Relationship between cerebrospinal fluid concentrations of orexin A/hypocretin-1 and body composition in humans

Short title: CSF orexin A concentrations and body composition

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Abstract

The hypothalamic neuropeptide orexin A (hypocretin-1) is a key signal in sleep/wake regulation and promotes food intake. We investigated the relationship between cerebrospinal fluid orexin A concentrations and body composition in non-narcoleptic human subjects with a wide range of body weight to gain insight into the role of orexin A in human metabolism. We collected cerebrospinal fluid and blood samples and measured body composition by bioelectric impedance analysis in 36 subjects (16 women and 20 men) with body mass indices between 16.24 and 38.10 kg/m^2 and an age range of 19 to 80 years. Bivariate Pearson correlations and stepwise multiple regressions were calculated to determine associations between orexin A and body composition as well as biometric variables. Concentrations of orexin A in cerebrospinal fluid averaged 315.6 ± 6.0 pg/ml, were comparable between sexes ($p>0.15$) and unrelated to age ($p>0.66$); they appeared slightly reduced in overweight/obese compared to normal-weight subjects ($p=0.07$). Orexin A concentrations decreased with body weight ($r=-0.38$, $p=0.0229$) and fat-free mass ($r=-0.39$, $p=0.0173$) but were not linked to body fat mass ($p>0.24$). They were inversely related to total body water ($r=-0.39$, $p=0.0174$) as well as intracellular ($r=-0.41$, $p=0.0139$) and extracellular water ($r=-0.35$, $p=0.0341$). Intracellular water was the only factor independently associated with cerebrospinal fluid orexin A concentrations ($p=0.0139$). We conclude that cerebrospinal fluid orexin A concentrations do not display associations with body adiposity, but are inversely related to intracellular water content. These cross-sectional findings suggest a link between orexin A signaling and the regulation of water homeostasis in humans.

The neuropeptide orexin A (hypocretin-1) is mainly expressed by neurons in the lateral hypothalamus; it promotes wakefulness and stabilizes arousal (1,2), while also displaying orexigenic properties (3,4). Orexinergic neurons connect to a broad network of central nervous regions including the hypothalamic arcuate nucleus, a central hub of metabolic control where peptidergic messengers such as proopiomelanocortin and neuropeptide Y interact to regulate feeding behavior (5). Orexin A stimulates food anticipatory behavior and food intake, especially with regard to reward-driven eating (6,7). The abundance of glucose sensors and receptors for leptin and ghrelin in orexinergic neurons (8) further indicates that orexin is essential for adapting the level of alertness to metabolic needs (9). Vice versa, impairments in orexinergic signaling might help explain why insufficient and impaired sleep predisposes to increases in food intake (10) and elevated body weight (11; for review see reference 12). The contribution of orexin A to metabolic function extends to glucose homeostasis: high doses of orexin A administered to rats promote hepatic glucose release and increases in blood glucose (13,14). Respective studies in mice indicate that the peptide bidirectionally fine-tunes hepatic gluconeogenesis by regulating autonomic balance, and point towards a role of the orexin system in the regulation of circadian blood glucose oscillations (15).

Adipose tissue interacts with orexin A in metabolic control (16). Leptin and ghrelin inhibit and, respectively, enhance orexin A signaling in the brain (17,18). Orexin knock-out (KO) in comparison to wild-type (WT) mice display sex-specific body weight and body composition changes (19,20). Adult and aged female orexin KO mice have elevated body weight, a higher proportion of fat, muscle and free fluid, whereas male orexin KO mice do not differ from WT counterparts in body weight but carry more body fat (20). By affecting the accumulation of brown adipose tissue, orexin A indirectly determines metabolic rate and thermogenesis (2,21). In rats, intracerebroventricular administration of high doses of orexin A induces lipolysis via

histamine receptor-mediated effects on sympathetic activation, whereas low doses have opposing effects (22).

Most insights into the function of orexin A derive from animal studies, while the lion's share of what is known about orexin A signaling and body weight regulation in humans concerns specific clinical aspects. Orexin A is deficient in patients with narcolepsy with cataplexy (23), a neurological disorder characterized by impaired sleep/wake regulation leading to excessive daytime sleepiness, and sudden episodes of partial or total loss of muscle tone. These patients, and also respective animal models of orexin A deficiency (19,24), show a tendency towards overweight (25), even though food-seeking behavior appears to be attenuated (26,27). Interestingly, circulating leptin levels in narcolepsy have been reported to be comparable to (28) or lower (29,30) than those of healthy controls. In order to investigate the interplay between central nervous orexin A signaling and body weight regulation, we investigated the relationship between body composition and cerebrospinal fluid (CSF) concentrations of orexin A in a sample of non-narcoleptic participants with a wide range of body-mass index (BMI). Considering the stimulatory role of orexin A in food intake control, we expected to find indicators linking CSF concentrations of the peptide to body fat content.

Methods

Participants

Forty-one Caucasian subjects (21 men and 20 women) aged between 19 and 80 years (mean age ± SEM, 52.98 ± 2.29 years) were included in the study. They had a BMI range of 16.24 to 38.10 kg/m^2 and a mean BMI ± SEM of 26.68 ± 0.70 kg/m^2. Fifteen subjects were normal-weight and 26 subjects were overweight or obese (BMI ≥ 25). Exclusion criteria were an anamnestic history of diabetes, congestive heart failure, liver or kidney disease, malignancy, signs of

inflammation, pregnancy, and any drugs influencing body weight like corticoids, diuretics or contraceptives. Five subjects (one woman and four men) were newly diagnosed with type 2 diabetes due to fasting plasma glucose levels >7 mmol/l according to the criteria of the American Diabetes Association. Since their exclusion did not essentially alter the results, their data were included in the analyses. Three subjects were excluded from analyses because of CSF orexin A concentrations below 110 pg/ml, which are indicative of orexin A deficiency (31), and two further subjects because of outlying values below the group average minus two standard deviations. None of these or the remaining subjects reported narcoleptic symptoms (e.g., sleep disturbances, excessive daytime sleepiness, catalepsy), or worked in shifts. All participants gave written informed consent to the study that conformed to the Declaration of Helsinki and was approved by the local ethics committee.

Collection of blood and CSF samples and assessment of body composition

After an overnight fast with caffeine restriction but unlimited water supply, subjects reported to the lab in the morning between 07:00 and 09:00 for simultaneous sampling of blood and CSF (1 ml) via lumbar puncture after local anesthesia (2 ml mepivacain-HCl 1%). Blood samples were immediately centrifuged, and plasma and CSF samples were frozen at -80°C until assay. We assessed BMI and waist-to-hip ratio and measured body composition by standard multifrequency bioelectric impedance analysis (BIA; BIA 2000-M, Data Input GmbH, Frankfurt, Germany). Frequencies of 1, 5, 50 and 100 Hz were employed and results analyzed with Eurobody software (Data Input GmbH, Frankfurt, Germany). This safe and non-invasive technique estimates total body water (TBW), extracellular water (ECW), intracellular water (ICW), fat mass (FM) and fat-free mass (FFM) using equations validated for different populations (for in-depth information see reference 32).

CSF concentrations of orexin A were measured by means of a commercially available [^{125}I] radioimmunoassay kit (Phoenix Pharmaceuticals, Belmont, CA, USA) with a detection limit of 50 pg/ml and an intra-assay variability below 10%. All samples were assayed in duplicate and measured in the same kit at the same time. Measurements were evaluated using a standard curve and concentrations of CSF orexin A were determined against a set of four internal standard CSF samples (see reference 33 for further details). In addition, plasma and CSF glucose concentrations were measured (Beckman Glucose Analyzer II; Beckman Instruments, Munich, Germany). Insulin was determined using a commercial competitive double-antibody RIA (Pharmacia Insulin RIA 100; Pharmacia Diagnostics, Upsalla, Sweden). Assay sensitivity was increased to a threshold of 1.8 pmol/l by using 100 µl of CSF, 50 µl of [^{125}I] insulin diluted with buffer at a ratio of 1:3, and 50 µl of insulin antiserum diluted at a ratio of 1:2 (incubation time of 3 h; intra-assay variation was <4.5%). Adiponectin concentrations in plasma and CSF were determined using a commercially available radioimmunoassay kit (Linco Research, St. Charles, MO) according to the manufacturer's protocol, with an intraassay coefficient of variation of 6.2%.

Statistical Analyses

Data of 36 subjects (four and ten normal-weight, 16 and six overweight or obese men and, respectively, women) entered analyses (Table 1). Bivariate Pearson correlations and stepwise multiple regressions were calculated to determine associations between CSF orexin A concentrations and relevant variables (age, body weight, waist and hip circumference, BMI, fat mass, total body water, intracellular water, extracellular water, body cell mass and CSF and plasma concentrations of glucose, insulin and adiponectin). Two-tailed t-tests were used to compare differences in CSF orexin concentrations between different groups (male/female,

lean/obese); interactions between sex and body weight status were analyzed by ANOVA. We conducted statistical analyses in R 3.2.4 (34) using the leaps package for stepwise multiple regressions, and considered a p-value <0.05 to be significant.

Results

The mean concentration of orexin A in CSF was 315.6 ± 6.0 pg/ml, with no differences between men and women (308.0 ± 6.0 pg/ml vs. 325.2 ± 5.8 pg/ml, t(32.8)=-17.24, 95% CI [-41.29, 6.81], p>0.15). There was a trend towards higher concentrations in normal-weight than overweight participants (330.5 ± 6.9 pg/ml vs. 306.1 ± 4.9 pg/ml, t(21.2)=24.36, 95% CI [-2.05, 50.78], p=0.07). We did not find indicators for interactions between sex and body weight status with relevance for CSF orexin A concentrations (F(1, 32)=0.06, p>0.8).

Orexin A concentrations in CSF decreased with body weight (r=-0.38, p=0.0229; Figure 1A and Table 1) and waist circumference (r=-0.36, p=0.0362). CSF orexin A concentrations were not significantly associated with fat mass (r=-0.20, p>0.24) nor BMI (r=-0.23, p>0.17). However, CSF orexin A levels decreased in proportion to increasing fat-free mass (r=-0.39, p=0.0173; Figure 1B), total body water (r=-0.39, p=0.0174) as well as extracellular (r=-0.35, p=0.0341) and intracellular water (r=-0.41, p=0.0139); Figure 1C). Step-wise multiple regression analyses indicated that intracellular water was the only factor independently associated with CSF orexin A concentration (adjusted R^2=0.15, B = -2.61, SE=1.00, β=-0.42, p=0.0139), explaining 17% of its variance. This outcome was still significant after adjusting for sex and age (adjusted R^2=0.09, B=-2.85, SE=1.38, β=-0.45, p=0.0481). The remaining parameters, including age, body cell mass, plasma and CSF glucose, insulin and adiponectin were not significantly associated with CSF orexin A (all p>0.09).

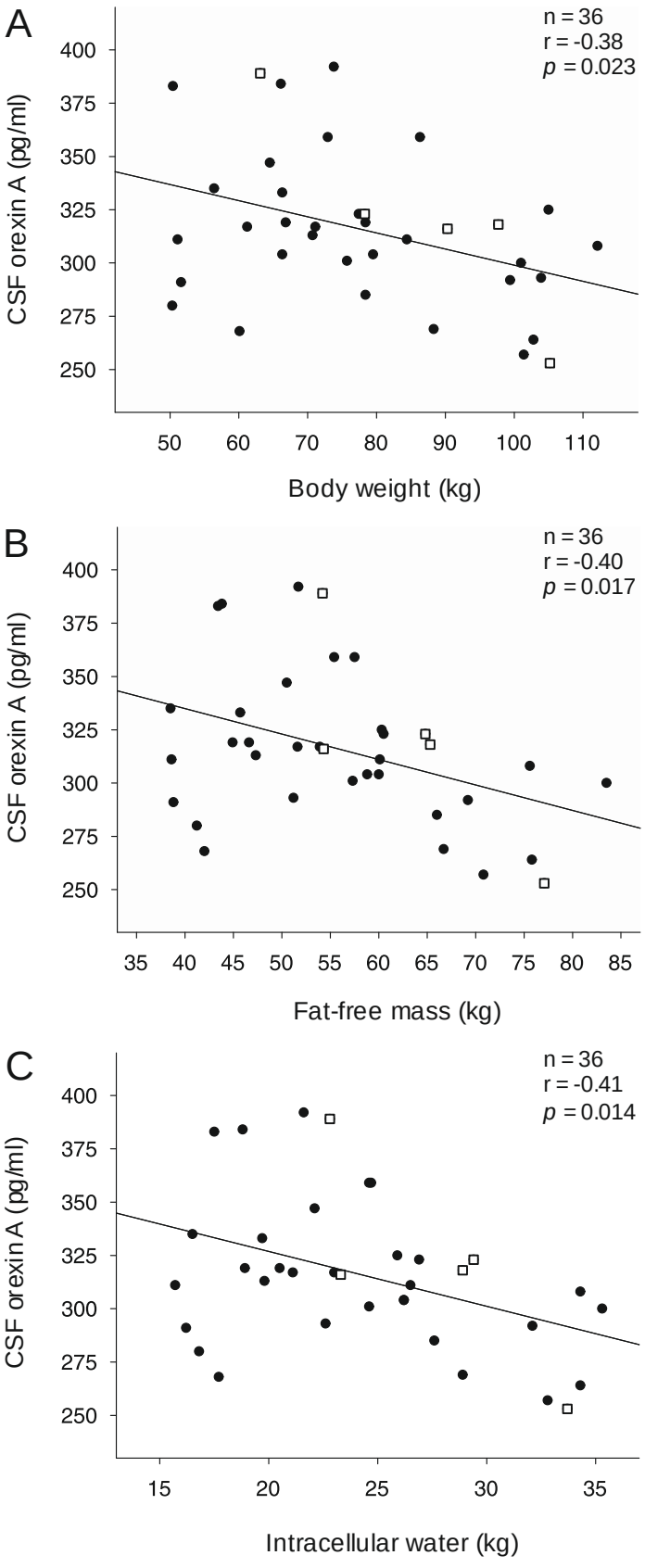

Figure 1. Individual CSF orexin A concentrations plotted against (A) body weight, (B) fat-free mass, and (C) intracellular water. Results of correlational analyses are indicated in the top right corners. Open squares indicate five subjects newly diagnosed with type 2 diabetes. When their data were excluded from analyses, intracellular water still was the only factor independently associated with CSF concentrations of orexin A (adjusted R^2=0.12, B = -2.33, SE=1.04, β=-0.39, p=0.034).

170

In exploratory analyses, we investigated water-related differences between body weight- and sex-specific subsamples. As expected, obese/overweight in comparison with normal-weight participants had higher amounts of total body water (43.9 ± 1.3 vs. 35.4 ± 0.8 l, t(33.3)=-10.49, 95% CI [-15.09, -5.89], p=0.0001), intracellular water (26.3 ± 0.8 vs. 20.4 ± 0.5 l, t(33.2)=-7.10, 95% CI [-10.05, -4.15], p<0.0001), and extracellular water (17.6 ± 0.5 vs. 14.9 ± 0.31 l, t(33.6)=-3.39, 95% CI [-5.13, -1.65], p=0.0004). We also found that the association between body water content and orexin A in CSF was more pronounced in the obese/overweight (r=-0.57, 95% CI [-0.80, -0.19], p=0.0059) than normal-weight participants (r=0.19, 95% CI [-0.38, 0.66], p>0.51; z-scores, -0.64 vs. 0.19, z=2.21, p=0.027). Respective z-score comparisons between sexes were not significant (p>0.14).

Table 1. Subject characteristics and correlations with CSF orexin A concentrations.

	Mean (SEM)	**Correlation with CSF orexin A (95% CI)**	***p* value**
Age (years)	53.4 (2.70)	-0.07 (-0.39, 0.26)	0.667
BMI (kg/m^2)	26.6 (0.81)	-0.23 (-0.52, 0.10)	0.172
Body weight (kg)	78.0 (3.0)	-0.38 (-0.63, -0.06)	0.023
Body fat mass (kg)	21.8 (1.77)	-0.20 (-0.50, 0.14)	0.244
Fat-free mass (kg)	56.2 (1.98)	-0.40 (-0.64, -0.08)	0.017
Body cell mass (kg)	29.0 (1.22)	-0.27 (-0.55, 0.07)	0.113
Total body water (l)	41.1 (1.45)	-0.39 (-0.64, -0.08)	0.017
Intracellular water (l)	24.4 (0.95)	-0.41 (-0.65, -0.09)	0.014
Extracellular water (l)	16.8 (0.52)	-0.35 (-0.61, -0.03)	0.034
Plasma glucose (mmol/l)	5.4 (0.19)	0.28 (-0.05, 0.56)	0.096
CSF glucose (mmol/l)	3.38 (0.08)	0.04 (-0.29, 0.36)	0.815
Plasma insulin (pmol/l)	81.1 (11.33)	-0.07 (-0.39, 0.26)	0.681
CSF insulin (pmol/l)	2.9 (0.21)	0.12 (-0.22, 0.43)	0.493

	Mean (SEM)	Correlation with CSF orexin A (95% CI)	*p* value
Plasma adiponectin (ng/ml)	12225 (867.35)	0.17 (-0.17, 0.47)	0.321
CSF adiponectin (ng/ml)	6.25 (1.25)	-0.05 (-0.37, 0.29)	0.788
Waist circumference (cm)	95.3 (2.60)	-0.36 (-0.62, -0.03)	0.036

Discussion

Our findings indicate that CSF orexin A concentrations and body weight are inversely related. This relationship is mediated by a significant negative association between total body water content and CSF orexin A concentrations, with intracellular water being the only independent predictor of CSF orexin A variation. Contrary to our expectations, body fat stores were not linked to CSF orexin A concentrations. This outcome argues against the assumption that in healthy, non-narcoleptic subjects, central nervous orexin A concentrations may be downregulated to counteract surplus energy intake, a conjecture based on the function of orexin A in the maintenance of arousal and the promotion of food-seeking behavior (35,36).

The negative correlation between central nervous orexin A and body water content ties in with emerging findings that suggest functional links between this neuropeptide and body water fluxes. Female orexin-KO rats in comparison to WT controls display greater amounts of adipose tissue, but also more free fluid (20). In the present study, the amount of intracellular water was the only independent predictor of the degree of orexin A variation, and the primary mediator of the adiposity-independent negative association between body weight and CSF orexin A concentrations. Studies in rodents suggest that crosstalk with anti-diuretic hormones such as arginine-vasopressin (AVP), which is known to activate orexinergic neurons (37), may establish a functional link between orexin A signaling and the regulation of body water. In line with this reasoning, intracerebroventricular administration of orexin A stimulates water intake, suggesting

that the orexinergic system mediates increases in locomotor activity emerging due to water deprivation or injections of AVP (37). In our study, circulating AVP concentrations were not assessed. However, since AVP release displays a diurnal rhythm with increased release during late night (38,39), care was taken to schedule CSF collection at the same time of day and to prevent fluid deprivation of our subjects. Therefore, it appears unlikely that the observed pattern was primarily due to state-dependent inter-individual differences in AVP tone. Absence of dehydration and over-hydration was also crucial for the validity of the multifrequency BIA approach to determine body composition (40), which, notably, has been reported in meta-analyses not to overestimate body water content (41). Thus, our findings raise the intriguing possibility that central nervous orexin A signaling plays a more substantial role in water homeostasis than previously assumed.

Interestingly, in our exploratory analyses in subsamples of subjects, the observed association between central nervous orexin A and body water seemed to be particularly strong in subjects with elevated body weight, although on average, body weight status (and also sex) did not differentially affect CSF orexin A values. Further work is necessary to assess the relevance of obesity for the relationship between central nervous orexin A and body water, not least considering the size of our sample and of the correlations we have detected. In accordance with previous studies (42), we did not find indicators that age differentially affects CSF orexin A concentrations in humans.

Our study did not yield evidence for a close relationship between CSF orexin A levels and body fat content, although we expected both parameters to be connected in light of reports that the adipokine leptin, as well as glucose, inhibit orexin A neurons (8), and that orexin A stimulates food intake in animal experiments (36). Orexin A moreover drives lipolysis in rodents via increases in autonomic activation (22), indicating a role of central nervous orexin A signaling in

the control of adipocyte metabolism. It is worth noting in this context that narcoleptic patients with orexin A deficiency tend to be overweight (25) and, in particular, display stronger elevations in body weight than respective patients with normal concentrations of orexin A (43). Future studies should address whether the relationship between body composition and CSF orexin A concentrations differs between narcoleptic patients, who were not included in the present experiments, and non-narcoleptic humans. Our results suggest that in the latter, body adiposity is not linked to central nervous orexin A concentrations. Investigations in larger samples of subjects that include more fine-grained phenotyping as well as analyses and interventions aimed at hydration status should extend the present experiments.

In conclusion, our cross-sectional findings indicate that in non-narcoleptic humans, central nervous orexin A concentrations do not show associations with body adiposity. Rather, they are inversely related to body water and in particular intracellular water content. These results point to a link between orexin A signaling and the regulation of water homeostasis, and underline the need for interventional investigations into the function of orexin A in the control of water, but also energy fluxes in humans.

Authors' contributions

JCPS and MH analyzed and interpreted the data and wrote the manuscript. MO and PB analyzed data and revised the manuscript for intellectual content. WK designed the study together with MH, acquired the data and revised the manuscript for intellectual content.

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Conflicts of interest: None.

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