A history of cranial radiotherapy is associated with a higher visceral to subcutaneous fat ratio in men with pituitary insufficiency

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Short running title: Fat distribution in cranial irradiated hypopituitary men

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ABSTRACT

Objective: Endocrine deficiencies, like GH and estrogen deficiency, are likely candidates to explain increased visceral to subcutaneous fat ratio in patients with pituitary insufficiency. However, recent reports pointed to cranial radiotherapy (CRT) as an additional determinant of an unfavorable fat distribution. Therefore, we determined the effect of CRT on abdominal fat distribution in men with treated pituitary insufficiency.

Design: Cross-sectional study

Methods: 35 Consecutive male subjects (16 men with and 19 men without CRT; respectively aged 62±12 and 56±14 yr, p = 0.175) visiting our Endocrine Outpatient Clinic for pituitary insufficiency were invited to participate. A standardized single slice abdominal CT-scan at the level of the fourth lumbar vertebra was performed to determine visceral fat area, subcutaneous fat area and visceral to subcutaneous fat ratio. In addition, we assessed body mass index, total fat percentage with bioelectrical impedance analysis, resting energy expenditure with indirect calorimetry, caloric intake with a diary and serum hormone concentrations.

Results: Subjects with CRT had a smaller subcutaneous fat area [225.1 (71.1 - 480.7) vs. 269.0 (133.2 - 59.9) cm², p = 0.022] and a higher visceral to subcutaneous fat ratio [0.79 (0.39 - 1.55) vs. 0.63 (0.23 - 0.88), p = 0.001] than subjects without CRT. Both groups were comparable for body mass index, waist-hip ratio, resting energy expenditure, and caloric intake. Importantly, serum hormone concentrations were similar.

Conclusion: In men treated for pituitary insufficiency, previous CRT is associated with a higher visceral to subcutaneous fat ratio.
INTRODUCTION

Patients with pituitary insufficiency are at increased risk for obesity. Although obesity in itself is associated with increased cardiovascular morbidity, especially the accumulation of visceral fat is an established risk factor for the development of cardiovascular disease and type 2 diabetes mellitus (1). The importance of these metabolic consequences highlights the need to identify factors responsible for changes in body fat distribution in patients with pituitary insufficiency. Traditionally, suboptimal hormone replacement therapy has been considered an obvious explanation in these patients, since the majority of pituitary controlled hormones affect body fat distribution. For instance, androgens are associated with increased visceral fat mass, with the inverse pattern for estrogens (2, 3), and excess of cortisol or a shortage of GH are associated with visceral obesity as well (4, 5). However, in addition to these endocrine explanations, other factors may be involved. Interestingly, excess visceral fat was recently reported in patients who had received cranial radiation therapy (CRT) as part of their treatment for acute lymphoblastic leukaemia (6, 7). Whether this relationship between CRT and body fat distribution is also present in patients with pituitary insufficiency is unclear. To study this, we decided to investigate the effect of CRT on body fat distribution in men currently receiving hormone replacement therapy for pituitary insufficiency.

SUBJECTS AND METHODS

Subjects

Consecutive patients visiting our Endocrine Outpatient Clinic (Academic Medical Center of the University of Amsterdam) with pituitary insufficiency, i.e. at least one anterior pituitary hormone deficiency, who had been treated for a (supra)sellar tumor between 1966 and 2007 were invited to participate. Only men were included, as previous studies have demonstrated that body composition is gender dependent and related to pre- and postmenopausal status of women. All patients were seen on a regular basis by an internist-endocrinologist for clinical and biochemical evaluation. Patients received conventional hormone replacement therapy consisting of L-thyroxine,
hydrocortisone, testosterone, recombinant human growth hormone (rhGH) and/or vasopressin analogues when indicated. Excessive production of pituitary hormones had not been present for at least 5 years in subjects with a hormone producing tumor. Hormone concentrations were measured in venous serum or plasma samples obtained between 9:00 and 10:00 a.m. after an overnight fast. IGF-1 was measured by an immunometric assay (Immulite® IGF-I, Diagnostic Products Corporation, Los Angeles, USA). Age specific reference values have previously been determined in a random population sample (n=296) (8).

The study protocol was approved by the Medical Ethics Committee of the Academic Medical Center and conducted in accordance with the Declaration of Helsinki. All subjects provided written informed consent prior to participation in the study.

**Physical examination and indirect calorimetry**

Physical examination included measurement of height (cm), weight (kg), waist circumference (at the midpoint of the costal margin and iliac crest) (cm) and hip circumference (at the level of the great trochanters with the legs close together) (cm). Body mass index (kg/m$^2$) and waist-hip ratio were calculated. Bioelectrical impedance analysis (Maltron BF906, Rayleigh, UK) was used to measure body composition.

Resting energy expenditure was measured over a 30-min period after an overnight fast by indirect calorimetry using the ventilated hood technique (Sensormedics model 2900; Sensormedics, Anaheim, USA). Resting energy expenditure was calculated from oxygen consumption and carbon dioxide production as described by Frayn (9).

**Abdominal fat measurement**

A standardized single slice abdominal CT-scan (Mx8000Quad, Philips Medical Systems, Best, The Netherlands) using 120kV, 100mAs and a slice thickness of 1 cm was performed. On the survey image, the level of the fourth intervertebral lumbar disc was selected, since the fat area in a slice at this level is a valid predictor of total abdominal fat in men (10, 11). The area of visceral fat and subcutaneous fat (both in cm$^2$) was determined by adding the area of the voxels with CT values within
the range of -170 to -30 Hounsfield units. Care was taken to exclude intracolonic contents with CT values within the same range (12).

**Caloric intake**

Energy intake was assessed using a food diary. This involved the patient recording their daily food and drink intake for two weekdays and one weekend day. To estimate daily caloric intake the food diaries were analyzed using www.dieetinzicht.nl. Data are expressed as total energy intake per kg bodyweight per day.

**Statistical analysis**

Statistical analyses were performed using SPSS for Windows (version 16.0, SPSS Inc, Chicago, IL). Normally distributed variables are presented as mean ± SD, and not normally distributed variables as median (range) and categorical variables as counts (percentages). Group differences in numerical variables were evaluated using the Student’s t-test for normally distributed variables and the Mann-Whitney U test for not normally distributed parameters. The Chi-square test was used to analyze differences between categorical data in both groups. If the sample size was small or cells had an expected count less than 5, the Fisher exact test was used. An analysis of covariance (ANCOVA) was used to determine the effect of CRT on fat distribution with ‘age’, ‘time between initial diagnosis and the present study’ and ‘ADH-deficiency’ as covariates. Simple bivariate correlation was performed using the Spearman rank correlation coefficient. A $p$-value of <0.05 was considered significant, using two-tailed tests.

**RESULTS**

**Subjects**

Thirty-five men were enrolled in the present study, of which 16 had been treated with CRT (mean age 62 ± 12 yr) and 19 men had not been treated with CRT (mean age 56 ± 14 yr). Within the CRT group, thirteen subjects received post-operative CRT, two received CRT following unsuccessful dopamine agonist treatment and one subject received CRT to prevent Nelson’s syndrome after bilateral
adrenalectomy. Total radiation doses administered to the CRT group ranged from 40 – 50 Gy (mean 45.2 ± 4.6 Gy).

The time between initial diagnosis and the present study in the group with CRT was longer than in the group without CRT (20 ± 9 vs. 11 ± 11 yr, \( p = 0.021 \)), but there was no difference between the groups in body mass index, waist-hip ratio, resting energy expenditure, caloric intake and hypothalamic-pituitary hormone deficiencies (table 1).

All ACTH-, TSH- and ADH-deficient patients were on stable doses of hydrocortisone, L-thyroxine and desmopressin, respectively. rhGH-therapy was given to 5 out of 10 CRT-patients with GH-deficiency and 11 out of 11 non-CRT patients with GH-deficiency. The remaining patients were classified as having an intact GH-IGF-1 axis based on an age specific IGF-1 between +/- 2SD.

Testosterone supplementation was given to 11 out of 14 CRT-patients with LH/FSH deficiency and 15 out of 17 non-CRT patients with LH/FSH deficiency. The majority of hypogonadal patients received transdermal testosterone therapy (61%). The remaining patients received intramuscular (19%), oral (12%) or buccal (8%) formulas of testosterone.

Serum testosterone, age-adjusted IGF-1 and fT4 levels were not different between patients with and without CRT, nor were urine production and urine osmolality. Hydrocortisone tablets were used two or three times daily by all ACTH-deficient patients. In the CRT group 7 patients received a hydrocortisone dosage of 20mg/day and 1 patient received 60 mg/day. In the non-CRT group 1 patient received 15mg/day, 11 patients received 20 mg/day and 2 patients received 30mg/day. The total hydrocortisone dosage did not differ between patients with and without CRT (table 1).

**Subcutaneous and visceral fat**

The visceral to subcutaneous fat ratio was 25.4% higher in men with CRT than in men without CRT [0.79 (0.39 - 1.55) vs. 0.63 (0.23 - 0.88), \( p = 0.001 \)]. This difference was caused by a smaller absolute median subcutaneous fat area of 43.8 cm\(^2\) in men with CRT [225.1 (71.1 - 480.7) vs. 268.9 (133.2 - 599.1) cm\(^2\), \( p = 0.022 \)], and an absolute median difference in visceral fat area of 44.4 cm\(^2\), although the latter was not statistically significant [201.1 (50.2 - 375.3) vs. 156.7 (55.3 - 300.2) cm\(^2\), \( p = 0.271 \)] (figure 1).
Factors influencing the visceral to subcutaneous fat ratio

‘Age’, ‘time between initial diagnosis and the present study’ and ‘ADH-deficiency’

Controlled for ‘age’, ‘time between initial diagnosis and the present study’ and ‘ADH-deficiency’, men with CRT had still a higher visceral to subcutaneous fat ratio than men without CRT ($F(1, 30) = 11.76, p = 0.002$).

Relationship between IGF-1 and body composition

We did not find a correlation between serum IGF-1 concentration and visceral to subcutaneous fat ratio ($r^2 = 0.007, p = 0.644$), nor between IGF-1 and, respectively, visceral or subcutaneous fat ($r^2 = 0.000, p = 0.987; r^2 = 0.004, p = 0.719$).

Hypopituitarism

In our study population, some men were untreated for their GH- or testosterone-deficiency. Because untreated hormonal deficiencies are implicated in the regulation of fat distribution, we analysed a subgroup containing men with intact and/or hormonal suppleted axes exclusively. In this separate analysis the difference in visceral to subcutaneous fat ratio in men with and without CRT was even more pronounced [0.97 (0.52 - 1.55) vs 0.57 (0.23 - 0.88), $p = 0.001$], again mainly accompanied by a smaller subcutaneous fat area [233.2 (71.1 - 480.7) vs 284.2 (167.5 - 599.1) cm$^2$, $p = 0.017$], although the visceral fat area tended to be larger in men with CRT [247.1 (50.2 - 375.3) vs 160.6 (55.3 - 300.2) cm$^2$, $p = 0.053$]. If we controlled for ‘age’, ‘time between initial diagnosis and the present study’ and ‘ADH-deficiency’, men with CRT had still a higher visceral to subcutaneous fat ratio than men without CRT ($F(1, 21) = 16.46, p = 0.001$).

Of note, in this analysis, there were no differences in hydrocortisone treatment regiment and doses between the groups (20 (20 – 60) vs 20 (20 – 30); $p = 0.702$), nor in serum testosterone, age-adjusted serum IGF-1, serum fT4, urine production or urine osmolality.

DISCUSSION

This is the first report on the relationship between CRT and body fat distribution measured by CT in patients with pituitary insufficiency caused by a tumour in the sella region. Our results indicate that
CRT is associated with a higher visceral to subcutaneous fat ratio in men with pituitary insufficiency. This finding is consistent with previous observations in survivors of childhood acute lymphoblastic leukaemia (6, 7).

Changes in body fat distribution in patients with pituitary insufficiency are usually attributed to hormonal deficiencies. However, our results suggest that CRT contributes to compartment-specific modulation of adipose tissue accumulation independent of pituitary deficiencies, since patients with and without CRT had a comparable degree of pituitary insufficiency. Additionally, in a smaller subgroup analysis containing men with intact or adequately supplemented hormonal axes, visceral to subcutaneous fat ratio was markedly higher in men with CRT. These observations add strength to the notion that CRT might affect body fat distribution independently of pituitary function.

An intriguing question is how CRT affects body fat distribution. The hypothalamus is an important regulator of food intake and energy balance (13), but we did not observe a difference in body mass index, resting energy expenditure or food intake. Apparently other mechanisms in the brain modulate body fat distribution independently of energy intake and expenditure. Recent experimental studies suggest that neurons in the ventral medial hypothalamus regulate visceral fat content independently of food intake by modulating the sympathetic tone (14). In addition, distinct subsets of preautonomic neurons within the hypothalamus have been shown to project separately to the visceral and subcutaneous fat compartment via the autonomic nervous system, revealing a neuroanatomical network by which the hypothalamus may control body fat distribution (15-17). As the hypothalamus is vulnerable to radiation injury (18), it is conceivable, but highly speculative at this stage, that CRT impairs hypothalamic nuclei involved in the regulation of body fat distribution, thereby altering body fat distribution in patients after CRT.

Also tumours invading the hypothalamus, in particular craniopharyngiomas, may inflict damage on the hypothalamus. On imaging the craniopharyngiomas in our series did not invade the hypothalamus. Clinical evidence of hypothalamic damage is poorly defined in the literature, although most clinicians would agree that the development of massive obesity in patients with craniopharyngioma is indicative of hypothalamic damage. In our study 1 out of the 5 patients with craniopharyngioma had developed hypothalamic obesity. None of the patients had poikylothermia or severe disturbances in their
sleep/wake rhythm indicating hypothalamic damage, whereas all patients with craniopharyngioma had diabetes insipidus. Considering the limitations in defining hypothalamic damage one patient should probably be classified as having hypothalamic damage based on clinical symptoms.

A limitation of this cross-sectional study is that the temporal relationship between CRT and body composition cannot be clearly established. In addition, we studied a relatively small sample, but nevertheless we were able to demonstrate a statistically significant difference in fat distribution between irradiated and non-irradiated patients. The CRT and non CRT groups were well matched for the majority of variables except ‘age’, ‘time between initial diagnosis and the present study’ and presence of ‘ADH-deficiency’. Statistically controlled for these factors, men with CRT had still a higher visceral to subcutaneous fat ratio. Therefore, it is unlikely that ‘age’, ‘time between initial diagnosis and the present study’ or ‘ADH-deficiency’ has significantly influenced our results.

In our series approximately 60 percent of patients had GH-deficiency. Usually GH is the first affected hormone, especially if pituitary insufficiency results from irradiation (19). However, in patients with pituitary insufficiency due to a macroadenoma, GH deficiency is not by definition the most prevalent pituitary hormone deficiency as demonstrated in a series of 444 patients with macroadenoma, scheduled for transphenoidal surgery (20). In our study 83% of patients had a macroadenoma, which may explain why GH deficiency was not the most prevalent pituitary hormone deficiency. The absence of GH-deficiency in our study was defined by an age specific IGF-1 between +/- 2SD. As the gold standard for the diagnosis of GH-deficiency is an insulin tolerance test or GHRH arginine test, consequently we may have underestimated the actual proportion of patients with GH-deficiency.

However, as age specific IGF-1 levels were comparable in both groups, the effect of potential GH-deficiency on the outcome is probably limited.

It is important to note that our results do not necessarily hold for women, as previous studies have demonstrated that body composition is gender dependent and even dependent on pre- and postmenopausal status of women. Therefore, further and larger studies in men and women are needed to clarify the association between CRT and body fat distribution in patients with pituitary insufficiency.
In conclusion, our study demonstrates that CRT in men with pituitary insufficiency is associated with a higher visceral to subcutaneous fat ratio. The outcome adds a new dimension to the several well-known long-term side effects of CRT such as development of pituitary hormone deficiencies, radiation induced necrosis, optic neuropathy and secondary malignant tumours (21, 22).
DECLARATION OF INTEREST

There is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

FUNDING

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References


18. Darzy, KH & Shalet, SM. Hypopituitarism as a consequence of brain tumours and radiotherapy. Pituitary 2005 8 203-211.


TABLE 1 Clinical characteristics

<table>
<thead>
<tr>
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<th>History of cranial radiotherapy</th>
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<tr>
<td></td>
<td>Yes (n=16)</td>
<td>No (n=19)</td>
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<tr>
<td>Age - yr</td>
<td>62 ± 12</td>
<td>56 ± 14</td>
<td>0.175</td>
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<td>Time between tumor diagnosis and this study - yr</td>
<td>20 ± 9</td>
<td>11 ± 11</td>
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<tr>
<td>Time between cranial radiotherapy and this study - yr</td>
<td>18.2 ± 8.8</td>
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<tr>
<td>Physical examination</td>
<td></td>
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<tr>
<td>Body mass index – kg/(height)^2</td>
<td>29.1 ± 4.4</td>
<td>30.7 ± 4.5</td>
<td>0.286</td>
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<td>Waist-to-hip ratio</td>
<td>0.95 ± 0.05</td>
<td>0.93 ± 0.04</td>
<td>0.097</td>
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<tr>
<td>Lean body mass - %</td>
<td>70.8 (39.5 - 77.1)</td>
<td>67.6 (49.2 - 78.3)</td>
<td>0.302</td>
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<tr>
<td>Resting Energy Expenditure – kcal/kg</td>
<td>17.3 ± 1.96</td>
<td>17.1 ± 1.76</td>
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<td>Caloric intake – kcal/day</td>
<td>1946 ± 467</td>
<td>1906 ± 491</td>
<td>0.805</td>
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<tr>
<td>Biochemistry §</td>
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<tr>
<td>Age adjusted IGF-1 - SD</td>
<td>-0.63 ± 1.16</td>
<td>-0.45 ± 1.34</td>
<td>0.686</td>
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<tr>
<td>Testosteron – nmol/L</td>
<td>13.4 (6.8 - 60.0)</td>
<td>12.8 (0.04 - 34.0)</td>
<td>0.659</td>
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<td>fT4 – pmol/L</td>
<td>14.9 ± 4.5</td>
<td>12.9 ± 2.4</td>
<td>0.132</td>
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<td>Osmolality urine – mOsm/kg</td>
<td>628.4 ± 153.1</td>
<td>556.8 ± 194.0</td>
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<td>Urine volume – mL/24h</td>
<td>1863 ± 539</td>
<td>1857 ± 899</td>
<td>0.378</td>
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<td>Histology – no. (%)</td>
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<tr>
<td>Macroadenoma - prolactonoma</td>
<td>5 (31.2)</td>
<td>5 (26.3)</td>
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<tr>
<td>Macroadenoma – GH producing</td>
<td>1 (6.2)</td>
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<tr>
<td>Macroadenoma – gonadotropinoma</td>
<td>1 (6.2)</td>
<td>1 (5.3)</td>
<td></td>
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<tr>
<td>Macroadenoma – non functioning</td>
<td>8 (50)</td>
<td>8 (42.1)</td>
<td></td>
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<tr>
<td>Craniopharyngioma</td>
<td>5 (26.3)</td>
<td></td>
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<tr>
<td>Microadenoma – ACTH producing</td>
<td>1 (6.2)</td>
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<tr>
<td>Previous therapy – no. (%)</td>
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<tr>
<td>Surgical treatment</td>
<td>13 (81.2)</td>
<td>16 (84.2)</td>
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<td>Hypothalamic-pituitary hormone deficiency – no. (%)</td>
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<tr>
<td>ACTH-deficiency*</td>
<td>8 (50)</td>
<td>14 (73.7)</td>
<td>0.149</td>
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<td>GH deficiency</td>
<td>10 (62.5)</td>
<td>11 (57.9)</td>
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<td>TSH deficiency</td>
<td>13 (81.2)</td>
<td>13 (68.4)</td>
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<td>LH/FSH deficiency</td>
<td>14 (87.5)</td>
<td>17 (89.5)</td>
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<td>ADH deficiency</td>
<td>1 (6.2)</td>
<td>7 (43.8)</td>
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Data are expressed as mean±SD, median (range) or number (percentage).

§ Reference values: testosterone 11.0 - 35.0 nmol/L; fT4 10.0 - 23.0; osmolality urine 300 - 900 mOsm/kg.

* Hydrocorisone tablets were used two or three times daily by all ACTH-deficient patients and their total daily dosage did not differ between patients with and without CRT (20 (20 - 60) vs 20 (15-30); p-value = 0.815).
**Figure 1.** Graphical representation of fat distribution in hypopituitary men with a history of cranial radiotherapy (CRT+) or without (CRT-). (A) visceral to subcutaneous fat ratio, (B) subcutaneous fat area, (C) visceral fat area. Horizontal lines represent median values. The Mann Whitney U test was used for comparison between both groups; *P*-values are shown in each graph.
Figure 1.