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Cerebrospinal Fluid Hypocretin 1 Deficiency, Overweight, and Metabolic Dysregulation in Patients with Narcolepsy

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SCIENTIFIC INVESTIGATIONS

Study Objectives: The possible relationship between cerebrospinal fluid (CSF) hypocretin and leptin levels, overweight, and association to risk factors for diabetes 2 in narcolepsy with cataplexy were compared to patients with idiopathic hypersomnia and controls.

Patients: 26 patients with narcolepsy, cataplexy, and hypocretin deficiency; 23 patients with narcolepsy, cataplexy, and normal hypocretin values; 11 patients with idiopathic hypersomnia; and 43 controls.

Measurements and Results: Body mass index (BMI), serum leptin, and HbA1C were measured in patients and controls; and CSF hypocretin 1 and leptin measured in all patients. Female and male patients with narcolepsy and hypocretin deficiency had the highest mean BMI (27.8 and 26.2, respectively), not statistically different from patients with narcolepsy and normal hypocretin or controls, but statistically higher than the patients with idiopathic hypersomnia ($p < 0.001$ and 0.011, respectively). The number of obese patients (BMI > 30) was increased in both narcolepsy groups. Serum and CSF leptin levels correlated positively to BMI in patients and controls, but not to CSF hypocretin concentrations. HbA1C was within normal levels and similar in all groups.

Conclusions: The study confirms a moderate tendency to obesity (BMI > 30) and overweight in patients with narcolepsy and cataplexy. Obesity was not correlated to hypocretin deficiency or reduced serum or CSF leptin concentrations. We suggest that overweight and possible metabolic changes previously reported in narcolepsy, may be caused by other mechanisms.

Keywords: Narcolepsy, hypocretin, overweight, leptin, body mass index (BMI), metabolism.

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BRIEF SUMMARY

Current Knowledge/Study Rationale: Several studies of patients with narcolepsy have reported increased BMI , which has been tentatively attributed to an effect of hypocretin-1 deficiency. To clarify whether increased BMI in narcolepsy is related to hypocretin-1 deficiency, this study compares BMI and leptin values of narcolepsy/cataplexy patients with normal CSF hypocretin-1 concentration to patients with low hypocretin values.

Study Impact: The study concludes with a moderate tendency to overweight and obesity in narcolepsy with cataplexy which is independent of CSF hypocretin levels, but may be connected to the narcoleptic condition by other mechanisms.

Narcolepsy is a lifelong disorder which usually starts between the age of 10 and 30 years, disturbing the regulation of sleep and wakefulness. The disease affects approximately 0.02% to 0.05% of the European population.[1-4] It is characterized by sleep attacks and excessive daytime sleepiness, cataplexy (attacks of muscle weakness/paralysis caused by laughter or sudden emotions), sleep paralysis, and hypnagogic hallucinations, as well as reduced sleep quality and other accessory symptoms. The diagnostic criteria were specified and revised in 2005 and include narcolepsy with cataplexy, narcolepsy without cataplexy, and narcolepsy secondary to medical conditions.[5]

Hypocretin is produced in the lateral parafornical area of hypothalamus and has properties as neuroexcitatory transmitters promoting wakefulness and motor activity.[6,7] Studies have demonstrated very low or unmeasurable CSF hypocretin in 70% to 90% of patients with narcolepsy with typical cataplexy and loss of hypocretin producing neurons.[8-12] In rodents, hypocretins have been suggested to stimulate food seeking behavior, indicating possible participation in complex homeostatic mechanisms regulated via hypothalamus, in addition to their major effect on wakefulness.[13,14]

Several studies have reported increased BMI and increased risk of type 2 diabetes in patients with narcolepsy, but the underlying pathophysiological mechanisms are still unclear.[15-19] Based on animal experiments and clinical reports, a possible association between food intake, energy balance, and the functions of hypocretin has been advocated.[13-15,19-21]

Previous studies have not compared the tendency to overweight in patients with narcolepsy and hypocretin deficiency to patients with narcolepsy and normal CSF hypocretin levels, to clarify the possible role of hypocretin deficiency in the development of obesity in narcolepsy. The present exploratory study was initiated to elucidate the possible association between overweight, metabolic parameters, and CSF hypocretin deficiency in a population of well characterized Norwegian patients with narcolepsy with and without hypocretin deficiency, compared to patients with idiopathic hypersomnia and controls.

MATERIALS AND METHODS

Patients

We used serum and CSF from a well-defined previously described cohort of patients.[8] Sixty-seven patients were eligible for the present study. All patients had been examined and interviewed by an experienced neurologist and diagnosed according to international diagnostic criteria.[5] The patients with narcolepsy also had an additional interview, with special emphasis on a detailed description of possible cataplexy. Polysomnography, MSLT, and typing for HLADQB1*0602 were performed in all patients. CSF hypocretin 1 levels were measured by radioimmunoassay (RIA) of extracted CSF, using a polyclonal antibody (Phoenix Pharmaceutical, St Joseph, MO, USA) as described previously.[8] CSF hypocretin values were defined as low when < 135 pg/mL, (one-third of the mean value of the controls), as specified in international diagnostic criteria.[5]

The patients were divided in 3 diagnostic categories:

1. **Narcolepsy with cataplexy and low CSF hypocretin** (26 patients). All had typical cataplexy and were HLADQB1*0602 positive. Mean CSF hypocretin concentration was 85 pg/mL, range 0-135 pg/mL.
2. **Narcolepsy with cataplexy and normal CSF hypocretin values** (23 patients). They all had ≥ 2 SOREM and mean sleep latency < 5 min on MSLT. They also had cataplexy, but the cataplectic attacks were generally less frequent and involved fewer muscle groups than in the patients with low CSF hypocretin. Seventy-five percent were HLADQB1*0602 positive.[8] Mean CSF hypocretin concentration was 460 pg/mL, range 221-595 pg/mL.
3. **Idiopathic hypersomnia** (11 patients). Thirty-six percent were HLADQB1*0602 positive. Mean CSF hypocretin concentration was 487 pg/mL, range 393-613 pg/mL. They had mean sleep latencies < 5 min on MSLT, but no SOREM periods.

Patients with narcolepsy without cataplexy were not included in the study, as the number was too small for statistical evaluation.

Controls

Forty-three age- and sex-matched healthy controls were recruited consecutively among blood donors in the blood bank of Oslo, for BMI measurement and plasma leptin analyses. Some unknown sampling bias in blood donors cannot be excluded, although they are supposed to represent the general population.

Leptin Analysis in Serum and CSF

Leptin was measured using a competitive radioimmunoassay (RIA) (Millipore Corp, Billerica, Ma, USA) employing ^{125}I-leptin as radioactive ligand, separating antibody from tracer by use of goat-anti-rabbit IgG and polyethylene glycol (PEG). The antibody against leptin showed no cross reaction with insulin, proinsulin, C-peptid, or glucagon. The lower sensitivity was 0.03 ng/L; the precision of the assays for intra- and interassay measurements were 3% and 10%, respectively. The samples were obtained between 09:00 and 12:00. The time of sampling was noted and related to the control material used in the Department for Medical Biochemistry, Oslo University Hospital, taking into account that the highest values of serum leptin are found from midnight until early in the morning.

HbA1C Measurements in Blood

HbA1C was measured in blood using a HPLC-based method according to the manufacturers, (Tosoh Corporation, Tokyo, Japan), utilizing 1 mL of whole blood. HbA1C was measured as a part of routine analysis carried out at the Department of Medical Biochemistry, Oslo University Hospital, Ullevaal. The normal range of HbA1C was from 4.0% to 6.0%. The intra- and interassay variation were 3.5% and 6%, respectively.

Statistics

SPSS version 18 was used to perform the statistical analysis. Nonparametric Mann-Whitney test was used to compare BMI and leptin values. For comparing numbers with obesity and overweight in each group, we used 2-sided χ^2 test. A significance level of 5% was used throughout.

Ethics

The study was approved by the Regional Committee of Medical Ethics, Health Region South East, in accordance with Norwegian laws and regulations, including informed verbal and written consent from patients and control persons.

RESULTS

Body Mass Index (BMI) (Table 1)

Mean BMI for patients and controls are shown in **Table 1**. Persons with BMI > 30 kg/m^2 are generally considered obese, while BMI between 25 and 29.9 kg/m^2 signifies overweight. Female and male patients with narcolepsy and CSF hypocretin deficiency had the highest mean BMI, but were not statistically different from narcolepsy patients with normal hypocretin values (**Figure 1**) or controls. However, all narcolepsy groups, irrespective of hypocretin values and gender, had a higher number of obese patients compared to controls, most marked in the females, reaching significance both for female patients with hypocretin deficiency and normal CSF hypocretin levels ($p = 0.003$ and 0.005, respectively). The number of obese patients among male patients with hypocretin deficiency was also statistically significantly higher than the controls ($p = 0.005$), but without statistically significant difference from male narcolepsy patients with normal hypocretin levels. The patients with idiopathic hypersomnia had lower mean BMI than the controls, but not reaching statistical significance ($p = 0.29$) with no obese and only 2 female patients being overweight.

Serum Leptin Concentrations

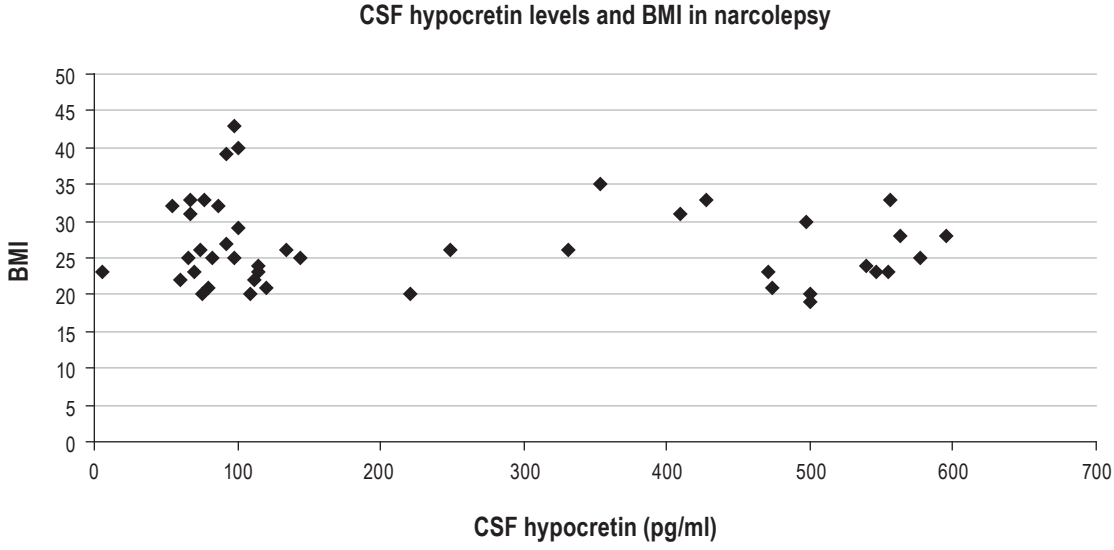
Serum leptin concentrations (**Figures 2A** and **B**) showed marked gender differences, with lower values in males, and an approximately 5 times higher increase per BMI unit in females than in males. This was also found in our controls and patients. Serum leptin and leptin/BMI ratios were therefore evaluated for each gender separately (**Table 1**). The moderately higher mean serum leptin in narcolepsy with low hypocretin values compared to patients with normal hypocretin values was not statistically significant ($p = 0.25$), but correlated to the higher

Table 1—Metabolic parameters in patients and controls

	No	Age ± SD	BMI ± SD	BMI 25-29.9 No (%)	BMI > 30 No (%)	Leptin (ng/mL)	Leptin/BMI ratio	HbA1C
Female								
NC. low hcrt	18	53 ± 4	27.8 ± 2.1	4 (28)	7 (39)	18.87 ± 7.69	0.68	5.6 ± 0.5
NC. norm. hcrt	13	45 ± 13	26.4 ± 3.5	4 (31)	4 (31)	15.88 ± 7.28	0.60	6.0 ± 0.3
IH	8	36 ± 11	22.1 ± 3.2	2 (25)	0	9.86 ± 4.37	0.44	5.7 ± 0.5
Controls	28	47 ± 14	24.6 ± 2.6	5 (21)	3 (10)	20.39 ± 4.47	0.83	5.9 ± 0.4
Male								
NC. low hcrt	8	51 ± 22	26.2 ± 2.8	4 (50)	2 (25)	4.98 ± 0.98	0.19	5.8 ± 0.2
NC. norm. hcrt	10	35 ± 8	24.7 ± 2.7	1 (10)	1 (10)	4.19 ± 2.73	0.16	5.7 ± 0.8
IH	3	41 ± 12	23.3 ± 0,5	0	0	3.42 ± 0.72	0.15	5.8 ± 0.4
Controls	15	53 ± 11	24.1 ± 0.6	5 (33)	0	4.06 ± 2.29	0.17	5.4 ± 0.4

Mean values ± SD for age, BMI, plasma leptin, and HbA1C ± SD are shown in female and male patients with narcolepsy, cataplexy and low CSF hypocretin values (NC. low hcrt), narcolepsy, cataplexy and normal hypocretin values (NC. norm. hcrt), idiopathic CNS hypersomnia (IH), and controls. Number of patients with overweight (BMI 25-29.9) and obesity (BMI ≥ 30) in each group is also shown.

Figure 1—BMI and CSF hypocretin values in 44 male and female patients with narcolepsy and cataplexy



Points above BMI 30 kg/m^2 represent obesity. There is no correlation between CSF hypocretin levels and BMI.

BMI. Mean leptin/BMI ratio was lower in both female narcolepsy groups compared to the controls (p = 0.44), and slightly higher in male narcolepsy patients with hypocretin deficiency, but it did not reach statistical significance. Patients with idiopathic hypersomia had lower serum leptin values and leptin/BMI ratios than controls, approaching statistical significance for serum leptin (p = 0.057) in female patients.

CSF Leptin Concentrations

The relationship between CSF and serum concentrations of leptin is shown in **Figures 3A** and **B**. The mean CSF/serum ratio was similar in all patient groups, with a mean ratio of 0.017, except in patients with leptin values > 25 ng/mL, who had a decreasing CSF/serum ratio. There was no difference in leptin CSF/serum ratio between hypocretin deficient patients and those with normal CSF hypocretin concentrations or idiopathic hypersomnia.

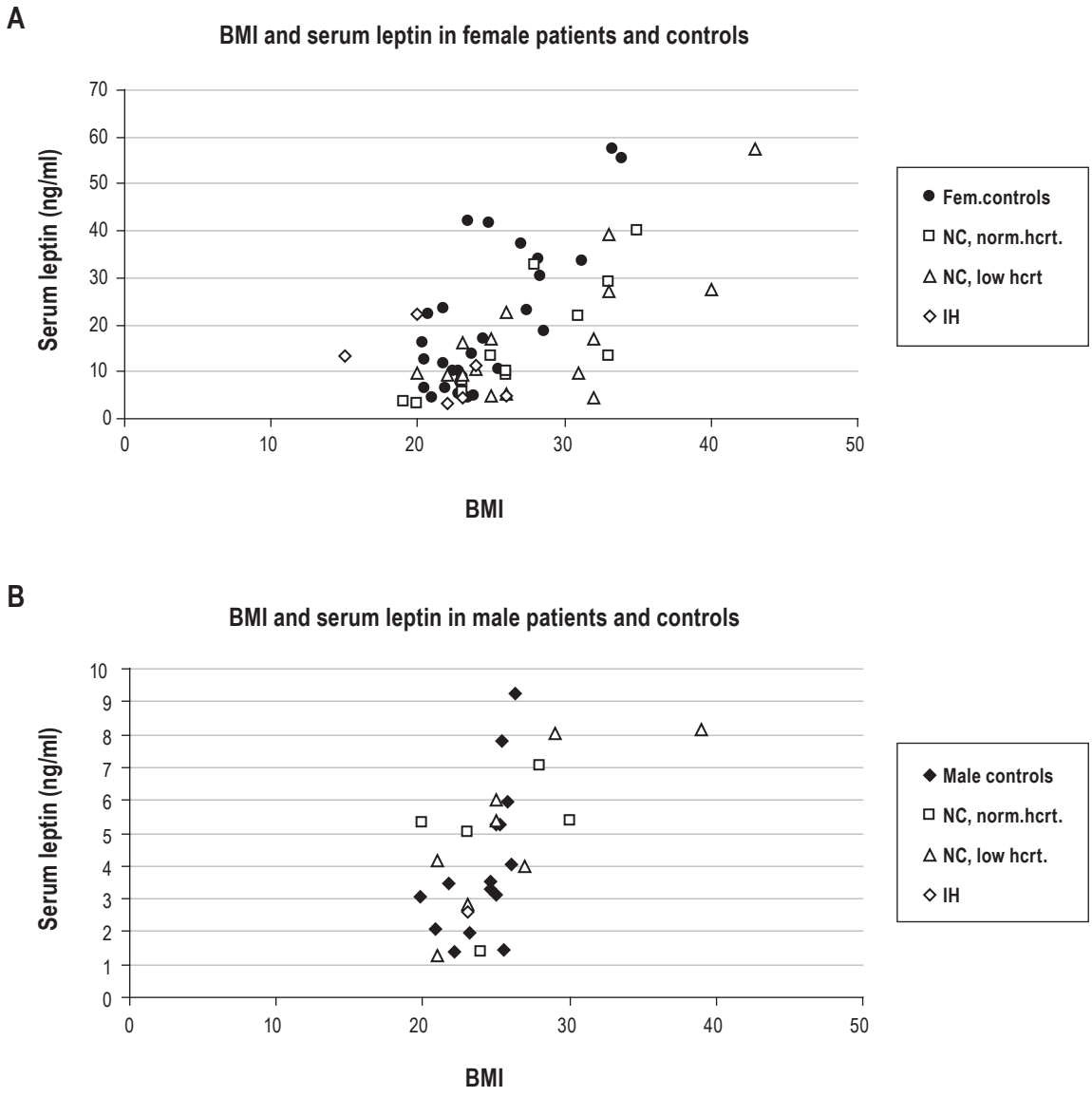
HbA1C

HbA1C concentrations were within normal limits in all patients and controls, without significant differences between the groups (**Table 1**).

DISCUSSION

The mean BMI in the patients with narcolepsy did not differ significantly from that of the controls, but a significantly higher number of patients with narcolepsy were obese compared to the controls. This may indicate that some patients with narcolepsy are particularly prone to develop obesity, although this may not be a general tendency in narcoleptic patients. Obesity was most prevalent in female patients with CSF hypocretin deficient narcolepsy, but an increased number with obesity was also found in the corresponding group of male patients and in female nar-

Figure 2



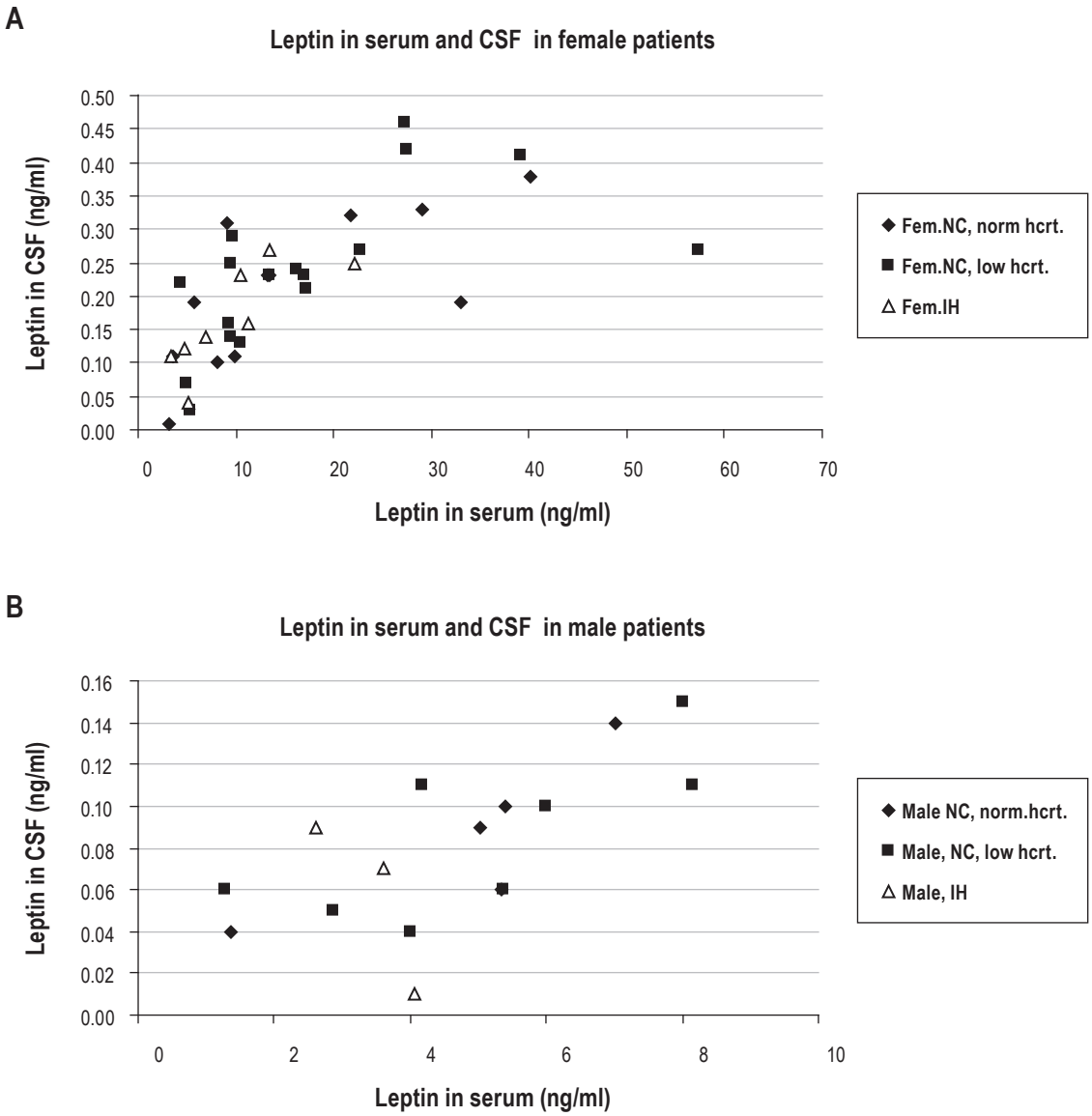
BMI and serum leptin in female **(A)** and male **(B)** patients with narcolepsy (NC) with low and normal CSF hypocretin (hcrt.) values and patients with idiopathic CNS hypersomnia (IH) and controls.

colepsy patients with normal hypocretin values. The prevalence of overweight in narcolepsy did not differ significantly from the controls.

The female patients with idiopathic hypersomnia had lower BMI than controls. The mean leptin/BMI ratio was also slightly lower than in the other groups. The male patients with idiopathic hypersomnia were too few for statistical evaluation, and the female patients were fewer and younger than the other patient groups, making the interpretation of these results uncertain; but the results are in accordance with observations in other studies.[19,22] Although total sleep time in our patients was not included in the study, both patients with narcolepsy and those with idiopathic hypersomnia suffered from hypersomnia as part of their disorder. Thus, our findings indicate a tendency to overweight and obesity in narcolepsy with cataplexy that is not correlated to hypersomnia in general or to CSF hypocretin deficiency, but may be connected to the narcoleptic condition by other mechanisms.

Leptin is produced in fatty tissues and has been shown to have an appetite-reducing effect, possibly affecting hypocretin-producing neurons.[23,24] Early studies reported reduced serum leptin in patients with narcolepsy,[25,26] but the results could not be confirmed in later studies of large patient populations.[22,27] It was also not confirmed in the present study, in which narcolepsy patients with normal and low CSF hypocretin concentrations had similar leptin/BMI ratios, not statistically different from the controls. Furthermore, there was no difference in the leptin concentrations reaching the CNS in patients with low and normal CSF hypocretin levels, as the CSF/serum ratio of leptin is the same in both narcolepsy groups. A possible reduced leptin

Figure 3



Leptin concentrations in serum and CSF in female **(A)** and male **(B)** patients with narcolepsy and cataplexy (NC) with normal and low CSF hypocretin (hcrt.) levels, and patients with idiopathic CNS hypersomnia (IH).

sensitivity due to defective hypocretin-producing neurons cannot explain obesity in narcolepsy, as the tendency to obesity was found both in hypocretin-deficient narcolepsy patients and patients with normal hypocretin values.

In the present study, mean HbA1C was within normal limits in patients and controls and did not reveal any increased risk of type 2 diabetes. In another recent study,[14] however, the results indicated the presence of metabolic syndrome in more than half of the patients with narcolepsy with cataplexy, in spite of lower daily food intake compared to patients with idiopathic hypersomnia. No patients with narcolepsy and normal hypocretin values were examined for comparison, so the possible role of hypocretin deficiency could not be determined. Other recent studies have indicated a tendency to mild eating disorders, mostly classified as EDNOS (eating disorder not otherwise specified), in patients with narcolepsy.[28,29] This could, however, not be confirmed in a larger study of 116 narcolepsy patients.[30] A possible relationship between eating disorders and overweight in narcolepsy therefore still remains unclear.

Other previous studies have indicated a relationship between short sleep duration and increased risk of diabetes 2, hypertension, and overweight.[31-33] Several metabolic and hormonal processes are altered during sleep and closely connected to the sleep-wake cycle. Although both idiopathic hypersomnia and narcolepsy are characterized by hypersomnia, narcolepsy is often characterized by fragmented sleep at night and daytime sleep attacks of short duration, whereas long sleep periods and increased amounts of deep NREM sleep are characteristic of idiopathic hypersomnia.[34,35] Fragmented and disrupted sleep in narcolepsy may possibly have an effect on body weight and

metabolic processes, but this was not evaluated in the present study, and may need further investigation.

In conclusion our findings indicate a tendency to overweight and obesity in narcolepsy with cataplexy, which is not correlated to CSF hypocretin deficiency or leptin levels, and not to hypersomnia in general, but may be connected to the narcoleptic condition by other mechanisms.

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