CLINICAL STUDY

Investigation of antihypothalamus and antipituitary antibodies in amateur boxers: is chronic repetitive head trauma-induced pituitary dysfunction associated with autoimmunity?

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Abstract

Objective: Current data clearly demonstrate that sports-related chronic repetitive head trauma due to boxing might result in hypopituitarism. However, the mechanism of sports-related traumatic brain injury-induced pituitary dysfunction is still unclear. In order to understand whether autoimmune mechanisms could play a role in the pituitary dysfunction due to sports-related head trauma, we investigated the presence of antipituitary antibodies (APAs) and antihypothalamus antibodies (AHAs) in amateur boxers.

Patients and design: Sixty-one actively competing ($n=44$) or retired ($n=17$) male boxers (mean age, 26 years; range, 17–53) who had been evaluated regarding pituitary functions previously were included in the study. In all boxers and in 60 age/sex-similar normal controls, AHAs and APAs were investigated by an indirect immunofluorescence method.

Results: AHAs were detected in 13 of 61 boxers (21.3%), and APAs were detected in 14 of 61 boxers (22.9%), but in none of the normal controls. Pituitary dysfunction was significantly higher in AHA-positive boxers (46.2%) than in AHA-negative boxers (10.4%) ($P=0.003$). There was a significant association between AHA positivity and hypopituitarism due to boxing (odds ratio: 7.37, 95% confidence interval 1.8–30.8). There was no significant association between APA positivity and hypopituitarism.

Conclusions: This study demonstrates for the first time the presence of AHAs and APAs in boxers who were exposed to sports-related head trauma. Moreover, the present investigation provides preliminary evidence that AHAs are associated with the development of pituitary dysfunction in boxers, thus suggesting that autoimmunity may have a role in the pathogenesis.

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Introduction

Traumatic brain injury (TBI), which is an important public health problem, has been recently recognized as a leading cause of pituitary dysfunction. Hypopituitarism due to TBI may be complete or partial, and 25–50% of the patients have been reported to have some degrees of pituitary dysfunction even after mild TBI (1–3). Road traffic accidents are the most common cause of TBI, which are responsible for more than half of the head injuries (4).

It has been reported that concussion is the main type of lesion after TBI, and in 40% of these patients,

concussion was found (5, 6). Concussion is an injury associated with sports including boxing, kickboxing, football, and ice hockey (7, 8). Current data clearly demonstrate that sports-related chronic repetitive head trauma due to amateur boxing and kickboxing might result in pituitary hormone deficiencies, in particular, isolated GH deficiency (9–11).

Several mechanisms have been suggested for the hypothalamic–pituitary dysfunction due to TBI, including hypoxic insult or direct mechanical injury to the hypothalamus, pituitary stalk, or the pituitary gland; compression from hemorrhage, edema, or increased intracranial pressure; and vascular injury to the

hypothalamus or the pituitary gland (2, 12). However, none of these mechanisms have been proven, and the mechanism of sports-related head trauma-induced pituitary dysfunction is still unclear. A possible role of autoimmunity has been suggested by studies conducted in animals, which demonstrated naturally occurring IgG autoantibodies against dying neurons in the injured brain of adult rats following a cortical lesion (13) and autoreactive antibodies against neurons and basal lamina in the serum of rats submitted to experimental TBI (14). Supporting these experimental findings, a significant association between antipituitary antibody (APA) positivity and hypopituitarism 3 years after TBI has been clearly demonstrated in a very recent clinical study (15).

Although the occurrence of APAs in patients with TBI has been demonstrated, there is no study investigating the presence of antihypothalamus antibodies (AHAs) and/or APAs in athletes exposed to chronic repetitive head trauma. In order to investigate whether autoimmune mechanisms could play a role in the pituitary dysfunction due to sports-related head trauma, we have planned this study with the aim of investigating the presence of APAs and AHAs in active or retired amateur boxers.

Subjects and methods

After obtaining permission from the Turkish Boxing Federation, we approached all amateur, elite boxers on the Turkish National Boxing Team. We included 61 actively competing ($n=44$) or retired ($n=17$) male boxers (mean age, 26 years; range, 17–53 years). Retired boxers were official trainers and were not actively boxing. The ethics committee of Erciyes University Medical School, Kayseri, Turkey, approved this study, and we obtained informed consent from each participant. None of the boxers reported any comorbid conditions or previous pituitary disorders, and none were currently taking any medications.

Assessment of pituitary function by basal hormone levels and dynamic tests

The data regarding pituitary functions of these subjects were published recently (11).

Gonadotropin (FSH/LH) deficiency was defined by both basal total and free testosterone levels below the normal range (total testosterone <134 ng/dl and free testosterone <11.5 ng/ml) in the presence of normal or low values of gonadotropins (16, 17). TSH deficiency was defined by low serum free thyroxine level (<7.7 pg/ml) without appropriate elevation in serum TSH (16, 17).

To assess GH–insulin-like growth factor 1 (IGF1) axis, GH-releasing hormone (GHRH)+GHRP-6 test and glucagon stimulation test were used, and to assess ACTH

deficiency, glucagon stimulation test was performed as described previously. The details of the tests and the cutoff values were published recently (10, 11).

Analytic methods of hormonal parameters

Serum GH levels were measured using IRMA with a commercial kit (DSL, Webster, TX, USA); intra-assay and inter-assay coefficients of variation (CV) were 3.1 and 5.9% respectively. The minimum detection limit was 0.01 g/l, and GH standards were calibrated according to the WHO reference standard 88/624. IGF1 level was measured by IRMA after formic acid–ethanol extraction (DSL); intra-assay and inter-assay CV values were 3.4 and 8.2% respectively.

All the other serum hormones were measured using RIA, IRMA, or chemiluminescent methods with the commercial kits.

Immunological evaluation

In 61 boxers and in 60 male age-similar normal controls (mean age, 25 years; range, 18–50 years), AHAs and APAs were investigated. In particular, AHAs were detected by an indirect immunofluorescence method on a cryostat section of a young baboon hypothalamus supplied by Biosystem, Italia, SRL (SanMartino, Buon Albergo VR, Italy), as described previously (18).

APAs were investigated by an indirect immunofluorescence method on a cryostat section of a young baboon pituitary gland supplied by Biosystem as described previously (19). The collaborators performing the immunological evaluation were blinded to the pituitary deficiency status of the boxers.

The control group was recruited from healthy volunteers from Turkey who had no traffic accident history, no combative sports history, and no previous hospitalization history due to head trauma. Additionally, if the controls declared any kind of simple head trauma including during childhood, they were not included in the control group.

Statistical analysis

Statistical analysis was performed using the SPSS 10.0 package for Windows (Chicago, IL, USA). All data were subjected to the Kolmogorov–Smirnov test for normality. The differences between the groups were compared by unpaired t -test, and the two groups that were not normally distributed were compared by the Mann–Whitney U test. The categorical data were shown as percentages, and compared with χ^2 test. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated. $P<0.05$ was considered statistically significant. In addition, Pearson's or Spearman's correlation analysis was performed to determine whether significant correlations existed between the chosen variables.

Results

The behavior of AHAs in all boxers and normal controls is depicted in Fig. 1A, whereas immunostaining of AHA-positive and AHA-negative sera is depicted in Fig. 1B. AHAs were detected in 13 of 61 boxers (21.3%), but in none of the normal controls. Among the 13 AHA-positive patients, 4 (30.7%) were weakly positive (titer=1/8), while 9 (69.3%) were strongly positive (titer ranging from 1/16 to 1/64). The behavior of APAs in all boxers and normal controls is depicted in Fig. 2A, whereas immunostaining of APA-positive and APA-negative sera is depicted in Fig. 2B. APAs were detected in 14 of 61 boxers (22.9%), but in none of the normal controls. Among the 14 APA-positive patients, 6 (42.8%) were weakly positive, while 8 (57.2%) were strongly positive.

Of the 61 boxers, 11 (18.3%) had hypopituitarism. Of the 44 active boxers, 3 (7%) had hypopituitarism, whereas 8 of 17 (47%) retired boxers had hypopituitarism. Baseline characteristics, deficient pituitary hormones, and individual AHA and APA titers in boxers with hypopituitarism are given in Table 1. The boxers with pituitary dysfunction were not on hormone replacement therapy during the study period. Among the 11 boxers with hypopituitarism, 6 boxers (54.5%) had AHA positivity and 3 boxers (27.3%) had APA positivity. All the AHA and APA titers in boxers with hypopituitarism were strongly positive (titer ranging from 1/16 to 1/64; Table 1). Additionally, when mean AHA and APA titers were compared between boxers with and without hypopituitarism, both AHA ($P<0.027$) and APA titers ($P<0.006$) were significantly higher in boxers with hypopituitarism.

Figure 1 (A) Antihypothalamus antibodies (AHAs) in boxers and in 60 normal controls. ●, boxers with hypopituitarism; ○, boxers without hypopituitarism. (B) Serum sample positive for antibodies to hypothalamic cells detected by immunofluorescence in a boxer (a) in comparison with a negative control serum (b).

Figure 2 (A) Antipituitary antibodies (APAs) in boxers and in 60 normal controls. ●, boxers with hypopituitarism; ○, boxers without hypopituitarism. (B) Serum sample positive for antibodies to pituitary cells detected by immunofluorescence in a boxer (a) in comparison with a negative control serum (b).

The comparison of the chronic repetitive head trauma-induced pituitary dysfunction development between AHA-positive and AHA-negative boxers is summarized in Table 2. Pituitary dysfunction was significantly higher in AHA-positive boxers (46.2%) than in AHA-negative boxers (10.4%) ($P=0.003$). The corresponding OR was 7.37 (95% CI 1.8–30.8), showing that the relative chance of having pituitary dysfunction after boxing increased 7.37-fold when the subject had positive AHAs. However, there was no significant difference in pituitary dysfunction development between APA-positive and APA-negative boxers (Table 2).

When we performed correlation analysis of AHA and APA titers versus baseline characteristics and hormonal parameters in 61 boxers (17 retired and 44 active boxers), there was no significant correlation (data not shown). In the subgroup analysis which included retired boxers, there was a significant negative correlation ($r = -0.537$, $P = 0.026$) between AHA titer ratio (higher titer ratio means low AHA titer) and retirement age, showing that high AHA titers are associated with high retirement age. Additionally, there was a significant positive correlation ($r = 0.632$, $P = 0.006$) between AHA titer ratio and peak GH response to GHRH + GHRP-6 test, showing that high AHA titers are associated with low GH response to GHRH + GHRP-6 test in retired boxers.

Discussion

This study demonstrates for the first time the presence of AHAs and APAs in active or retired amateur boxers who were exposed to sports-related head trauma. Moreover, the present investigation provides preliminary evidence

Table 1 Baseline characteristics of boxers with hypopituitarism and individual antihypothalamus antibodies (AHA) and antipituitary antibodies (APA) titers.

No.	Age (years)	Boxing status	Boxing duration (years)	Retirement age (years) ^a	Number of bouts ^b	Deficient pituitary hormones	AHA titer	APA titer
1	20	Active boxer	7	—	1080	ACTH	1/16	Absent
2	19	Active boxer	3	—	330	ACTH, GH	1/64	Absent
3	21	Active boxer	10	—	1000	ACTH	Absent	Absent
4	44	Retired boxer	10	28	1000	ACTH, GH	Absent	1/64
5	47	Retired boxer	14	28	2240	ACTH, GH	1/32	1/64
6	44	Retired boxer	12	30	2400	GH	1/64	Absent
7	49	Retired boxer	12	27	960	GH	Absent	Absent
8	50	Retired boxer	11	26	1100	GH	1/32	Absent
9	40	Retired boxer	9	25	1800	GH	Absent	Absent
10	37	Retired boxer	8	25	800	GH	Absent	1/64
11	42	Retired boxer	11	28	880	GH	1/32	Absent

Retired boxers were official trainers and were not actively boxing.

^aMean years since retirement in 17 retired boxers was 16 years (range, 8–28 years).

^bTotal number of championships and training fights throughout their career.

that AHAs are associated with the development of pituitary dysfunction in boxers.

After TBI, a substantial number of patients have been reported to have some degrees of pituitary dysfunction, and isolated pituitary hormone deficiencies are more frequent than multiple hormone deficiencies (1–3, 20). Several possible mechanisms have been suggested for hypothalamic–pituitary dysfunction due to TBI, including hypoxic insult or direct mechanical injury to the hypothalamus, pituitary stalk, or the pituitary gland; compression from hemorrhage, edema, or increased intracranial pressure; and vascular injury to the hypothalamus or the pituitary gland (2, 12). In an elegant histopathological study, Salehi *et al.* have investigated the pituitary specimens of 42 TBI patients who died within 1 week of a motor vehicle accident. They have demonstrated acute pituitary infarcts of varying sizes in nearly half of the patients, clearly suggesting that in the acute phase of TBI, vascular damage is the most likely explanation for the early pituitary abnormalities (21). A possible role of autoimmune process involving the hypothalamic–pituitary region triggered by head trauma has been suggested by

studies conducted in animals. In an experimental study, naturally occurring IgG autoantibodies against dying neurons in the injured brain have been detected in adult rats following a cortical lesion, and it has been proposed that autoantibody binding may be involved in the phagocytosis and removal of the injured neurons (13). Furthermore, autoreactive antibodies against neurons and basal lamina have been found in serum following experimental TBI in rats. The authors concluded that the presence of autoreactive antibodies against neurons and basal lamina after TBI could play a pathogenic role in the delayed neuron degeneration (14).

In human studies, the presence of APAs and AHAs has been shown in patients with Sheehan's syndrome even many years after the onset of hypopituitarism. The authors concluded that an autoimmune process involving both the hypothalamus and pituitary gland may contribute to late pituitary dysfunction (22). Moreover, APAs can be present not only in patients with suspected autoimmune pituitary diseases, but also in those with pituitary diseases secondary to other non-autoimmune-specific causes, such as pituitary adenoma, and in some healthy subjects. However, in these cases, APAs are usually present at low titers (<1:8), and they can be considered negative; on the contrary, in the sera of patients with lymphocytic hypophysitis, only when they are present at a high titer (>1:8) are they considered positive, as has been observed already for other autoantibodies (23). Although the nature and the clinical significance of these autoantibodies are still controversial, they are considered as markers of pituitary impairment when they are detected at a high titer and are undetectable (<1:8) in healthy control subjects (19, 24). Apart from childhood GH deficiency, APAs have been associated with adulthood GH deficiency with or without related autoimmune diseases, and with autoimmune thyroiditis with normal pituitary function (19, 25–27). However, the association of high titers of APAs (>1:8)

Table 2 Comparison of pituitary dysfunction development between AHA-positive and AHA-negative boxers and between APA-positive and APA-negative boxers.

Pituitary dysfunction (PD)	AHA-positive boxers (n=13)	AHA-negative boxers (n=48)
AHA-positive and AHA-negative boxers		
With PD	6 (46.2%) ^a	5 (10.4%)
Without PD	7 (53.8%)	43 (89.6%)
APA-positive and APA-negative boxers		
With PD	3 (21.4%)	8 (17.0%)
Without PD	11 (78.6%)	39 (83.0%)

AHA, antihypothalamus antibodies; APA, antipituitary antibodies
 $\chi^2=0.14$ and $P=0.707$; Data are given as a number and as a percentage of each category.

^a $\chi^2=8.84$ and $P=0.003$.

with GH deficiency and inverse correlation between titer of antibodies and response of GH to insulin tolerance test were observed previously (19). Recently, APAs, specifically against GH-producing cells, were found in 30% of children with idiopathic GH deficiency, prompting the authors to confirm that GH deficiency may be caused by an autoimmune process involving the pituitary gland (26). The first clinical study demonstrating the presence of APAs in TBI patients 3 years after head trauma was published by our collaborative group (15). Twenty-nine patients with TBI, mainly due to road traffic accidents, were included in that study, and pituitary dysfunction development ratio was significantly higher in APA-positive patients (46.2%) than in APA-negative patients (12.5%) ($P=0.04$). There was a significant association between APA positivity and hypopituitarism due to TBI (OR: 2.25, 95% CI 1.1–4.6) (15). We re-analyzed and evaluated the association between AHA positivity and hypopituitarism in these 29 TBI patients, but could not find any significant association ($P=0.9$; unpublished data).

The pattern of head trauma in sports-related TBI, boxing and kickboxing in particular, is characterized by chronic repetitive head trauma with low intensity. In a current study conducted in our clinic, investigation of pituitary function in amateur national boxers revealed that the retired boxers had a high rate of pituitary dysfunction (11). While the emerging evidence clearly demonstrates that athletes dealing with contact sports have a substantial risk for pituitary dysfunction, the mechanism of sports-related head trauma-induced pituitary dysfunction is unclear. In the present study, the first important point is the presence of AHAs (21.3%) and APAs (22.9%) in amateur boxers. But the only significant association was present between AHA positivity and hypopituitarism due to boxing ($P=0.003$). Pituitary dysfunction development was 7.37-fold higher (95% CI 1.8–30.8) in AHA-positive boxers than in AHA-negative boxers. The second important point in this study is the significantly higher rate of pituitary dysfunction in retired boxers, and AHA positivity was also significantly higher (29.4%) in retired boxers than in active boxers (18.1%) ($P<0.05$). Although the retired boxers had not been exposed to sports-related head trauma for nearly 16 years, six of eight retired boxers with hypopituitarism had high titers of APAs and/or AHAs (Table 1). Moreover, indicating the importance of chronic exposure to the head trauma, correlation analysis in retired boxers revealed that high AHA titers are significantly associated with high retirement age, and that high AHA titers are associated with low GH reserve in retired boxers. The present results suggest that the activation of hypothalamic–pituitary autoimmunity, as evidenced by the presence of AHAs at high titers, may have an impact on the development of pituitary dysfunction after sports-related chronic repetitive head trauma. The possible mechanisms of the activation of

the hypothalamic–pituitary autoimmunity after TBI or boxing remain to be clarified. Generally, in patients after moderate and severe TBI, necrotic, ischemic, and hypoxic changes could be present not only at the pituitary level but also at the hypothalamic level (2, 28); GH-GHRH and corticotropin-releasing hormone (CRH) neurons could be highly vulnerable to injury in these cases (28, 29). With this in mind, it is tempting to speculate that head trauma may trigger an ongoing cascade of vascular and histopathological alterations involving mediators of inflammatory process, thus favoring the immune system activation which can contribute to late pituitary dysfunction. It is important to emphasize that there may not be a unifying hypothesis that early pituitary hormone deficits after TBI or sports-related head trauma may be due to vascular injury and late pituitary hormone deficits may be immune mediated. In the present study, 3 of the 11 boxers with hypopituitarism had neither APA nor AHA positivity. Additionally, previous 1-year and 3-year prospective studies showed that in some patients pituitary function may improve over time after TBI (1, 20). In fact, it has been clearly demonstrated that during the natural course of lymphocytic hypophysitis, APAs could sometimes spontaneously disappear and spontaneous partial or total pituitary recovery and/or mass resolution can occur (24, 30). Prospective screening of the antibody-negative boxers may reveal whether they recover or not. In particular, generally in patients with TBI, as a result of the primary injury, brain edema and circulatory disturbance may occur (31–33). Inflammatory mediators (cytokines, in particular interleukin 6, free radicals, amino acids, and nitric oxide) may lead to the acceleration of neuronal cell necrosis (32, 34). The infundibular hypothalamic–pituitary structure due to its peculiar anatomical and vascular characteristics may be very vulnerable to these necrotic, ischemic, and hypoxic changes that are present after TBI (29). Therefore, the release of sequestered pituitary or hypothalamic antigens from necrotic hypothalamic–pituitary system after TBI or sports-related head trauma may trigger an autoimmune response, and could lead to late post-traumatic hypopituitarism. Interestingly, while there was a significant association between APA positivity and hypopituitarism in TBI patients (15), only significant association was present between AHA positivity and hypopituitarism in boxers. An interesting point emerging from our data is the high prevalence of GH and/or ACTH deficiency and the absence of diabetes insipidus in all AHA-positive patients. This seems to indicate that these antibodies may be directed toward GHRH- and CRH-secreting cells more than toward AVP-secreting cells. However, an appropriate study using a double immunofluorescence method is needed to clarify which cells are the targets of these antibodies at hypothalamic level, and such a study is in progress.

The cross-sectional design of the present study is the most important limitation. To understand whether

AHAs are good clinical markers for the development of pituitary dysfunction, a long-term prospective investigation is necessary, especially in AHA-positive boxers with normal pituitary function. Additionally, the characterization of the hypothalamic cells targeted by these antibodies, which is in progress, could strengthen the present findings. Moreover, due to the cross-sectional design of the study, we could conclude that the presence of AHAs may contribute to the development of hypopituitarism in boxers, but at present, it is difficult to claim that this relationship is causal.

In conclusion, the presence of AHAs and APAs was demonstrated in active or retired amateur boxers who were exposed to sports-related head trauma. In contrast to previous findings in TBI patients, only positivity for AHAs was associated with hypopituitarism in boxers, implying that pattern and type of the head trauma may have an impact on the level of the hypothalamic-pituitary injury. Therefore, this preliminary study, showing the presence of these antibodies at high titers in some boxers with impairment of pituitary function, suggests that autoimmunity may contribute to the development of sports-related head trauma-induced hypopituitarism. However, longitudinal studies with a high number of boxers are needed to investigate the possible disappearance of APAs over time in those with pituitary dysfunction, and to clarify the predictive value of AHAs as markers of pituitary dysfunction and for the possible clinical implications. The presence of the possible association between autoimmunity and boxing-induced hypopituitarism may provide a new point of view in this field, and promote further clinical and experimental studies.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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References

- 1 Aimaretti G, Ambrosio MR, Di Somma C, Gasperi M, Cannavo S, Scaroni C, Fusco A, Del Monte P, De Menis E, Faustini-Fustini M, Grimaldi F, Logoluso F, Razzore P, Rovere S, Benvenga S, Uberti EC, De Marinis L, Lombardi G, Mantero F, Martino E, Giordano G & Ghigo E. Residual pituitary function after brain injury-induced hypopituitarism: a prospective 12-month study. *Journal of Clinical Endocrinology and Metabolism* 2005 **90** 6085–6092.
- 2 Kelly DF, Gonzalo IT, Cohan P, Berman N, Swerdloff R & Wang C. Hypopituitarism following traumatic brain injury and aneurysmal subarachnoid hemorrhage: a preliminary report. *Journal of Neurosurgery* 2000 **93** 743–752.
- 3 Tanriverdi F, Senyurek H, Unluhizarci K, Selcuklu A, Casanueva FF & Kelestimur F. High risk of hypopituitarism after traumatic brain injury: a prospective investigation of anterior pituitary function in the acute phase and 12 months after trauma. *Journal of Clinical Endocrinology and Metabolism* 2006 **91** 2105–2111.
- 4 Annegers JF, Grabow JD, Groover RV, Laws ER, Elveback LR & Kurland LT. Seizures after head trauma: a population study. *Neurology* 1980 **30** 683–689.
- 5 Hillier SL, Hiller JE & Metzger J. Epidemiology of traumatic brain injury in South Australia. *Brain Injury* 1997 **11** 649–659.
- 6 Servadei F, Verlicchi A, Soldano F, Zanotti B & Piffer S. Descriptive epidemiology of head injury in Romagna and Trentino. Comparison between two geographically different Italian regions. *Neuroendocrinology* 2002 **21** 297–304.
- 7 Powell JW. Cerebral concussion: causes, effects, and risks in sports. *Journal of Athletic Training* 2001 **36** 307–311.
- 8 Zazryn TR, Finch CF & McCrory P. A 16 year study of injuries to professional kickboxers in the state of Victoria, Australia. *British Journal of Sports Medicine* 2003 **37** 448–451.
- 9 Kelestimur F, Tanriverdi F, Atmaca H, Unluhizarci K, Selcuklu A & Casanueva FF. Boxing as a sport activity associated with isolated GH deficiency. *Journal of Endocrinological Investigation* 2004 **27** RC28–RC32.
- 10 Tanriverdi F, Unluhizarci K, Coksevim B, Selcuklu A, Casanueva FF & Kelestimur F. Kickboxing sport as a new cause of traumatic brain injury-mediated hypopituitarism. *Clinical Endocrinology* 2007 **66** 360–366.
- 11 Tanriverdi F, Unluhizarci K, Kocyigit I, Tuna IS, Selcuklu A, Casanueva FF & Kelestimur F. Brief communication: pituitary volume and function in competing and retired male boxers. *Annals of Internal Medicine* 2008 **148** 827–831.
- 12 Yuan XQ & Wade CE. Neuroendocrine abnormalities in patients with traumatic brain injury. *Frontiers in Neuroendocrinology* 1991 **12** 209–230.
- 13 Stein TD, Fedynyshyn JP & Kalil RE. Circulating autoantibodies recognize and bind dying neurons following injury to the brain. *Journal of Neuropathology and Experimental Neurology* 2002 **61** 1100–1108.
- 14 Rudehill S, Muhallab S & Wennersten A. Autoreactive antibodies against neurons and basal lamina found in serum following experimental brain contusion in rats. *Acta Neurochirurgica (Wein)* 2006 **148** 199–205.
- 15 Tanriverdi F, De Bellis A, Bizzarro A, Sinisi AA, Bellastella G, Pane E, Bellastella A, Unluhizarci K, Selcuklu A, Casanueva FF & Kelestimur F. Antipituitary antibodies after traumatic brain injury: is head trauma-induced pituitary dysfunction associated with autoimmunity? *European Journal of Endocrinology* 2008 **159** 7–13.
- 16 Burke CW. The pituitary megatest: outdated? *Clinical Endocrinology* 1992 **36** 133–134.
- 17 Lamberts SW, de Herder WW & van der Lely AJ. Pituitary insufficiency. *Lancet* 1998 **352** 127–134.
- 18 De Bellis A, Colao A & Di Salle F. A longitudinal study of vasopressin cell antibodies, posterior pituitary function, and magnetic resonance imaging evaluations in subclinical autoimmune central diabetes insipidus. *Journal of Clinical Endocrinology and Metabolism* 1999 **84** 3047–3051.
- 19 De Bellis A, Bizzarro A & Conte M. Antipituitary antibodies in adults with apparently idiopathic growth hormone deficiency and in adults with autoimmune endocrine diseases. *Journal of Clinical Endocrinology and Metabolism* 2003 **88** 650–654.
- 20 Tanriverdi F, Ulutanaca H, Unluhizarci K, Selcuklu A, Casanueva FF & Kelestimur F. Three years prospective investigation of anterior pituitary function after traumatic brain injury: a pilot study. *Clinical Endocrinology* 2007 **68** 573–579.

- 21 Salehi F, Kovacs K, Scheithauer BW, Pfeifer EA & Cusimano M. Histologic study of the human pituitary gland in acute traumatic brain injury. *Brain Injury* 2007 **21** 651–656.
- 22 De Bellis A, Kelestimur F, Sinisi AA, Bellastella G, Tanriverdi F, Unluhizarci K, Bizzarro A & Bellastella A. Anti-hypothalamus and anti-pituitary antibodies may contribute to perpetuate the hypopituitarism in patients with Sheehan's syndrome. *European Journal of Endocrinology* 2008 **158** 147–152.
- 23 Falorni A, Laureti S & De Bellis A. Italian Addison network study: update of diagnostic criteria for the etiological classification of primary adrenal insufficiency. *Journal of Clinical Endocrinology and Metabolism* 2004 **89** 1598–1604.
- 24 De Bellis A, Bizzarro A & Bellastella A. Pituitary antibodies and lymphocytic hypophysitis. *Best Practice & Research. Clinical Endocrinology & Metabolism* 2005 **19** 67–84.
- 25 Bensing S, Fetissov SO, Mulder J, Perheentupa J, Gustafsson J, Husebye ES, Oscarson M, Ekwall O, Crock PA, Hokfelt T, Hulting AL & Kampe O. Pituitary autoantibodies in autoimmune polyendocrine syndrome type 1. *PNAS* 2007 **104** 949–954.
- 26 De Bellis A, Salerno M, Conte M, Coronella C, Tirelli G, Battaglia M, Bellastella G, Bizzarro A & Bellastella A. Antipituitary antibodies recognizing growth hormone (GH)-producing cells in children with idiopathic GH deficiency and in children with idiopathic short stature. *Journal of Clinical Endocrinology and Metabolism* 2006 **91** 2484–2489.
- 27 Manetti L, Lupi I, Morselli LL, Albertini S, Cosottini M, Bogazzi F, Bartalena L & Martino E. Prevalence and functional significance of antipituitary antibodies in patients with autoimmune and non-autoimmune thyroid diseases. *Journal of Clinical Endocrinology and Metabolism* 2007 **92** 2176–2181.
- 28 Ferriero DM. Neonatal brain injury. *New England Journal of Medicine* 2004 **351** 1985–1995.
- 29 Popovic V. GH deficiency as the most common pituitary defect after TBI: clinical implications. *Pituitary* 2005 **8** 239–243.
- 30 Caturegli P, Newschaffer C, Olivi A, Pomper MG, Burger PC & Rose NR. Autoimmune hypophysitis. *Endocrine Reviews* 2005 **26** 599–614.
- 31 Bondanelli M, Ambrosio MR, Zatelli MC, De Marinis L & degli Uberti EC. Hypopituitarism after traumatic brain injury. *European Journal of Endocrinology* 2005 **152** 679–691.
- 32 Gaetz M. The neurophysiology of brain injury. *Clinical Neurophysiology* 2004 **115** 4–18.
- 33 Greenwald BD, Burnett DM & Miller MA. Congenital and acquired brain injury. 1. Brain injury: epidemiology and pathophysiology. *Archives of Physical Medicine and Rehabilitation* 2003 **84** 3–7.
- 34 Gionis D, Ilias I, Moustaki M, Koutras DA & Mastorakos G. Hypothalamic–pituitary–adrenal axis and interleukin-6 activity in children with head trauma and syndrome of inappropriate secretion of antidiuretic hormone. *Journal of Pediatric Endocrinology and Metabolism* 2003 **16** 49–54.

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