Hypothalamic Gliosis by MRI and Visceral Fat Mass Negatively Correlate with Plasma Testosterone Concentrations in Healthy Men

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Objective: This study aimed to determine whether a relationship was evident between gliosis in the mediobasal hypothalamus (MBH) and plasma testosterone concentrations in men.

Methods: A total of 41 adult men (aged 18-50 years) from 23 twin pairs underwent fasting morning blood draw and brain magnetic resonance imaging. T2 relaxation time was used to quantify gliosis in the MBH and control areas in the putamen and amygdala. Plasma concentrations of testosterone and 17β-estradiol were measured by liquid chromatography-tandem mass spectrometry. Body composition including visceral adiposity was measured by dual x-ray absorptiometry.

Results: A negative association was found between MBH T2 relaxation time and plasma concentrations of both free and total testosterone ($r = -0.29, P < 0.05$ and $r = -0.37, P < 0.01$, respectively). Visceral adiposity exhibited a negative correlation with plasma total testosterone concentration ($r = -0.45, P = 0.001$) but a positive correlation with MBH T2 relaxation time ($r = 0.24, P = 0.03$). The negative correlation between plasma total testosterone and MBH T2 relaxation time remained significant after adjustment for visceral adiposity, age, BMI, and insulin resistance.

Conclusions: In healthy men across a range of BMIs, MBH gliosis was associated with higher visceral adiposity but lower endogenous testosterone. These findings suggest that MBH gliosis could provide novel mechanistic insights into gonadal dysfunction in men with obesity.

Introduction

A striking interdependence exists between obesity and hypogonadism in men. Men with low circulating levels of testosterone may be at increased risk of developing obesity, insulin resistance, and type 2 diabetes (1-3). In turn, obesity increases risk for gonadal dysfunction in both men and women, which manifests as alterations in plasma sex steroid concentrations and reduced fertility (4,5). Such findings suggest a close, potentially bidirectional relationship between obesity and hypogonadism, but the mechanisms underlying the observed interdependence remain poorly understood.

Among men with obesity, a well-established association exists between higher BMI and increased risk of hypogonadotropic hypogonadism (HH)
Our group and others have previously demonstrated that, in rodent models, high-fat diet feeding rapidly leads to a reactive inflammatory process within the mediobasal hypothalamus (MBH) called hypothalamic gliosis that precedes the onset of diet-induced obesity (8-10). Gliosis is a physiologic, adaptive response to injury in the central nervous system; however, if sustained, gliosis can lead to hypothalamic injury and loss of pro-opiomelanocortin (POMC) neurons in the MBH (10), which are key regulators of body weight and energy homeostasis (10,11). The MBH is the anatomical locus of the infundibular nucleus (also known as the arcuate nucleus in rodents), which, importantly, houses several neuron populations, including those that regulate gonadal function and reproduction (kisspeptin/neurokinin B/dynorphin [KNDY] neurons), as well as neurons that regulate energy homeostasis (POMC and neuropeptide Y/agouti-related protein [NPY/AgRP] neurons). Neurons that produce GnRH also reside within the MBH; although GnRH neurons are found in the infundibular nucleus in humans, they reside outside of the arcuate nucleus in rodents. Gliosis in the MBH therefore could prove a novel mechanism contributing to comorbid obesity and hypogonadism in men.

Gliosis is detectable by magnetic resonance imaging (MRI). Clinical studies have used visual identification of increased T2 signal intensity (brightness) to qualitatively detect gliosis in brain regions including the hypothalamus (12,13). In contrast, quantitative MRI methods can be used as a research strategy to measure T2 signal through a standardized approach. Thus, longer T2 relaxation time (a measure of T2 signal) in the MBH is a validated index of gliosis in this region and correlates with findings of gliosis on histology in both rodents and humans (8,9,14). Longer MBH T2 relaxation times have positively associated with both BMI and insulin resistance in clinical studies (14,15). Corroborating these findings, BMI also was shown to correlate with postmortem evidence of MBH gliosis (16). MRI is therefore an important technology to assess hypothalamic gliosis in vivo in humans and thereby better understand relationships among hypothalamic gliosis, obesity, and associated comorbidities.

Using quantitative MRI, we measured MBH T2 relaxation time (the index of hypothalamic gliosis) and plasma free and total testosterone concentrations in a cohort of men. We hypothesized that MBH gliosis would be associated with lower plasma testosterone concentrations and further predicted that this relationship would be independent of BMI.

Methods
Subjects
Subjects were otherwise healthy men aged 18 to 50 years with normal body weight, overweight, or obesity (BMI 19-45 kg/m²) recruited from the Washington State Twin Registry for a study of genetic influences on obesity in which subjects with obesity were oversampled (17). Subjects for the present analyses included male subjects who had both baseline plasma samples available for sex steroid measurement and brain MRI images. A total of 41 adult men from 23 twin pairs met these inclusion criteria and were included in the current analyses. One subject was subsequently excluded from analyses involving visceral adiposity for an improbably low value for visceral fat mass; data for this subject were included in the remaining analyses. The University of Washington Human Subjects Division approved all study procedures. All subjects provided written informed consent for participation.

Study protocol
The study protocol has been published previously (18). Briefly, each subject presented in the morning for anthropomorphic measures including height and body weight, and a fasting blood draw was performed at 8:00 AM. Subjects underwent brain MRI and body composition assessment via dual x-ray absorptiometry (DXA) scan.

Laboratory analyses
Blood samples were collected in EDTA tubes containing protease inhibitor cocktail (Sigma-Aldrich, St. Louis, Missouri), aprotinin (Sigma-Aldrich), and dipeptidyl peptidase-4 inhibitor (Millipore, Billerica, Massachusetts). Following collection, each sample was cold centrifuged at 4°C, aliquoted, and then stored at ~80°C until analysis. Plasma glucose concentration was measured using an automated assay (Roche Modular P Chemistry autoanalyzer, based on the hexokinase method; Roche Diagnostics Inc., Indianapolis, Indiana). Plasma insulin concentration was measured using automated assay ( Tosoh 2000 analyzer, a two-site immune-enzymometric assay; Tosoh Bioscience Inc., San Francisco, California). Insulin resistance was determined using homeostatic model assessment of insulin resistance (HOMA-IR) (19). All assays were performed in duplicate, and samples from each twin pair were processed in the same batch. Plasma total testosterone and 17β-estradiol concentrations were measured by liquid chromatography-tandem mass spectrometry using an AB Sciex QTRAP 5500 tandem quadrupole mass spectrometer (AB Sciex, Foster City, California) through modified methods described previously (20). The intra-assay coefficients of variation were < 5% for measurement of both testosterone and 17β-estradiol. Sex hormone-binding globulin (SHBG) concentrations were quantified by enzyme-linked immunosorbent assay (ELISA) (R&D Systems, Minneapolis, Minnesota) and used to calculate free testosterone using a standard albumin concentration of 43 g/L (21).

Body composition
DXA (on either General Electric Lunar Prodigy or iDXA using a correction factor) was used to measure body composition. Estimates of total, subcutaneous, and abdominal visceral adipose tissue mass were determined using EnCORE software (platform version 16.2; GE Healthcare, Madison, Wisconsin) (22).

MRI image acquisition and analyses
Methodology for MRI image acquisition and analysis has been previously published (14). All MRI imaging was performed on a 3-T Philips Achieva scanner (version 3.2; Philips Medical Systems, Best, The Netherlands) using a 32-channel head radio-frequency coil. Sequences included T1-weighted scan and quantitative multislice/multiecho T2-weighted sequence with 16 echoes (interecho spacing 10 milliseconds) (repetition time [TR]/echo time [TE]/number of signals averaged [NSA]: 2000/20-170/1). Images were acquired to span the area from the optic chiasm through the mammary bodies. Nine to twelve slices were acquired for each subject (slice thickness = 2.5 mm; interstice gap = 0.2 mm).
With an in-plane pixel resolution of 0.7 to 0.75 mm, this resulted in a voxel size of 1.313 mm³. Calculations of T2 relaxation time were determined using the signal decay curve of the 16 echoes on a pixel-by-pixel basis and then displayed on a parametric map as quantitative T2 relaxation time in order to evaluate tissue T2 relaxation time by region. As in previous studies, the coronal slice immediately posterior to the optic chiasm encompassing the rostral arcuate/infundibular nucleus was identified for each subject (23). Coefficients of variation for the left and right hypothalami were 7.6% and 7.9%, respectively. Reference regions of interest (ROIs) in the putamen and amygdala were identified within this same coronal slice. ROIs were initially placed on high-resolution coronal images for ease of identifying anatomical structures and then transferred to a T2 parametric map. Mean ± SD T2 relaxation time per ROI was recorded (OsirIX imaging software, version 5.6, Bernex, Switzerland).

**Statistical analysis**

Because twins were analyzed as individuals for the current study, generalized estimating equations were used to account for the non-independence of the twin sample. Robust standard errors were used to account for heteroscedasticity in the data. T2 relaxation time measurements in control regions (amygdala and putamen) were included as covariates in all models of MBH T2 relaxation time to control for scan-related variability in T2 signal. Scatterplots were generated using a mean-centering approach to reflect adjusted statistical models. Data are presented as mean ± SD. Statistical analyses were performed using Stata software version 12.1 (College Station, Texas) and SPSS Statistics software version 25 (IBM Corp., Armonk, New York), and graphs were created using GraphPad Prism 5 (GraphPad Inc., La Jolla, California).

**Results**

**Subject characteristics**

Subjects ranged in age from 18 to 48 years and BMI from 20.0 to 40.0. Mean free and total testosterone concentrations were in the normal range (Table 1). Mean HOMA-IR was consistent with mild insulin resistance (24), and mean percent body fat was 31.1% ± 7.4%.

**T2 relaxation time in MBH exhibits negative correlation with plasma testosterone concentrations in men**

Longer T2 relaxation time in the MBH was negatively associated with plasma concentrations of both total and free testosterone (Figure 1A-B). These relationships remained and were minimally attenuated when models were adjusted for age (total testosterone \( r = -0.36, P < 0.01 \); free testosterone \( r = -0.26, P = 0.06 \)). A significant association also was found between longer MBH T2 relaxation time and lower plasma SHBG concentration (Figure 1C). In contrast, no correlation was found between MBH T2 relaxation time and plasma 17β-estradiol concentration (Figure 1D).

BMI was negatively correlated with plasma concentrations of both total testosterone \( r = -0.33, P < 0.01 \) and free testosterone \( r = -0.27, P < 0.05 \) and was positively associated with MBH T2 relaxation time \( r = 0.30, P = 0.02 \). Notably, the negative correlation between MBH T2 relaxation time and plasma total testosterone concentration remained significant after adjustment for BMI \( (r = -0.33, P = 0.02) \), and the correlation between MBH T2 relaxation and plasma free testosterone persisted as a strong trend \( (r = -0.26, P = 0.058) \). A correlation between higher BMI and lower plasma SHBG concentration was evident as a trend \( (r = -0.23, P = 0.07) \), and, after adjustment for BMI, the negative correlation between MBH T2 relaxation time and plasma SHBG was attenuated \( (r = -0.27, P = 0.08) \).

Similarly, the negative correlation between MBH T2 relaxation time and plasma total testosterone concentration remained significant after adjustment for insulin resistance as quantified by HOMA-IR \( (r = -0.32, P = 0.02) \). The negative correlation between MBH T2 relaxation time and plasma free testosterone concentration again was evident as a trend after adjustment for insulin resistance \( (r = -0.24, P = 0.09) \).

**TABLE 1 Cohort characteristics**

|                      | Mean ± SD (N = 41) | BMI < 30 (N = 20) | BMI ≥ 30 (N = 21) |
|----------------------|--------------------|-------------------|-------------------|
| Age (y)              | 29.7 ± 10.3        | 26.7 ± 9.3        | 32.4 ± 10.6       |
| BMI (kg/m²)          | 29.7 ± 5.8         | 24.6 ± 3.1        | 34.5 ± 2.6        |
| <25 (13)             |                    |                   |                   |
| 25-29 (7)            |                    |                   |                   |
| 30-35 (13)           |                    |                   |                   |
| >35 (8)              |                    |                   |                   |
| Body weight (kg)     | 97.1 ± 23.8        | 76.4 ± 12.2       | 116.7 ± 12.5      |
| Free testosterone nmol/L (reference 0.32-0.49 nmol/L) | 0.44 ± 0.13 | 0.47 ± 0.14 | 0.40 ± 0.13 |
| Total testosterone nmol/L (reference 8.4-29.4 nmol/L) | 16.5 ± 5.7 | 18.4 ± 5.2 | 14.6 ± 5.6 |
| SHBG (nmol/L)        | 20.6 ± 9.6         | 23.2 ± 9.7        | 18.1 ± 9.1        |
| Estradiol (pmol/L) (reference 57.6-156.4 pmol/L) | 102.7 ± 33.2 | 89.2 ± 21.8 | 115.6 ± 37.3 |
| HOMA-IR              | 2.2 ± 1.7          | 1.2 ± 0.7         | 3.1 ± 1.9         |
| Total body fat mass (kg) | 30.5 ± 13.3      | 19.5 ± 6.8        | 41.0 ± 8.6        |
| Visceral fat mass (g) | 1,382 ± 968        | 661 ± 615         | 2,035 ± 739       |
| T2 relaxation time, mediobasal hypothalamus (ms) | 95.2 ± 6.0 | 94.2 ± 6.3 | 96.3 ± 5.6 |
| T2 relaxation time, putamen (ms) | 59.8 ± 4.3 | 60.9 ± 4.0 | 58.7 ± 4.3 |
| T2 relaxation time, amygdala (ms) | 82.6 ± 3.8 | 83.8 ± 3.7 | 81.7 ± 3.7 |
Visceral adiposity is inversely related to MBH gliosis and plasma testosterone concentrations

Longer T2 relaxation time in the MBH was associated with greater visceral fat mass (Figure 2A). A positive correlation also was evident between MBH T2 relaxation time and total body fat mass, although this association did not achieve statistical significance (Figure 2B). Visceral adiposity was negatively associated with plasma concentrations of both total testosterone (Figure 2C) and free testosterone (Figure 2D), and these relationships were independent of age (total testosterone $r = -0.45$, $P < 0.001$; free testosterone $r = -0.39$, $P = 0.001$). Nonetheless, after adjustment for visceral fat mass, a significant, negative correlation between plasma total testosterone concentration and MBH T2 relaxation persisted and was minimally attenuated ($r = -0.34$, $P = 0.01$). The negative correlation between MBH T2 relaxation time and plasma free testosterone concentration similarly remained evident ($r = -0.28$, $P = 0.03$). Strikingly, the negative correlation between plasma total testosterone concentration and MBH T2 relaxation time remained significant after adjustment for BMI, insulin resistance, and visceral fat mass ($P = 0.01$).

Discussion

Our findings demonstrate associations of MBH gliosis with lower plasma testosterone and higher visceral adiposity in otherwise healthy men across a wide spectrum of BMIs. The tendency for men exhibiting greater evidence of MBH gliosis to have lower endogenous circulating testosterone was not explained by other factors, including age, higher BMI, or greater visceral adiposity. In contrast, no relationship was seen between MBH gliosis and circulating 17β-estradiol concentration. Notably, a negative correlation between MBH gliosis and circulating SHBG concentration was also evident but appeared attributable, at least in part, to BMI. These data therefore offer novel, preliminary evidence that MBH gliosis may be related to the high prevalence of hypogonadism in men with obesity, although its possible roles as a cause or effect of hypogonadism cannot be discerned from these cross-sectional analyses. Furthermore, our findings suggest that visceral adipose deposition may be a marker of not only cardiometabolic risk but gonadal dysfunction in men.

No prior clinical studies, to our knowledge, have examined the relationship between MBH gliosis and gonadal function. However, rapidly accumulating evidence derived from both preclinical and clinical studies has indicated that MBH gliosis plays a role in the pathogenesis of obesity. In rodent models of diet-induced obesity, MBH gliosis arises early after exposure to high-fat feeding and precedes the evolution of obesity (10,25-27). Moreover, genetic or pharmacologic inhibition of MBH gliosis reduces obesity progression, suggesting a causal role for MBH gliosis in obesity pathogenesis (27,28). Clinical studies to date have demonstrated positive correlations between MBH gliosis and BMI as well as insulin resistance, independent of BMI (14,15). Radiographic
evidence of hypothalamic gliosis has been shown to correlate not only with BMI but also with genetic factors and host microbiome composition (15). In postmortem analyses, subjects with obesity exhibited greater microglial activation in the hypothalamus relative to subjects of normal body weight, and this degree of activation correlated with BMI (16). Interestingly, in a parallel rodent model of diet-induced obesity, the same authors demonstrated that the phenotype of activated microglia in the hypothalamus is highly dynamic and changes over time in response to high-fat feeding (16). Thus, clinical evidence supports a possible pathogenic role for MBH gliosis in human obesity and insulin resistance. Importantly, the negative correlation found between MBH gliosis and plasma testosterone concentration in the current study was independent of adiposity, suggesting that MBH gliosis may be an independent risk factor for male hypogonadism, which is plausible given the established role of neurons within the MBH in regulation of reproduction.

The MBH houses the infundibular nucleus (known as the arcuate nucleus in rodents), the center of appetite and energy expenditure regulation as well as reproductive function. In rodents with susceptibility to diet-induced obesity, chronic exposure to high-fat feeding led to gliosis in the arcuate nucleus, which, in turn, was associated with decreased neurogenesis, loss of synapses, and dysregulation of POMC and NPY/AgRP neuron signaling because of disruption of the blood–brain barrier (29). Thus, one mechanism through which reactive gliosis could lead to HH is impairment of POMC and NPY/AgRP neuron function, both of which have been implicated in the regulation of GnRH neurons (30). Alternatively, gliosis could disrupt GnRH and/or KNDy neuron function directly, through parallel processes to those described for POMC and NPY/AgRP neurons, such as perikaryal ensheathment and reduced neurogenesis. However, these potential effects of gliosis have yet to be shown specifically for GnRH or KNDy neurons. GnRH neurons also receive direct, regulatory signals from glial cells (31), so changes in glial activation state further could impede normal, pulsatile GnRH secretion through altered signaling via these regulatory inputs. Finally, peripheral metabolic signals, including leptin, ghrelin, and insulin, are key regulators of GnRH neuron function, and changes in the production of these peripheral signals because of diet and/or obesity also could contribute to impaired gonadal function (30). Similarly, the central action of these mediators could be altered in the setting of obesity, irrespective of their peripheral production, because of insulin resistance, gliosis, or other obesity-related changes. Notably, this regulation is likely mediated indirectly, as GnRH neurons exhibit negligible expression of the insulin and leptin receptors (30). Consistent with this idea, neuron-specific deletion of the insulin receptor led to a phenotype of diet-induced obesity and infertility in both male and female mice (32). Thus, multiple potential mechanisms could explain either the co-occurrence of obesity, MBH gliosis, and hypogonadism or a direct, causal relationship between MBH gliosis and gonadal dysfunction.

As our findings reflect cross-sectional data, prospective studies will be essential for establishing whether MBH gliosis precedes and predicts

Figure 2 Higher visceral adipose mass is associated with both greater hypothalamic gliosis and lower plasma testosterone concentrations. Scatterplot and regression lines showing correlations between mean bilateral MBH T2 relaxation time and (A) visceral fat mass and (B) total body fat mass, as well as correlations between visceral fat mass and plasma concentrations of both (C) total and (D) free testosterone. Correlation P values were determined through generalized estimating equations. Data shown in panels A and B are adjusted for T2 relaxation times in control regions in the putamen and amygdala.
gonadal dysfunction with progressive obesity. Obesity-associated HH is reversible, as surgical weight-loss interventions result in improved gonadal function with regard to both normalized plasma testosterone and improved semen quality in men (6,33,34). MBH gliosis also may be reversible; in a rodent model of diet-induced obesity, MBH gliosis was reversed after animals were transitioned back to a regular chow diet (8). Thus, additional work also is needed to determine whether regression of MBH gliosis after weight loss in men predicts restoration of gonadal function. Finally, this line of work will require parallel preclinical studies to determine mechanistically how the magnitude and/or duration of gliosis in the MBH may lead to dysregulated function of GnRH-secreting neurons.

The current findings also demonstrate strong correlations between visceral fat mass, but not total body fat mass, and both MBH gliosis and low testosterone concentrations. Visceral adiposity has been implicated in the evolution of obesity-related sequelae, including insulin resistance, metabolic syndrome, and nonalcoholic fatty liver disease (35-37). Recently, decreases specifically in visceral fat have been shown to correlate with improvements in gonadal function following metabolic surgery (38). Increased visceral adiposity, MBH gliosis, and gonadal dysfunction could result in parallel from common mechanisms, including hyperleptinemia, systemic cytokine production, dietary factors, and insulin resistance (14,39-41). Alternatively, the possibility also exists that visceral adiposity may contribute directly to obesity-related hypogonadism, an effect that could be mediated, in part, through elicitation of hypothalamic gliosis. The current analyses also demonstrate an association between greater MBH gliosis and lower plasma SHBG concentration, although this association was partially attributable to BMI. Hepatic lipid accumulation, insulin resistance, cytokines, and a diet high in monosaccharides have been mechanistically linked to obesity-associated decreases in circulating SHBG concentration (42), which contribute to but do not fully explain obesity-associated hypogonadism (40). POMC neurons regulate hepatic insulin sensitivity (43), providing one plausible mechanism through which MBH gliosis could give rise to hepatic insulin resistance and, consequently, diminished SHBG production. The relevance particularly of altered insulin sensitivity and glucose metabolism to the evolution of HH in men with obesity is underscored by findings that men with obesity and diabetes had both a higher prevalence of HH and an accelerated decline in circulating free testosterone concentration compared with men with obesity but without diabetes (44). Thus, common pathways may underlie many of these obesity-associated traits, and the possible contribution of these pathways to the negative association between MBH gliosis and circulating testosterone concentration may account for our finding that this association was not explained by obesity alone. Future mechanistic work is needed to fully delineate the relationships among these diverse facets of reproductive and metabolic dysregulation.

The present analyses have several limitations. Because this study was cross-sectional in design, the directionality of the relationship between MBH gliosis and circulating testosterone cannot be determined. The cohort size was relatively small, and findings require validation in additional, larger cohorts, as well as longitudinal analyses to establish the temporal relationships among obesity, MBH gliosis, and HH in men. Importantly, increased T2 relaxation time is not specific to gliosis and also, for example, increases consequent to edema or infection. However, as subjects were otherwise healthy men, these alternative pathologies are unlikely to be present. Moreover, increased T2 signal in the MBH has previously been shown to correlate with postmortem evidence of gliosis (14). Nonetheless, although our MRI findings indicate tissue changes within the MBH, histologic analyses would be required to definitively show that these radiographic findings truly reflect gliosis and not an alternative form of tissue pathology. Although our analyses benefitted from the broad range of BMIs and adiposity among subjects, additional work is needed to establish whether the relationship between MBH gliosis and plasma testosterone varies as a function of subject body weight, body fat mass, age, or other clinical variables. Most subjects in this cohort had testosterone concentrations in the eugonadal range, and the present findings must be extended to subjects with true obesity-related HH. Similarly, the relationship between MBH gliosis and HH will need to be verified for both biochemical evidence of hypogonadism and hypogonadism manifesting with clinical symptoms. Finally, these analyses were performed exclusively in men, and future work will be dedicated to establishing whether increases in MBH gliosis similarly are evident in women with obesity-associated reproductive dysfunction.

Conclusion

Our analyses demonstrate an association between hypothalamic gliosis and diminished endogenous testosterone concentrations in men. These data suggest a potential new mechanism whereby as yet undefined dietary exposures may evoke both excess adiposity and impairment in gonadal function. Insights gained from delineating the overlapping, central mechanisms of energy balance and gonadal dysregulation could inform novel clinical approaches to the management of obesity and its associated comorbidities.

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