ORIGINAL RESEARCH

Physiologic hyperleptinemia in obesity does not affect vasopressin secretion in acute hypo- or hyperosmolality

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Abstract

Purpose. Abnormal water excretion after ingestion of a water load has been described in obesity. We hypothesized that AVP secretion is abnormal in obese subjects in acute hypo- and hyperosmolality and that the hormone leptin is partly responsible for this.

Methods. We studied the relation between leptin, AVP and serum osmolality in two separate tests: (1) after ingestion of a water load (20 ml/kg lean body mass plus 5 ml /kg of adipose tissue) and (2) after *iv* hypertonic saline (5% NaCl) at a rate of 0.1 ml/kg lean body mass/minute for 120 min in ten subjects of normal weight (BMI >20 and < 25 kg/m²; controls) and ten obese females (BMI>30 kg/m²). Obese subjects were tested before (98.6 ± 9.3 kg) and after weight loss (90.2 ± 8.5 kg).

Results. In the water load experiment, obese subjects excreted a smaller percentage of the water load than controls. Weight loss restored the ability to excrete the water load in the obese. In the water load and hypertonic saline infusion experiment, plasma AVP concentrations and the area under the curve (AUC) for AVP concentration were not different in obese from normal weight women. Baseline leptin concentration was not correlated with baseline AVP or the change in AVP during the experiment in any of the groups. Weight loss did not change AVP responses in obese subjects.

Conclusion. AVP secretion in response to acute hypo- and hyperosmolality is not different in normal weight and obese subjects. There is no correlation between leptin and AVP in normal weight or obese subjects.

Obese subjects are unable to suppress plasma vasopressin (AVP) concentration after ingestion of a water load.¹ Acute salt loading by intravenous hypertonic saline infusion in obese males induces a delayed and attenuated increase in AVP concentration compared with normal weight males.¹ The cause of this presumed abnormal osmoregulation in obesity is unknown.

Leptin, a protein encoded by the *ob*-gene, is produced predominantly in adipose tissue.^{2,3} Leptin is involved in the regulation of food intake and energy expenditure.⁴ Fasting serum leptin levels are correlated to body mass index and percentage body fat.⁵ Obesity is associated with elevated leptin levels and weight loss and starvation with a decrease in serum leptin concentration.⁶⁻⁹

The influence of leptin on neurohumoral function has been studied in animals. Intracerebroventricular (icv) administration of leptin causes an increase of plasma vasopressin in rats¹⁰ and rabbits.¹¹ Expression of vasopressin messenger ribonucleic acid in rat supraoptic and paraventricular nuclei increases in response to icv leptin administration.¹⁰ If the observed neurohumoral actions of leptin are also present in physiological circumstances, leptin may be partly re-

sponsible for the inability of obese subjects to fully excrete a water load.

In this study, we compared changes in plasma vasopressin concentration in response to acute hypo- and hyperosmolality in obese and normal weight subjects and in obese subjects before and after weight loss. Acute hypo-osmolality was induced by the ingestion of a water load, and acute hyperosmolality by intravenous administration of hypertonic saline for 120 min. The two experiments were separated by at least three days. We tested if baseline AVP concentration and AVP changes following water or hypertonic saline administration were correlated to plasma leptin concentration.

Methods

Ethics approval

The study protocol was approved by the ethics committee of Leiden University Medical Center. All subjects gave oral and written informed consent. The studies were performed according to the standards set by the Declaration of Helsinki.

Subjects

Ten obese (body mass index (BMI) ≥ 30 kg/m²) and ten normal weight (BMI > 20 and < 25 kg/m²) healthy female volunteers were recruited by advertisements in local press. Inclusion criteria were a premenopausal state and age 18 to 40 yr. Exclusion criteria were the use of medication, including oral contraceptives, a history of hypertension, endocrine, cardiovascular or renal disorders, smoking and fluctuations of $>5\%$ body weight in the three months preceding the study. All subjects were studied in the follicular phase of the menstruation cycle. Before experiments, sodium in- and output in all subjects was brought into a steady state. Three days before the study days, all subjects started using standardized meals, containing approximately 120 mmol sodium per day. They received oral

and written diet prescriptions for the three days preceding the experiment. Subjects were requested to abstain from drinking caffeine-containing and alcoholic beverages for 24 hr before the study day. Room temperature at the study location was kept at 23-25°C and humidity at 25-65%.

Oral water load test

After an overnight fast and water deprivation, starting at 10:00 p.m., subjects presented at 7:30 a.m. at the study location. They were asked to void urine if possible. Subjects rested in a supine position with the head at approximately 30°, sitting only for voiding. The antecubital vein was cannulated for taking blood samples. After 60 min equilibrium, baseline blood and urine samples were taken. At time 0 the water load (Bar-le-duc “natural mineral water”, containing sodium 10.6 mg l⁻¹ and potassium 0.6 mg/l) was ingested by mouth as rapidly as tolerated, with a maximum ingestion time of 4 min. In obese subjects the excess weight consists primarily of adipose tissue, which has a lower hydration fraction. Therefore, the water volumes administered were calculated on the basis of 20 ml/kg lean body mass plus 5 ml/kg of adipose tissue. Body composition was measured by bioelectrical impedance analysis using Bodystat 1500. After the ingestion of the water load, subjects remained in supine position for three hours for completion of the measurements (time 0 to 180 min).

Total fasting and Hypertonic saline infusion

After an overnight fast and water deprivation, starting at 10:00 p.m., subjects presented at 7:30 a.m. at the study location. They were asked to void urine if possible. Subjects rested in a supine position with the head at approximately 30°, sitting only for voiding. The antecubital vein was cannulated for taking blood samples. After an equilibrium period, baseline blood and urine samples were taken. Then, a 5% NaCl infusion was started at 0.1 ml/kg lean body mass per minute for 120 min (time 0 to 120 minutes). Subjects re-

mained in the supine position for an additional 60 min to complete measurements (time 120-180 min). No foods or beverages were allowed during the entire experimental period. Following completion of the test, subjects were offered free fluid intake and a meal before leaving the study location. One of the investigators (MJC) clinically assessed them, to make sure they were in a good physical condition before leaving the room.

Low energy diet

After obese subjects had completed the experiment, they started, on the first day of their menstrual cycle, to use a very low energy diet, consisting of 500 kcal (Profiel drinkmaaltijd, Nutricia, The Netherlands) and approximately 2000 ml of caloric-free fluids daily for one menstrual cycle. This diet was composed of 29% protein, 55% carbohydrates, 16% fat, vitamins and minerals. Every week the obese subjects were seen at the outpatient clinic by one of the investigators (MJC) to be encouraged to adhere to the diet and for recording of physical complaints, if present. At the first day of the next menstrual cycle they started using a weight maintaining diet for three days, containing approximately 120 mmol sodium per day and free fluid intake. Then, both tests were repeated. Again, these tests were separated by three days, in which they used standardized meals provided by the investigators.

Evaluation of the subjects

Before the study, weight, height, bioelectrical impedance analysis and age of all subjects were recorded. BMI was calculated according to the formula: weight/height² (kg/m²). These measurements were repeated in obese subjects following the period they used a very low energy diet. Bioelectrical impedance was measured at time -60 minutes. Body weight was measured using a scale (Seca Alpha, model 770), at time 0, 60, 120 and 180 min.

Laboratory measurements

Venous blood was collected for determination of plasma vasopressin and leptin concentrations in chilled EDTA tubes. Blood was centrifuged immediately at 4°C and 3600 rpm for 10 minutes and the plasma stored at -20°C until assay. Blood in serum tubes were taken for measurements of glucose and sodium concentration. Urine volumes were monitored every hour during the water load experiment.

For measurement of serum sodium and glucose concentration, a Hitachi 747 analyzer (Boehringer Mannheim, FRG) was used. Plasma AVP concentration was measured by radioimmunoassay after extraction of 5 ml plasma on ODS-silica columns (Incstar, Stillwater, MN, USA). Recovery ranges between 92.7 and 97.6%, the intra-assay variation at several levels was between 7.1 and 9.3%, and the inter-assay variation was between 8.2 and 10.3%. The lower limit of detection is 0.3 ng/l. All samples of one subject were assayed in the same assay procedure. Plasma leptin concentrations were determined with a standardized radioimmunoassay (Linco Research, St Charles, MO, USA). The lower limit of detection is 0.5 µg/l. The limit of linearity for human leptin is 100 µg/l. The intra-assay coefficients of variation range from 6-7% over the leptin concentration range of 3-80 µg/l and the inter-assay coefficients of variation are 10.2%, 5.3% and 7.2% for the concentrations of 3.9, 11.3 and 63.8 µg/l. Serum sodium concentration was measured to calculate serum osmolality using the formula:

$$\begin{aligned} &\text{Effective serum osmolality} \\ &= 2 \times \text{serum [sodium]} \text{ (mosmol/kg)} \end{aligned}$$

Statistical and power analysis

Data are presented as mean ± standard deviation, except for plasma AVP and leptin concentrations. Plasma AVP and leptin concentrations were not normally distributed and are therefore shown as median (25-75 percentile). Within-group analyses were performed using paired Students' *t*-test for normally distributed

TABLE 1. Baseline characteristics of controls and obese subjects before and after weight loss

	Controls	Obese	
		before WL	after WL
Age (yr)	29.7 ± 7.9	30.9 ± 7.9	
Weight (kg)	65.3 ± 7.3	98.6 ± 9.3*	90.2 ± 8.5*†
Height (m)	1.70 ± 0.07	1.68 ± 0.06	
BMI (kg/m ²)	22.8 ± 1.7	35.6 ± 2.7*	32.2 ± 2.7*†

Data are presented as mean ± SD. WL, weight loss. * denotes $P < 0.001$ compared with controls, † denotes $P < 0.001$ compared with obese before diet

TABLE 2. Volume of ingested water and total urine volume in 3 hr following water ingestion

	Controls	Obese	
		before WL	after WL
Water load (ml)	1040 ± 105	1308 ± 115*	1250 ± 98*†
Urine volume (ml)	1213 ± 188	1217 ± 267	1272 ± 275

Data are presented as mean ± SD. WL, weight loss.

* $P < 0.001$ compared with controls, † $P < 0.001$ compared with obese before diet

parameters and Wilcoxon signed-ranks test for not normally distributed parameters. Within-group multiple comparisons of normally distributed parameters were tested for significance using analysis of variance. Inter-group comparisons were made using an unpaired t-test for normally distributed and a Mann-Whitney U test for not normally distributed parameters. Correction for multiple testing was applied according to Bonferroni Holm procedure. The associations between plasma vasopressin and leptin concentrations, body weight and body mass index were tested using Spearman correlation coefficient. Correlation coefficients for subgroups were calculated as the mean of individual correlation coefficients (95% confidence interval)

To have a 80% chance of detecting a difference in mean suppressed plasma vasopressin concentration of 1 ng/l (estimated standard deviation is 0.8 ng/l at vasopressin levels of 0.3-1.0 ng/l) between obese and normal weight women at the 5% significance level using the unpaired Students' *t*-test, ten subjects in each group were required. Statistical and power analyses were performed by one of the investigators (MJC) in

collaboration with R. Wolterbeek (Dpt of Medical Statistics, Leiden University).

Results

24-hr Urine sodium excretion at the day before testing was 120 ± 38 mmol/day for controls, 157 ± 38 mmol/day for obese before diet and 165 ± 50 mmol/day for obese after diet (ns). Baseline characteristics for controls and obese subjects before and after weight loss are shown in table 1. Obese subjects had before and after weight loss a significantly higher body weight and body mass index than controls.

Water load experiment

While obese subjects ingested a greater amount of water both before and after using a very low energy diet, urine volume in the three hours after water ingestion was not different between the groups (Table 2). Cumulative urine volumes following ingestion of a water load are shown as percentages of ingested water load (fig. 1). Controls excreted a greater urine volume in three hours than the water volume they had ingested ($P < 0.05$). The urine volume excreted by obese subjects was not different from the volume of water they had ingested before or after weight loss. Obese subjects before weight loss excreted a smaller percentage of the water load than controls (fig. 1).

Serum osmolality was not different between controls, obese before and after weight loss at any time (fig. 2). In all groups serum osmolality decreased at 60 min after water ingestion. In controls, serum osmolality returned to baseline after 180 min, while in obese subjects before and after weight loss, serum osmolality remained lower than at baseline.

The effect of water loading on plasma AVP and leptin concentrations is shown in tables 3 and 4. Plasma AVP concentration was not different in normal weight and obese subjects at any time. When the experiment was repeated in obese subjects after weight loss, there was no difference in plasma leptin (Table 3) and AVP concentrations in response to water load-

FIGURE 1. Cumulative urine volume as percentage of ingested water load in controls, obese subjects before weight loss and weight loss

WL, weight loss. Solid line represents controls, dotted line obese subjects before weight loss (WL) and interrupted line obese after WL.

* $P < 0.05$ for obese before WL compared with controls

FIGURE 2. Effective serum osmolality following ingestion of a water load in controls and in obese subjects before and after weight loss

Solid line represents controls, dotted line obese subjects before weight loss (WL) and interrupted line obese after WL. No differences between the groups

ing compared with before weight loss (data not shown). The area under the curve (AUC) of AVP (0-180 min) was not different in normal weight subjects (194 ± 99 ng*min/l), obese subjects before weight loss (232 ± 79 ng*min/l), and obese subjects after weight loss (201 ± 94 ng*min/l). The AUCs for 0-60 min and 0-120 min were not different either, between the groups (data not shown).

Baseline AVP concentration did not correlate with baseline leptin concentration (fig. 3). Following ingestion of a water load, plasma AVP concentration was not correlated with plasma leptin concentration in any of the groups (controls: $r = 0.09$ (95% confidence interval 0.65); obese before diet: $r = -0.01$ (0.83); obese after diet: $r = 0.09$ (0.77). The change in AVP concentration after water ingestion was at no time

TABLE 3. Plasma AVP concentration (ng/l) in relation to the time following ingestion of a water load (left columns) and during hyper-tonic saline infusion (right columns) in normal weight and obese subjects

Time (min)	Water Ingestion		NaCl 5% Infusion	
	Controls	Obese	Controls	Obese
Baseline	1.4 (0.9-2.0)	1.2 (0.7-1.6)	1.3 (1.0-1.6)	1.4 (1.1-1.8)
30	1.0 (0.4-2.5)	1.8 (0.7-2.0)	1.5 (1.0-2.4)	1.3 (1.1-2.0)
60	1.4 (0.7-2.5)	1.3 (1.0-1.7)	2.3 (1.4-6.5)	2.1 (1.8-2.8)
90	0.7 (0.2-1.3)	1.5 (1.0-1.7)	2.8 (1.8-3.9)	2.9 (2.1-3.4)
120	0.8 (0.5-1.5)	1.0 (0.2-1.6)	4.2 (2.6-6.4)	2.9 (2.0-3.5)
180	0.9 (0.3-1.7)	1.4 (0.5-2.1)	3.9 (2.6-8.1)	3.6 (1.8-14.1)

Data are presented as median (25-75 percentiles).

TABLE 4. Plasma leptin concentration ($\mu\text{g/l}$) in relation to the time following ingestion of a water load or hypertonic saline infusion in normal weight and obese subjects

Time (min)	Water Ingestion		NaCl 5% Infusion	
	Controls	Obese	Controls	Obese
0	13.1 (12.3-16.8)	37.5 (32.9-52.7)	11.6 (10.6-13.6)	35.3 (28.5-44.2)
60	12.0 (10.2-17.1)	37.2 (29.0-50.4)*	10.7 (9.7-11.8)	34.3 (26.2-41.3)‡
180	12.1 (11.0-16.8)*	33.8 (29.2-45.8)†	10.0 (8.1-11.8)	29.9 (23.3-41.0)‡

Data are presented as median (25-75 percentiles).

* $P < 0.05$, † $P < 0.01$ and ‡ $P < 0.001$ compared with leptin-0 within the same groups

point correlated to baseline leptin concentration in any of the groups (data not shown).

Hypertonic saline infusion

In table 5 are shown the volumes of hypertonic saline administered intravenously and the sodium. Urine sodium excretion in 3 hours following hypertonic saline administration was not different between the groups (Table 5). Serum osmolality increased in all groups during infusion ($P < 0.001$ in all groups, fig. 4). There were no differences between the groups at any time.

There were no differences in plasma AVP concentrations between controls and obese subjects before or after weight loss at any time (Table 3). The area under the curve (AUC) of AVP (0-180 min) was not different in controls ($742 \pm 112 \text{ ng} \cdot \text{min/l}$), obese before weight loss ($587 \pm 128 \text{ ng} \cdot \text{min/l}$) and obese after weight loss ($754 \pm 226 \text{ ng} \cdot \text{min/l}$). The AUCs of AVP 0-60 and 0-120 min were not different between the groups (data not shown). Plasma leptin concentration decreased significantly during hypertonic saline infusion in obese, but not in normal weight subjects (table 4).

In all groups there was a strong positive correlation between effective serum osmolality and plasma

FIGURE 3. Plasma AVP and leptin concentration before ingestion of a water load

Every symbol denotes plasma AVP concentration in relation to leptin concentration of one individual. Correlation coefficients between AVP and leptin are shown in the right upper quadrant.

FIGURE 4. Effective serum osmolality during and after hypertonic saline infusion

Solid line represents controls; dotted line represents obese subjects before weight loss and interrupted line obese after weight loss. No differences between the groups.

TABLE 5. Volumes of hypertonic saline and amount of NaCl administered intravenously

	Controls	Obese	
		before WL	after WL
5% NaCl (ml)	541 ± 47	615 ± 69*	622 ± 61†
NaCl (mmol)	461 ± 34	530 ± 51	530 ± 51

Data are presented as mean ± SD. WL, weight loss,

* $P < 0.05$ compared with controls, † $P < 0.01$ compared with controls

AVP concentration (controls: r 0.738 (95% CI 0.564-0.912); obese before diet: r 0.696 (95% CI 0.516-0.876); obese after diet r 0.836: (95% CI 0.664-0.999)). Delta AVP (0-180 min) was not correlated to baseline leptin concentration in any of the groups. Delta AVP during the hypertonic saline infusion (0-180 min) was not correlated to baseline body weight in any of the groups (data not shown).

Discussion

The results of this study show that AVP concentration is not different between obese and normal weight subjects in acute hypo- or hyperosmolality. Obese subjects were unable to fully excrete a water load in three hours following ingestion, while normal weight persons excreted a urine volume exceeding the ingested water load. Significant weight loss did not influence the AVP responses to hypo- or hyperosmolality in obese. There was no correlation between leptin and AVP in normal weight or obese subjects in baseline conditions or in acute hypo- or hyperosmolality.

We found, like others¹, that obese females were unable to excrete a water load fully within 3 hours after ingestion. Weight loss restored the ability to fully excrete the water load in our experiment. This is in contrast to previous findings of a persistent inability to excrete a water load in obese males, despite weight loss due to total fasting. Gender may partly account for this different findings.¹² The cause of delayed water excretion in obese subjects is not known. In animal experiments, it has been shown that intracerebroventricular administration of leptin increases AVP secre-

tion and plasma AVP concentration.^{10,11} Leptin receptors are found in animals centrally in the cerebral cortex, the hippocampus, the thalamus and the hypothalamus¹⁰ and in several peripheral tissues.¹³ There is increasing evidence that leptin regulates additional neuroendocrine, immunological and metabolic functions, besides the well-known effect on energy expenditure and satiety.¹³ We hypothesized that increased plasma leptin concentrations in obese subjects may be one of the factors causing defective water excretion, possibly by influencing plasma AVP levels. As it was impossible at the time of this study to administer leptin to humans in the Netherlands, we were not able to test whether leptin in pharmacological concentrations would influence AVP concentrations in humans. Therefore, we chose to manipulate leptin by inducing weight loss in a patient group with clear obesity (BMI > 30 kg/m²) and to compare obese subjects also to normal weight subjects. This provided us with the opportunity to test in vivo the effect of normal, medium high and high leptin concentrations in humans on AVP concentrations at baseline and after osmotic suppression or stimulation. AVP is known to be rapidly eliminated in a biphasic way, with short half-life values of 0.9 and 5.4 minutes in rabbits.¹⁴ We manipulated the plasma AVP concentration by orally administering a weight-based water load and by intravenous administration of hypertonic saline, in order to observe potential differences in plasma AVP concentration in normal weight and obese subjects in acute hypo- and hyperosmolality.

Given the similar baseline AVP concentration in obese and normal weight and the similar changes in serum osmolality in both experiments, we achieved optimally comparable conditions to compare AVP responses in the groups. In the hypertonic saline experiment, plasma AVP concentration increased in all groups to the same rate and extent, and the area under the curve for AVP was the same in all groups. There was a strong positive correlation between serum osmolality and plasma AVP concentration, suggesting intact osmoregulation in all groups. This is in contrast

with the slow and decreased AVP response during hypertonic saline infusion in obese men and the absent correlation between osmolality and AVP, as described by Drenick et al¹. In addition, our finding of similar AVP concentrations in obese and normal weight subjects during hypo-osmolality is in contrast with previous findings¹. Drenick et al¹ found that grossly obese males had inadequate AVP suppression following ingestion of a water load. Prolonged total fasting (mean 37 days) resulted in restoration of the ability to adequately suppress AVP concentration in response to water load. In our experiments, weight loss did not affect AVP responses in obese subjects.

Leptin appeared not to affect AVP concentration in these physiological circumstances. We observed that baseline leptin concentration was not correlated to baseline AVP concentration. Following ingestion of a water load, plasma AVP concentration was not correlated to plasma leptin concentration, nor was leptin correlated to delta AVP following water drinking. This implied that, although centrally administered leptin in pharmacological doses stimulated AVP secretion in animal experiments, leptin in physiological concentrations does not influence AVP secretion or concentration in humans in a water loading or hypertonic saline infusion experiment. The impaired water excretion in obese subjects cannot be explained by inadequate AVP suppression, due to a central effect of leptin. Impaired water excretion in obesity may be related to a renal mechanism, which may be independent of circulating plasma AVP concentrations. It may be hypothesized that there is increased sensitivity for AVP in obese subjects by a receptor or post-receptor effect, possibly mediated by local intra-renal modulating factors such as prostaglandins, increased peritubular osmolality or upregulation of renal aquaporins. However, the contrary is found in obese Zucker rats, with a mutation in the gene for the leptin receptor, leading to hyperphagia and obesity. These rats had renal dysregulation of salt and water excretion. Targeted proteomics in these animals showed down regulation of aquaporin channels in renal collecting ducts and apical urea

transporters.¹⁵ This has never been studied in humans. Leptin does not appear to have a direct effect on intra-renal hemodynamics, leading to impaired water excretion, as leptin administration does not cause a change in urine flow rate¹⁶ or glomerular filtration rate in rats.¹⁷

In conclusion, obese subjects have intact osmoregulation and a normal AVP response during acute hypo- and hyperosmolality, despite physiological hyperleptinemia.

Author contributions

M.J. Coenraad

Contributed to the conception and design of the study, performed the tests and the interpretation of data, drafted the article and approved the final version of the manuscript

M.Frölich

Contributed to the conception and design of the study, performed all chemical analyses and interpretation of the data. Drafted part of the manuscript and revised critically the manuscript. She approved the final version for submission.

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Contributed to the conception and design of the study, was closely involved in interpretation of the data. Revised and supervised drafting of the article and approved the final version of the manuscript

The experiments were performed at Leiden University Medical Centre.

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