CLINICAL STUDY

Hyperleptinaemia rather than fasting hyperinsulinaemia is associated with obesity following hypothalamic damage in children

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Abstract

Background: Obesity following hypothalamic damage is often severe and resistant to lifestyle changes. Disruption of hypothalamic feedback mechanisms that maintain energy homeostasis may be responsible for this intractable obesity. Adipocytokines including insulin and leptin are also known to be important regulators of appetite and weight.

Objective: To investigate the role of insulin, leptin, adiponectin and resistin in the aetiology of hypothalamic obesity (HO).

Design: This was a cross-sectional study of three groups of children, those with HO, congenital hypopituitarism (CH) and simple obesity (SO).

Results: A total of 69 children (HO = 28, CH = 18, SO = 23) had leptin, resistin, adiponectin and insulin measured. Although fasting hyperinsulinaemia and insulin resistance were demonstrated, no differences in insulin or insulin resistance were seen between the groups. The HO group, however, had higher levels of leptin, adiponectin and resistin, which persisted even after adjusting for fat mass, compared with the other groups (P < 0.05).

Conclusion: No differences in fasting hyperinsulinaemia or insulin resistance were seen between the groups; however, leptin levels are elevated, even after adjusting for fat mass, suggesting that an element of leptin resistance is associated with HO. This is consistent with the inability of leptin to act on the hypothalamus, either due to transport across the blood–brain barrier or dysfunctional receptors. The lack of response to leptin may be more important in the development of obesity in these individuals, and the fasting hyperinsulinaemia is a result of the increased adipose tissue rather than the cause of the weight gain.

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Introduction

With advances in the treatment of cranial tumours and increasing numbers of childhood cancer survivors, more patients are experiencing the late effects of cancer. One of these is obesity, which remains a significant problem, particularly in patients with tumours of the hypothalamic and pituitary regions (1). Both insulin hypersecretion and insulin resistance are thought to be involved in the aetiology of hypothalamic obesity (HO). Bray and Gallagher first described the elevated fasting insulin levels in four patients with HO compared with age- and weight-matched controls (2). Damage to the ventromedial hypothalamus (VMH) in rats leads to hyperinsulinaemia and hyperphagia, although the exact pathogenesis remains unclear (3). Hyperinsulinaemia has also been confirmed in more recent studies in patients with hypothalamic damage (4). Insulin hypersecretion is thought to occur due to increased vagal tone to the pancreas, as a result of the damage to the hypothalamic nuclei.

Primary hyperinsulinaemia due to increased vagal tone has also been suggested by Lustig and colleagues (5, 6) and others (7, 8) as a cause of obesity in patients with HO.

Leptin in animal studies reduces food intake and promotes weight loss (9–12). Increased levels, however, are seen in obese individuals and those following craniopharyngioma despite increasing adiposity (4).

Circulating adipocytokines, such as adiponectin and resistin, influence insulin sensitivity, and an increase in adipose tissue will lead to changes in the production of adipocytokines (13–15). Adiponectin is produced predominantly by peripheral adipose tissue, and resistin is produced by visceral adipose tissue. Adiponectin improves insulin sensitivity whereas resistin worsens it in rodent studies (16, 17), with conflicting results in humans (18–20). Although a recent study by Meigs and colleagues did demonstrate that insulin resistance

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in humans was inversely related to adiponectin and positively related to resistin (21).

Both leptin and insulin, as well as adiponectin and resistin, have receptors within the hypothalamus, through which they exert their effect to reduce appetite and maintain energy homeostasis (22).

The aim of this study was to investigate the role of insulin and leptin, together with resistin and adiponectin, which also influence insulin sensitivity in obesity, following hypothalamic damage.

Materials and methods

Subjects

This was a cross-sectional study. Subjects were recruited through the Endocrine and combined Endocrine-Oncology Brain Tumour and Dietetic clinics at the Birmingham Children’s Hospital. Ethical approval for the study was obtained from the South Birmingham Ethics Committee.

Height and weight was measured and body mass index (BMI) was calculated. Height, weight and BMI are expressed as SDS, calculated using British reference data (Cole et al. 1990).

Obesity was defined as a BMI greater than the International Obesity Task Force proposed age- and sex-specific cut-off points, corresponding to an adult BMI greater than 30 kg/m² (23).

Three groups of children were studied and compared with the following.

(i) Hypothalamic obesity

This is defined as developing obesity after the diagnosis of a hypothalamic lesion, or secondary hypothalamic damage as a result of surgery or radiotherapy, or both. At least one pituitary hormone deficiency was also used in the definition of HO. All HO subjects were at least 2 years post-operative.

(ii) Congenital hypopituitarism (CH)

This is defined as children with at least one primary pituitary hormone deficiency, without evidence of a cranial tumour.

(iii) Simple obesity (SO)

This is defined as obesity without any medical cause.

Where required, the HO and CH groups were on standard pituitary hormone replacement therapy including hydrocortisone (8–10 mg/m²).

Blood samples

Fasting blood samples were taken in all subjects and taken straightaway to the laboratory, where the plasma and serum if not analysed immediately, were separated and stored at −20 °C. Leptin, adiponectin and resistin levels were measured using human serum adipocyte LINOpolex kit (CAT#HADCYT-61K 2005; Millipore, Billerica, MA, USA).

Glucose was analysed by the Olympus AU640, using an enzymatic u.v. method (hexokinase) and insulin levels were measured using Mercodia Kit (manual ELISA method; Olympus UK Ltd, Middlesex, UK).

Insulin resistance was calculated using the homeostatic model assessment (HOMA) as described by Matthews (24).

Body composition

Dual energy X-ray absorptiometry (DXA) scans were performed using a Lunar Prodigy machine to assess body composition and body fat distribution.

Statistical analysis

This was performed using computer software (SPSS 12, Chicago, IL, USA). Where data were normally distributed, results are presented as mean ± s.e.m. For non-normally distributed data, the median and range have been shown.

Normally distributed data were compared between the groups using ANOVA, together with univariate analysis (general model using stepwise linear regression) and post hoc tests (Tukey).

Insulin resistance exhibited a log-normal distribution, and log transformation of the data was employed to produce a normal distribution; parametric tests were then used.

Statistical significance was taken as P < 0.05 or less using two-tailed probability tables.

Results

A total of 69 children (HO = 28, CH = 18, SO = 23) were recruited, and had leptin, resistin, adiponectin and insulin measured.

Auxological and fat mass details are presented in Table 1. Median age (range) is expressed in decimal years. Auxological data were not normally distributed and non-parametric statistical analysis was used. For age, height SDS, weight SDS and BMI SDS, the data are expressed as median (range). Fat mass data are expressed as mean (s.e.m.; Table 1). The primary diagnoses for the HO are shown in Table 2. Of the CH group, nine had septo-optic dysplasia and the remainder had CH. Pituitary hormone deficiencies in the HO and CH groups are shown in Table 3.

There was no statistical difference in age and sex between the groups. Significant differences were seen in height SDS and weight SDS between the groups, with the SO being taller and heavier compared with the other groups (P < 0.01). No differences were seen between the HO and CH groups. The SO had a higher BMI SDS.
compared with the other groups, but this was not statistically different compared with the HO and CH groups.

No statistical differences were seen in total fat mass or fat mass distribution between the groups.

**Leptin**

Leptin levels were elevated in the HO group compared with the other groups, although this did not achieve statistical significance ($P \approx 0.07$). Statistically significant differences were seen between the groups when leptin was adjusted for fat mass ($P \approx 0.01$; Table 3; adjusted means HO 3420 pg/ml vs CH 2795 pg/ml vs SO 2240 pg/ml; new total body fat 29 120 g; Fig. 1). These differences also persisted when leptin levels were adjusted for sex, age and puberty.

No differences in leptin levels were seen between the CH and SO groups.

**Adiponectin**

The HO group had significantly higher adiponectin levels compared with the other groups ($P < 0.05$; Table 3). This difference persisted even when adiponectin levels were adjusted for total fat mass, sex and age ($P < 0.05$; adjusted means; HO 3240 pg/ml vs CH 2795 pg/ml vs SO 2240 pg/ml; new total body fat 29 120 g; Fig. 1). These differences remained when leptin levels were also adjusted for sex, age and puberty.

No differences in leptin levels were seen between the CH and SO groups.

**Resistin**

Significant differences were found between the groups ($P < 0.05$). Resistin levels were significantly increased in the HO group compared with the CH and SO groups ($P < 0.05$ and $P < 0.01$ respectively; Table 3). Resistin levels remained elevated in the HO group when adjusted for sex, age and fat mass (adjusted means for fat mass: HO 7560 pg/ml vs CH 4590 pg/ml vs SO 4260 pg/ml). These differences also persisted after adjusting for puberty.

**Glucose**

Fasting glucose levels were higher in SO compared with the HO and CH groups, although only the HO were significantly lower than the SO group ($P < 0.01$; Table 3). No patient was diabetic or had impaired fasting glycaemia on fasting glucose measurements. There was no significant correlation between glucose and fat mass.

**Insulin**

No significant differences were seen in fasting insulin levels between the groups ($P = 0.33$). Differences were also not seen when insulin levels were adjusted for sex, age, puberty and fat mass.

The median (range) of the HO, CH and SO groups are shown in Table 3.

A cut-off value of $>132$ pmol/l was used to indicate fasting hyperinsulinaemia (25). Using this cut-off, a total of 20 (29%) patients were hyperinsulinaemic: HO = 7 (26% of HO group), CH = 5 (28%) and SO = 8 (35), with no significant differences between the groups ($P = 0.680$).

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**Table 1** Auxological and fat mass data.

<table>
<thead>
<tr>
<th>Median (range)</th>
<th>Brain tumour</th>
<th>Congenital hypopituitarism</th>
<th>Obese</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total numbers</td>
<td>28</td>
<td>18</td>
<td>23</td>
</tr>
<tr>
<td>Male numbers</td>
<td>13</td>
<td>10</td>
<td>9</td>
</tr>
<tr>
<td>Age (years)</td>
<td>12.5 (4–18)</td>
<td>9.5 (5–18)</td>
<td>12 (6–15)</td>
</tr>
<tr>
<td>Height SDS</td>
<td>0.11 (–3.1–2.3)</td>
<td>–0.06 (–3.3–1.7)</td>
<td>1.7 (0.2–3.7)</td>
</tr>
<tr>
<td>Weight SDS</td>
<td>2.42 (–0.4–4.5)</td>
<td>1.97 (–1.8–3.9)</td>
<td>3.4 (2–5)</td>
</tr>
<tr>
<td>BMI SDS</td>
<td>2.72 (2–4.9)</td>
<td>2.43 (–0.12–3.7)</td>
<td>3.21 (2.2–4.7)</td>
</tr>
<tr>
<td>Fat mass (g)</td>
<td>31 595 (2292)</td>
<td>21 164 (3080)</td>
<td>33 460 (2642)</td>
</tr>
<tr>
<td>Fat mass arms (%)</td>
<td>9.29 (0.22)</td>
<td>9.05 (0.39)</td>
<td>9.55 (0.24)</td>
</tr>
<tr>
<td>Fat mass legs (%)</td>
<td>39.31 (0.84)</td>
<td>39.48 (1.09)</td>
<td>40.53 (0.93)</td>
</tr>
<tr>
<td>Fat mass trunk (%)</td>
<td>47.97 (0.90)</td>
<td>46.18 (1.43)</td>
<td>46.65 (0.98)</td>
</tr>
<tr>
<td>Fat mass/height (kg/m)</td>
<td>19.67 (1.05)</td>
<td>15.00 (1.91)</td>
<td>21.25 (1.44)</td>
</tr>
</tbody>
</table>

Auxological data are presented as median (range) for non-normally distributed variables. Fat mass data are presented as mean (S.E.M.) for normally distributed variables. (1) $P$ values of the difference between HO and SO. (2) $P$ values of the difference between CH and SO. * $< 0.01$.

**Table 2** Primary diagnoses for hypothalamic obesity.

<table>
<thead>
<tr>
<th>Brain tumour</th>
<th>Number of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glioma</td>
<td>9</td>
</tr>
<tr>
<td>Craniopharyngioma</td>
<td>7</td>
</tr>
<tr>
<td>Germinoma</td>
<td>5</td>
</tr>
<tr>
<td>Astrocytoma</td>
<td>3</td>
</tr>
<tr>
<td>Medulloblastoma</td>
<td>2</td>
</tr>
<tr>
<td>Histiocytosis</td>
<td>1</td>
</tr>
<tr>
<td>Retinoblastoma</td>
<td>1</td>
</tr>
</tbody>
</table>

**Table 3** Percentage of pituitary hormone deficiencies in hypothalamic obesity (HO) and congenital hypopituitarism (CH) groups.

<table>
<thead>
<tr>
<th>Group (%)</th>
<th>GH</th>
<th>TSH</th>
<th>ACTH</th>
<th>Sex steroid therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>HO</td>
<td>83</td>
<td>94</td>
<td>56</td>
<td>28</td>
</tr>
<tr>
<td>CH</td>
<td>100</td>
<td>100</td>
<td>77</td>
<td>8</td>
</tr>
</tbody>
</table>

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There was a significant correlation between insulin levels and fat mass ($P < 0.01$, Spearman correlation $= 0.588$).

**Insulin resistance**

Insulin resistance was calculated using HOMA (Table 3). The SO were more insulin resistant compared with the other groups, although this did not achieve statistical significance ($P = 0.27$). The differences between the groups became less significant ($P = 0.97$) when insulin resistance was adjusted for sex, age, pubertal status and fat mass.

Using an adult cut-off for insulin resistance of $> 2.5$ (26), HO = 6 (22% of group), CH = 5 (27%) and SO = 8 (34%) patients were insulin resistant. Although there were a higher percentage of patients with insulin resistance in the SO group, this was not statistically significant. A higher cut-off for insulin resistance of 3.5 has been suggested in adolescents (27), but again this yielded similar numbers in each group (HO = 4, CH = 2 and SO = 3).

**Discussion**

Earlier studies of HO have usually been retrospective, or used longitudinal data and without appropriate control groups (4, 28–32). We have compared children with HO with CH and SO to control for hormonal deficiencies and the effects of adipose tissue on insulin and adipocytokine secretion.

**Leptin**

Previous studies of HO (33), including those by Pinto and colleagues (4), were longitudinal studies measuring leptin levels pre- and post-operatively. Their studies, reporting elevated leptin levels in craniopharyngioma patients did not compare leptin levels with obese controls, whereas our study has demonstrated differences in leptin between hypothalamic and simple obese individuals. Even after adjusting for fat mass, the HO group had significantly raised leptin levels, which were higher by 22 and 50% in the HO group compared with the CH and OB groups respectively, suggesting that there is an element of leptin resistance, which is greater in HO.

Leptin resistance has been described in obese subjects and is thought to be due to saturated leptin transport across the blood–brain barrier or abnormal signalling (34, 35). Another hypothesis for leptin resistance is thought to be due to persistently elevated levels of leptin leading to elevated levels of SOCS3, an intracellular protein which inhibits the action of leptin (36).

The concept of the hypothalamus being insensitive to leptin has also been suggested by Roth (37), whose study of 14 patients demonstrated elevated leptin levels in patients with craniopharyngioma, and Patel and colleagues (2002), who also reported raised leptin levels following hypothalamic damage (38), but low leptin binding activity, indicating high free leptin levels, and suggesting a defective action of leptin within the hypothalamus.

**Insulin**

Elevated fasting insulin and C peptide levels were seen in our HO group, with 26% of the group having hyperinsulinaemia, although this was no different to the other groups. Pinto and colleagues also demonstrated a rise in insulin levels following surgery for craniopharyngioma, again suggesting that increased insulin secretion resulted in weight gain (4). Previous studies implicating hyperinsulinaemia as a cause of HO have used non-obese controls or longitudinal data (4, 32). Our data demonstrated no differences in fasting insulin levels between hypothalamically obese patients and patients with simple obesity, in agreement with the study by Srinivasan and colleagues, which also confirmed raised levels and insulin resistance in HO, with no significant differences compared with obese controls (39).

The raised fasting insulin levels, which were seen in all groups, may be the result of insulin resistance secondary to the increased adipose tissue, rather than a primary effect. A recent study by Lustig and colleagues has suggested that it is important to assess insulin sensitivity/secretion in obese individuals, as this will determine pharmacotherapy (40). Some animal studies have demonstrated no differences in fasting insulin levels between hypothalamic obese patients and patients with simple obesity, in agreement with the study by Srinivasan and colleagues, which also confirmed raised levels and insulin resistance in HO (41, 42). King and colleagues have suggested postprandial insulin levels are more important in HO than fasting levels (8, 43).
Insulin has a central action within the hypothalamus, and intact insulin receptors are required for controlling appetite and weight (44, 45). There is also an interaction between insulin and leptin at the hypothalamic level, and abnormal leptin action may influence insulin activity (46). Transport of insulin into the hypothalamus is saturable (47), and the raised insulin levels may be due to impaired transport of insulin across the blood-brain barrier.

It is not clear whether it is the insulin levels that are driving the weight gain or the increasing adiposity which leads to hyperinsulinaemia. The weight gain following hypothalamic damage is usually rapid and severe, and if insulin was driving the obesity, differences in insulin secretion would be expected to be seen between our study groups.

**Insulin resistance**

Insulin resistance was demonstrated in the brain tumour group, although this was no different to the other groups. Using adolescent and adult cut-offs for insulin resistance (HOMA > 3.5 and > 2.5 respectively) (48, 49), similar levels of insulin resistance were demonstrated in all groups. Previous studies have not described insulin resistance and have predominantly studied insulin levels, although it is likely that the subjects in these studies were also insulin resistant. Hypothalamic damage results in the accumulation of adipose tissue, which will reduce insulin sensitivity and increase insulin secretion, suggesting that it may not be the insulin driving the weight gain, and that hyperinsulinaemia is a result of the weight gain.

**Adiponectin and resistin**

Our data demonstrated elevated levels of both adiponectin and resistin in the HO group, which have not been previously described. As adiponectin and resistin have opposing effects on insulin sensitivity, it is unclear why both should be raised in the HO group.

While differences in fat distribution might explain the raised resistin levels, as the HO group had more truncal fat and hence more visceral fat, this would not explain the raised adiponectin levels. Significantly elevated resistin and adiponectin levels were seen in the HO group, although differences in fat distribution between the groups were not significant. This would imply that the adipose tissue and its distribution is not solely responsible for adiponectin and resistin secretion.

Previous studies have shown resistin impairing glucose homeostasis and leading to insulin resistance (19, 50, 51), although not all human studies have confirmed this (18, 20). Resistin levels would therefore be expected to be greatest in the OB group, as this group had the highest insulin levels and insulin resistance, although they were not significantly different compared with the other groups. The resistin levels, however, were significantly higher in the HO group, suggesting that they may be raised due to mechanisms other than insulin resistance. The hypothalamic action of resistin is unclear, but animal studies have demonstrated that central administration of resistin reduces food intake in the short term (52, 53). Resistin receptors are present within the hypothalamus, in particular the arcuate and ventromedial nuclei (53, 52).

Adiponectin receptors are also expressed in the brain, and i.v. administration of adiponectin leads to a rise in CSF levels. Animal studies, using intracerebroventricular administration of adiponectin, have demonstrated reductions in weight, which is primarily due to increased thermogenesis (54).

The raised adiponectin and resistin levels may be due to disruption of the central feedback mechanisms as a result of ineffective and damaged hypothalamic receptors. Another possible explanation is that the raised adiponectin levels are counteracting the action of resistin.

**Conclusion**

Obesity following HO is severe and produces significant disability.

Although hyperinsulinaemia has been implicated in the aetiology of HO, our data demonstrated no differences in fasting insulin levels between hypothalamic and simple obese individuals, suggesting that fasting hyperinsulinaemia is not driving weight gain in HO; however, it is still possible that elevated post-prandial insulin levels may be involved in the aetiology of this obesity.

Leptin levels, together with adiponectin and resistin, were significantly raised in individuals following hypothalamic damage and it appears that the inability of leptin to act on the hypothalamus, either due to transport across the blood–brain barrier or dysfunctional receptors resulting in defective feedback mechanisms, may be more important in the development of obesity in these individuals.

**Declaration of interest**

M G S, R G G and J M W K received grant support (September 2001 to March 2005) from Novo disk, Pfizer, Birmingham Children’s Hospital Research and Development Department and the Ella Brown Foundation. Current address for R G G: Children’s Brain Tumour Research Centre, Queen’s Medical Centre, Nottingham, UK.

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