; P = 0.0009 and 0.001 respectively), and significantly greater in the acromegalic patients (median 6021 ng/ml, range 5194–7133 ng/ml) than in the controls (P = 0.00004). There was no significant difference between the controls and the GH-treated patients with GHD. No correlation was found between serum IGF-I and the area of sweat gland glomeruli, epidermal thickness and SSR respectively in any of the groups alone or in all the groups combined.
Discussion

We evaluated skin histomorphology in 32 patients with GH disorders as compared with that in 13 control individuals and found significant changes in epidermal thickness and eccrine sweat glands. This is in line with our hypothesis of the human skin being a GH target organ.

This hypothesis originates from the finding that in situations of stress, impaired sweating capacity, as seen in GHD (3, 4), is associated with a risk of hyperthermia (6, 7, 13). The impact of the decreased sweating ability is also demonstrated by the fact that some patients with GHD are poikilothermic (13). Conversely, acromegaly is associated with excess sweating and increased SSR (4, 8). The changes in GHD may be a result of atrophy of the eccrine sweat glands because of lack of stimulation of either GH or IGF-I, or both. Alternatively, it could be a reduction in sweat gland function.

In this study, the finding of decreased SSR in the untreated GHD group was not surprising (3). The GH-treated patients with GHD had increased SSR compared

Figure 1 General views of skin biopsies from: (A) control, (B) patient with untreated GHD and (C) patient with acromegaly (original magnification ×20 for each). Close-ups of epidermis from: (D) control, (E) patient with untreated GHD and (F) patient with acromegaly (original magnification ×50 for each). Close-ups of eccrine sweat glands from: (G) control and (H) patient with untreated GHD (original magnification ×20 for each).
with the untreated patients, suggesting some effect of GH treatment, but they still showed a decreased SSR compared with the controls. This may suggest that, despite a seemingly adequate treatment (IGF-I and IGFBP-3 concentrations within or above normal limits), the treatment effect on SSR was not sufficient. The decreased SSR in the untreated GHD group corresponded to the finding of a decreased area of the glomeruli of the sweat glands compared with the controls. We found no differences in the area of the sweat gland glomeruli between the GH-treated patients and the controls, despite differences in SSR between the two groups. One explanation for these findings may be that GH exerts both a structural and a functional effect on the sweat glands, and that not all effects are fully restored, despite long-term GH replacement in patients with GHD. Sweat gland size is known to decrease with age (14). In the present study, significant differences were found in the area of sweat gland glomeruli in untreated patients with GHD as compared with controls, with a difference in median size of almost 100%. The median difference in age was only 10 years between the two groups, both comprising young individuals, and the difference in glomerulus area is therefore unlikely to have been due to differences in age.

All the patients with GHD in the present study had CO-GHD. The impact of GHD in these patients may not be the same in patients with AO-GHD. In a previous study involving patients with AO-GHD of mean duration 8.6 years (range 2–27 years) since diagnosis, we found that SSR was similar to controls in female patients, but lower compared with controls in male patients. Eighteen months of GH replacement therapy did not effect SSR in either group (4). In the present study, only male patients were present in the untreated GHD group. In accordance with the findings of the previous study, reduced SSR was found in these patients after 18 months of GH treatment (4). The GH-treated patients with CO-GHD had, however, a significant effect of treatment compared with the untreated patients, even though the SSR did not reach the level attained by the controls. We suspect that this apparent discrepancy

Figure 2 (A) Area of sweat gland glomeruli, (B) epidermal thickness determined as mean of 10 random measurements, (C) results of pilocarpine sweat test expressed as sweat secretion rate and (D) serum IGF-I concentrations, in patients with untreated GHD, GH-treated GHD, acromegalis and controls. *P < 0.03 compared with controls; †P = 0.03 compared with GH-treated; ‡P = 0.01 compared with acromegalic patients. Horizontal bars indicate medians.
between CO-GHD and AO-GHD is in part related to duration of treatment (10 years, range 4–21 years, in the present study compared with 18 months in the previous study). As in the AO-GHD study, the impact of other pituitary deficiencies on SSR and sweat gland morphology cannot be ruled out. All patients deficient in other pituitary hormones had, however, been receiving stable substitution therapy since diagnosis, with comparable replacements in all groups.

The finding that patients with GHD (both untreated and GH-treated) also have a thin epidermis compared with healthy controls supports the notion of a permanent consequence of GHD, despite treatment and despite a small but non-significant improvement in those who are GH-treated compared with those who are untreated (Fig. 2B). A slight, non-significant, increase in skin thickness as determined by skinfold thickness has previously been demonstrated in normal elderly men treated with GH (15). We cannot exclude that other pituitary deficiencies may affect epidermal thickness, as it is well known that treatment with high doses of glucocorticoids may decrease skin thickness. This is, however, mostly caused by reduced collagen formation and is not influencing the epidermis (16). Furthermore, the influence of glucocorticoids on skin thickness is most common during regular glucocorticoid treatment and not in substitution therapy. Hypothyroidism and hypogonadism are also known to cause reduced skin thickness. As previously stated, however, all hormone-deficient patients were receiving adequate substitution therapy. Epidermal thickness has been shown to decrease with age at a magnitude of approximately 6–7% per decade (17). The epidermal thickness found in the controls in the present study is comparable to that previously reported in healthy individuals of similar age (17). In the present study, the GH-treated patients were comparable in age with the controls. The untreated patients with GHD were older compared with the controls, but the difference in epidermal thickness was of a magnitude that could not be explained by differences in age alone.

Increased sweating and greasy skin are well-known features of acromegaly (8). In the present study population, we found no significant change in SSR, size of sweat glands or of size of sebaceous glands in acromegalic patients compared with the controls. These negative findings are in contrast with our previous findings of abnormal SSR in both treated and untreated acromegaly (4). This discrepancy may be explained by the small number of patients in the present study and the small number of sweat glands present in the histological specimens in the biopsies of the acromegaly group (n = 7). In addition, increased sweating was found clinically only in 65–88% of untreated acromegalic patients (8), giving a wide range of SSR in this group of patients.

The finding of relatively thin epidermis in the acromegalic patients compared with the controls, albeit not significant, may seem surprising, considering that clinically these patients have very thick skin. This finding has, however, also been reported by other authors using H&E staining (18). Furthermore, this group showed an abnormal glycosaminoglycan composition in the dermis of acromegalic patients, by means of tissue digestion, and increased glycosaminoglycan deposition as determined by staining with colloidal iron (18). The finding of increased glycosaminoglycan deposition may correspond to our finding of the presence of a dense border of connective tissue under the epidermis as determined by H&E staining. The tendency towards thin epidermis most probably originates from increased cell turnover as determined by tritiated amino acid incorporation (19), and the clinical finding of thick skin therefore reflects increased dermal thickness. Holt & Marks (19) further showed, by H&E staining, an increased size of the epidermal cells but a decreased number of cells compared with the controls. In our material the dermis in the patients with acromegaly was so thick that it extended throughout the depth of the biopsy sample, rendering exact measurements impossible. In the present study, the age difference between the acromegalic patients and the controls was rather large, and the finding of a tendency towards thinner epidermis in the acromegalic patients may therefore simply have been a reflection of the age difference (17).

The presence of the GH receptor in human skin and its appendages, including eccrine sweat glands, suggests a direct effect of GH (10, 11), but the exact mechanism by which GH exerts its effect is unknown. However, studies using PCR have shown an abundance of IGF-I receptor mRNA in human skin biopsies (20), which could suggest an IGF-I-mediated action on skin.

We conclude that GH, either directly or via IGF-I may have both a structural and a functional effect on human skin and its appendages, and that patients with GHD have histomorphological changes in skin compared with controls. Importantly, these changes are not fully reversed despite long-term and adequate GH treatment in patients with CO-GHD.

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