Meal-induced compositional changes in blood and saliva in persons with bulimia nervosa1–3

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ABSTRACT

Background: Binge eating episodes in persons with bulimia nervosa may to some extent be a result of disturbed sensations of hunger and satiety. It has been hypothesized that abnormal appetite sensations may be due to bulimia nervosa–related alterations in the release of hormones that are known to be involved in the physiologic regulation of appetite and metabolism.

Objective: The objective was to investigate whether circulating concentrations of the appetite-regulating peptides leptin and ghrelin and markers of metabolism (glucose and insulin) are different in persons with bulimia nervosa than in controls before and after intake of a meal and whether these changes may be reflected in saliva.

Design: Twenty women with bulimia nervosa and 20 age- and sex-matched healthy controls participated. After an overnight fast, the subjects ate a standardized carbohydrate-rich breakfast. Whole saliva and blood were collected, and visual analogue scales for hunger and satiety were completed once before and continuously for 5 h after the breakfast.

Results: A lower pre- and postprandial whole saliva flow rate was found in subjects with bulimia nervosa, which might have been attributable to a concomitant intake of potentially xerogenic medication. Subjects with bulimia nervosa experienced reduced hunger, which could not be explained by pre- or postprandial alterations in circulating ghrelin, leptin, insulin, or glucose concentrations.

Conclusions: There were no apparent differences in the composition of blood and saliva between bulimia nervosa and control subjects, and meal-induced compositional changes in blood were not directly mirrored in saliva composition. Am J Clin Nutr 2008; 87:12–22.

KEY WORDS Bulimia nervosa, saliva, glucose, insulin, leptin, ghrelin, hunger, satiety

INTRODUCTION

Bulimia nervosa is an eating disorder characterized by episodic binge eating and the practice of inappropriate purging behaviors, such as self-induced vomiting and laxative and diuretic abuse. Persons with bulimia nervosa also experience a sense of lack of control over eating during the binge episodes, and self-evaluation is unduly influenced by body shape and weight (1). It has been suggested that binge-eating episodes, which are described as chaotic and uncontrollable, may be a result of impairment in achieving sufficient satiety at the end of a meal (2–4).

Although many clinical features have been described, little is known about biomarkers of the pathophysiology related to eating disturbance. Ghrelin and leptin are peptides involved in the homeostasis and regulation of appetite in healthy individuals. Ghrelin, which is a “hunger hormone,” is high in the fasting state and decreases in blood after food intake (5–7); meals that are particularly rich in carbohydrates, as opposed to fat, suppress ghrelin concentrations (8, 9). However, the function of leptin as a short-term satiety signal is uncertain (10). In general, the leptin concentration does not change significantly in response to a meal (ie, within 3–4 h), but is thought to be a satiety factor that regulates body weight through modulation of feeding behavior and energy expenditure (11). Thus, it has been suggested that binge-eating behavior in bulimia nervosa may be related to alterations in circulation concentrations of ghrelin and leptin. In general, normal responses of markers of metabolism, glucose and insulin, have been found in bulimia nervosa (12, 13), but reduced glucose and insulin concentrations after purging have also been reported (14). It remains an open question whether leptin, ghrelin, glucose, and insulin responses to a meal are altered in bulimia nervosa and whether meal-induced changes in...
salivary leptin, ghrelin, glucose, and insulin in bulimia nervosa reflect those of blood.

In the present study we hypothesized that distorted sensations of hunger and satiety in bulimia nervosa are mirrored by altered concentrations of leptin, ghrelin, glucose, and insulin at baseline and in response to a carbohydrate-rich meal. Additionally, we hypothesized that such meal-induced blood changes are reflected in saliva.

SUBJECTS AND METHODS

Subjects

Forty women participated in the study. Twenty women were diagnosed with bulimia nervosa (Diagnostic and Statistical Manual-IV) criteria; 1) (BN group), and 20 women were healthy controls with no history of eating disorders (control group). The women with bulimia nervosa were recruited partly from a psychiatric practice in Copenhagen (n = 12); the remaining participants with bulimia nervosa were recruited through an ongoing psychological research project at the University of Copenhagen (n = 3) and via a notice at the Faculty of Health Sciences, University of Copenhagen (n = 5). A structured interview concerning the DSM-IV criteria for bulimia nervosa was performed to diagnose the participants with bulimia nervosa. The recruitment of participants with bulimia nervosa was difficult because the study involved the intake of a standardized meal, which is considered a major challenge for many persons with bulimia nervosa. The control subjects were recruited via a notice at the Faculty of Health Sciences, University of Copenhagen. Written informed consent was given by all participants before enrolling in the project, which was approved by the local ethical committees of Copenhagen and Frederiksberg (KF 03-003/04).

Study design

The participants arrived at the Department of Human Nutrition, University of Copenhagen, at 0730 after having fasted overnight. The participants were standardized with respect to intake of water in the morning, and all participants attended the study during days 0–5 in their menstrual cycle, except for 4 participants in the BN group with amenorrhea. The participants rested in a supine position for ≥15 min before a catheter (BD Venflon) was inserted in a forearm vein. The participants rested for 10 min after insertion of the catheter, and the first blood sample was drawn into K2EDTA-coated tubes (BD Vacutainer; Becton Dickinson, Franklin Lakes, NJ) and another 3.5-mL blood sample was drawn into serum tubes (BD Vacutainer). The EDTA-coated tubes were kept on ice and immediately centrifuged, whereas serum tubes were kept at room temperature for 30 min and allowed to clotting before centrifugation. All blood samples were centrifuged at 4000 g and 4 °C for 15 min, and plasma and serum aliquots were stored at −80 °C until analyzed. On analysis, all samples were measured in duplicate.

Saliva samples and flow rate

Whole saliva was collected by the draining method into a preweighed plastic cup for a period of 10 min. The participants were instructed to rinse their mouths thoroughly in tap water, make an initial swallow, and then to lean forward and passively drain their saliva into the cup without swallowing during the collection procedure (15). After 10 min the plastic cup was weighed again, and the salivary flow rate (mL/min) was estimated by dividing the volume of the saliva sample (1 g saliva = 1 mL) by the collection time (in minutes). Saliva was centrifuged at 4000 × g and 4 °C for 15 min and stored in aliquots at −80 °C until analyzed. On analysis, all samples were measured in duplicate.

Visual analogue scale

The sensation of hunger and satiety was rated on a VAS of 100 mm in length with words anchored in each end expressing the most positive and the most negative rating. The questions and ratings were as follows: “How hungry do you feel?” (I am not hungry at all = 0 mm; I have never been more hungry = 100 mm) and “How satisfied do you feel?” (I do not feel satisfied at all = 0 mm; I have never felt more satisfied = 100 mm). The participants could not discuss or compare their ratings with one another.

DXA scanning

After voiding, the participants were weighed to the nearest 0.1 kg (model WB-110MA; Tanita UK Ltd, Middlesex, United Kingdom). The body composition of each participant was measured by dual-energy X-ray absorptiometry (DXA).
Kingdom) while wearing only underwear. Height was measured to the nearest 0.5 cm and body composition by DXA scanning (Lunar Prodigy Advance, GE Healthcare, Brabant, Belgium) followed. Participants were asked to wear light clothing and to remove all removable metal items during the scan. The participants were placed on the scan table in the supine position with their arms placed along their thighs. To maintain position during the scanning, the participants were mummy-wrapped in a broad cotton belt closed with Velcro. The whole-body DXA scan provided data for lean body mass, fat mass, bone mass, and bone mass density. The percentage of body fat was computed as 

\[
\frac{\text{fat mass}}{\text{fat mass} / \text{lean mass} + \text{bone mass}} \times 100.
\]

Laboratory analysis

Glucose concentration

The concentration of glucose in whole saliva and serum was measured enzymatically with a commercially available kit (EnzyPlus, Diffchamb, Sweden) based on the formation of NADPH. Serum (200 μL) was diluted in Millipore water (1.8 mL). In a 96-well plate, 10 μL diluted serum was mixed with 100 μL of solution 1 (an aqueous solution of triethanolamine buffer, NADP, ATP, and magnesium sulfate supplied by the manufacturer) and 190 μL Millipore water. The absorbances \((A_1)\) were measured spectrophotometrically at 340 nm in a microplate absorbance reader (Tecan Sunrice, version 1.02). The change in \(A \ (A_2 - A_1)\) for samples was calculated, and the glucose concentration in all unknowns was determined by standard curves [d-glucose; Merck (8346), Germany] in the range 30–400 mg/L for serum. The glucose in whole saliva was measured by the same method; however, saliva samples were not diluted before measurement, and 15 μL of sample was applied in each well. Glucose standard curves for determining glucose in whole saliva were constructed in the range of 10–400 mg/L.

Insulin, leptin, and ghrelin concentrations

The concentrations of insulin, leptin, and ghrelin in whole saliva, serum, and plasma were measured with commercially available immunologic assays. Thus, the concentration of insulin in serum and whole saliva was measured with a commercially available enzyme-linked immunosorbent assay [insulin enzyme-linked immunosorbent assay (ELISA), no. EIA-2935; DRG Diagnostics, Marburg, Germany] based on the direct sandwich technique. Insulin standard curves were constructed in the range of 6.25–100 mIU/mL with the use of standards supplied by the manufacturer.

The leptin concentration in whole saliva and in EDTA-plasma was determined with a commercially available ELISA (human Leptin ELISA, no. E07; Medignost, Marburg, Germany) also based on the ‘sandwich’ technique. Leptin standard curves were constructed in the range of 1–100 ng/mL by standards supplied by the manufacturer.

The concentration of total ghrelin in whole saliva and plasma was determined with a commercially available immunologic assay (Ghrelin Total RIA Kit, catalog no. GHRT-89HK; Linco Research, St Charles, MO) based on a competitive technique that uses a 125I-labeled ghrelin tracer. Ghrelin standard curves were constructed.
constructed in the range of 117–7500 pg/mL standard supplied by manufacturer.

Output in whole saliva

When analyzing an actual saliva concentration of a given substance one must take into consideration that the saliva flow rate is not constant over time, but varies with the stimulatory condition of the gland. Because the flow rate tended to differ between the groups, we also chose to calculate salivary release (output) of glucose, insulin, leptin and ghrelin. Outputs of glucose, insulin, leptin, and ghrelin in whole saliva were calculated as the concentration \( \times \) the flow rate, which resulted in an output with the following units: \( \mu \text{g/min}, \mu \text{IU/min}, \text{ng/min}, \) and \( \text{pg/min} \), respectively.

Statistical analysis

The aim of the statistical analysis was to evaluate differences between the BN group and the control group and to study whether blood concentrations of glucose, insulin, leptin, and ghrelin correlated with the concentrations and output in whole saliva.

All variables were compared between the 2 groups with a 2-sample t test for means. To further assess the differences in glucose and hormones between the BN group and the control group, multiple regression analyses were performed. Because smoking habits differed markedly between the groups, and because smoking is suggested to cause insulin resistance (16), this variable was included as a covariable in the multiple statistical analyses concerning glucose and the hormones. The intake of medication also differed between the groups, but was only included as a covariable in the multiple regression analyses concerning whole saliva flow, because the drugs that were taken by the BN group were known to cause oral dryness (17). However, the effect of intake of medication on glucose and hormones was tested as well (multiple regression) and showed no relation between intake of medication and glucose and hormones in the 2 groups. Relations between height and whole saliva flow rate have been suggested (18); thus, height was included in these analyses as well. Residuals were graphically investigated to check the assumptions. No discrepancy of normality and constant variance was detected.

Differences between the groups during the entire test period were assessed by analysis of variance for repeated measurements (SAS PROC MIXED; SAS Institute Inc, Cary, NC); smoking habits was included as a covariable in the analyses concerning glucose, hormones, and VAS, and intake of medication and height in the analyses concerning whole saliva flow rate.

Correlations between VAS and glucose and hormones as well as between concentrations of glucose and hormones and whole saliva concentrations and output of glucose and hormones were assessed by Pearson’s correlation coefficient at different times and by analysis of variance for repeated measurements (SAS PROC MIXED) for the overall relation during the entire period. Statistical analysis was performed in SAS (version 9.1 for WINDOWS). All analyses were performed at a 5% level of significance.

RESULTS

Study groups

One subject with bulimia nervosa did not wish to participate on the test day, which left 19 persons in the BN group. The participants were aged 18–33 y; the mean (±SD) age was 24.0 ± 4.2 y in the BN group and 23.1 ± 2.4 y in the control group. Body mass index (BMI), fat mass, lean mass, bone mass, and percentage body fat were not significantly different between groups (Table 2). Three subjects in the BN group had depression, 2 had a history of allergy, and 1 had a prolonged QT syndrome. One subject in the control group had polycystic ovary syndrome. The presence of disease other than bulimia nervosa did not affect body composition. Also, body composition in the 4 participants from the

<table>
<thead>
<tr>
<th>TABLE 2</th>
<th>Characteristics of the subjects in the bulimia nervosa (BN) and control groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>BN group (n = 19)</td>
<td>Control group (n = 20)</td>
</tr>
<tr>
<td>Age (y)</td>
<td>24 (18–33)</td>
</tr>
<tr>
<td>Current smokers (n)</td>
<td>10</td>
</tr>
<tr>
<td>Daily physical activity (n)</td>
<td>19</td>
</tr>
<tr>
<td>Exercise (h/wk)</td>
<td>4 (0–12)</td>
</tr>
<tr>
<td>Other diseases (n)</td>
<td>6</td>
</tr>
<tr>
<td>Oral contraceptive use (n)</td>
<td>10</td>
</tr>
<tr>
<td>Daily medication use (n)</td>
<td>8</td>
</tr>
<tr>
<td>Potential xerogenic medication use (n)</td>
<td>8</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>21.5 (13.5–26.7)</td>
</tr>
<tr>
<td>Fat mass (kg)</td>
<td>17.8 (2.9–3.4)</td>
</tr>
<tr>
<td>Lean mass (kg)</td>
<td>41.1 (27.5–53.6)</td>
</tr>
<tr>
<td>Bone mass (kg)</td>
<td>2.5 (1.5–3.3)</td>
</tr>
<tr>
<td>Percentage body fat (%)</td>
<td>27.5 (9.2–42.2)</td>
</tr>
</tbody>
</table>

1 Obtained with a 2-sample t test for means.
2 Mean; range in parentheses (all such values).
3 Walking and bicycling to or from work or educational institution.
4 Sporting activities practiced during leisure time.
5 Prolonged QT syndrome (n = 1), depression (n = 3), and allergy (n = 2).
6 Polycystic ovary syndrome (n = 1).
7 Does not include oral contraceptive use, but does include antidepressants, antihistamines, \( \beta \)-blockers, and laxatives (in a dose above that recommended).
8 Reference 17. Includes antidepressants, antihistamines, \( \beta \)-blockers, and laxatives (in a dose above that recommended.).
The practice of daily physical activity and exercise in the 2 groups was not significantly different. The use of oral contraceptives was not significantly different between groups, whereas the daily intake of other medications and the number of current smokers differed between groups (Table 2). The intake of medication consisted of antidepressants (selective serotonin reuptake inhibitors), antihistamines, β-blockers, or combinations thereof.

The mean number of cigarettes smoked was 6/d, and no subjects were smoking >14 cigarettes/d. There was no significant difference between the numbers of cigarettes smoked by the smokers in the 2 groups. Because of the obvious differences between groups in medication use and smoking, they were included as co-variables in the statistical analyses, as previously described.

The participants with bulimia nervosa were all of the purging subtype, with binge episodes followed by self-induced vomiting. Almost half (47%) of the BN group was practicing self-induced vomiting every day or several times a day (n = 7 and n = 2, respectively). In the other half (53%) of the BN group, self-induced vomiting was often practiced several times a week. Thus, the mean (±SD) frequency of self-induced vomiting and binge eating was 0.9 ± 0.5 and 0.8 ± 0.5 episodes/d, respectively. The mean duration of bulimia nervosa was 6.4 ± 4.6 y. None of the participants had a previous history of anorexia nervosa.

Preprandial saliva flow rate

The preprandial unstimulated whole saliva flow was 38% lower (P = 0.010) in the BN group than in the control group (Table 3). However, when adjusted for medication use and height, there were no significant differences in saliva flow rate between the groups. Thus, the participants from the BN group who took medication had a 65% lower whole saliva flow rate at baseline (0.09 ± 0.09 mL/min) than did the control group (P = 0.001).

Preprandial glucose and hormones in blood and saliva

Glucose, insulin, leptin, and ghrelin were also measurable in preprandial whole saliva, although concentrations differed between blood and saliva (Table 3). Glucose and leptin concentrations in saliva were lower than in blood, whereas insulin and ghrelin were found in the same range of concentrations in blood and saliva.

Preprandial concentrations of glucose in whole saliva were significantly higher in the BN group than in the control group, regardless of smoking; whereas the leptin output in whole saliva was only significantly lower in the BN group than in the control group when adjusted for smoking (Table 3).

Preprandial concentrations of glucose, insulin, leptin, and ghrelin in blood; concentrations of insulin, leptin, and ghrelin in saliva; and whole saliva output of glucose, insulin, and ghrelin did not differ significantly between the BN group and the control group, and smoking had no effect on this result (Table 3).

Meal-induced changes in saliva flow rate

The effect of a standardized breakfast on whole saliva flow rate was somewhat similar in the 2 groups (Figure 2). After intake of the standardized breakfast, there was a significant increase in the whole saliva flow rate 15 min after the meal, which was followed by an immediate decrease to a level not significantly different from the baseline level. However, the decrease in the whole saliva flow rate >15 min after the meal was more pronounced in the control group than in the BN group (Figure 2). Although the whole saliva flow rate was lower in the BN group than in the control group throughout the postprandial period, this
difference was not significant when the effects of medication use and height were taken into account.

Meal-induced changes in blood and saliva composition

Glucose

Serum. The effect of a standardized breakfast on serum glucose concentrations did not differ significantly between the BN and control groups, regardless of smoking status (Figure 3).

Saliva. In saliva the glucose concentration increased >10-fold in both groups immediately after intake of a standardized breakfast (Figure 3). The saliva glucose concentration was higher in the BN group than in the control group throughout the period when the effect of smoking was included ($P = 0.035$). However, when salivary flow was taken into consideration, the salivary glucose output did not differ significantly between the groups. In both groups the salivary glucose concentration returned to the baseline level within 1 h.

Correlations between blood and saliva glucose. Glucose concentrations in blood and saliva did not correlate significantly (Table 4). The glucose concentration in serum correlated with glucose release, output, in whole saliva after intake of the standardized breakfast ($P = 0.024$) (data not shown). The measurements just after the meal (15 and 45 min) were excluded because glucose measured in whole saliva at that stage was due to contamination of the oral cavity with glucose contained in the diet (Table 4).

Insulin

Serum. The effect of a standardized breakfast on serum insulin concentrations did not differ significantly between the BN group and the control group. In both groups the serum insulin concentration increased ≈10-fold in response to the meal. During the following hours the serum insulin concentration decreased, reaching the baseline level ≈4 h and 45 min after intake of the breakfast (Figure 3). There was a general significantly positive correlation ($P < 0.001$) between insulin and glucose concentrations in serum (data not shown).

Saliva. A similar effect of the breakfast was seen in the saliva insulin concentration in both groups. However, for both groups
Correlations between blood and saliva concentrations of glucose, insulin, leptin, and ghrelin in the bulimia nervosa (BN) and control groups by time before and after breakfast

<table>
<thead>
<tr>
<th>Glucose</th>
<th>Total (n = 49)</th>
<th>BN group (n = 19)</th>
<th>Control (n = 20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>−45 min</td>
<td>r = −0.06, P = 0.721</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>75 min</td>
<td>r = 0.16, P = 0.347</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>105 min</td>
<td>r = −0.06, P = 0.708</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>165 min</td>
<td>r = 0.26, P = 0.116</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>225 min</td>
<td>r = 0.25, P = 0.125</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>285 min</td>
<td>r = 0.33, P = 0.046</td>
<td>P = 0.1082</td>
<td>—</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Insulin</th>
<th>Total (n = 49)</th>
<th>BN group (n = 19)</th>
<th>Control (n = 20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>−45 min</td>
<td>r = 0.21, P = 0.226</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>15 min</td>
<td>r = 0.15, P = 0.370</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>45 min</td>
<td>r = 0.24, P = 0.145</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>105 min</td>
<td>r = 0.37, P = 0.019</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>165 min</td>
<td>r = 0.11, P = 0.513</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>225 min</td>
<td>r = 0.30, P = 0.070</td>
<td>P = 0.1192</td>
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<table>
<thead>
<tr>
<th>Leptin</th>
<th>Total (n = 49)</th>
<th>BN group (n = 19)</th>
<th>Control (n = 20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>−45 min</td>
<td>—</td>
<td>r = 0.48, P = 0.037</td>
<td>r = −0.33, P = 0.160</td>
</tr>
<tr>
<td>15 min</td>
<td>—</td>
<td>r = 0.31, P = 0.197</td>
<td>r = −0.01, P = 0.955</td>
</tr>
<tr>
<td>45 min</td>
<td>—</td>
<td>r = 0.45, P = 0.050</td>
<td>r = 0.01, P = 0.983</td>
</tr>
<tr>
<td>75 min</td>
<td>—</td>
<td>r = 0.36, P = 0.125</td>
<td>r = −0.06, P = 0.801</td>
</tr>
<tr>
<td>105 min</td>
<td>—</td>
<td>r = 0.54, P = 0.016</td>
<td>r = −0.08, P = 0.755</td>
</tr>
<tr>
<td>165 min</td>
<td>—</td>
<td>r = 0.44, P = 0.059</td>
<td>r = −0.19, P = 0.433</td>
</tr>
<tr>
<td>225 min</td>
<td>—</td>
<td>r = 0.51, P = 0.027</td>
<td>r = −0.20, P = 0.408</td>
</tr>
<tr>
<td>285 min</td>
<td>—</td>
<td>r = 0.62, P = 0.005</td>
<td>r = −0.09, P = 0.710</td>
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<table>
<thead>
<tr>
<th>Ghrelin</th>
<th>Total (n = 49)</th>
<th>BN group (n = 19)</th>
<th>Control (n = 20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>−45 min</td>
<td>r = 0.18, P = 0.337</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>15 min</td>
<td>r = 0.11, P = 0.530</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>45 min</td>
<td>r = 0.38, P = 0.028</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>75 min</td>
<td>r = 0.20, P = 0.266</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>105 min</td>
<td>r = 0.47, P = 0.005</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>165 min</td>
<td>r = 0.21, P = 0.213</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>225 min</td>
<td>r = 0.20, P = 0.243</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>285 min</td>
<td>r = 0.02, P = 0.921</td>
<td>P = 0.0802</td>
<td>—</td>
</tr>
</tbody>
</table>

1 Glucose concentrations measured in whole saliva at 15 and 45 min were excluded because of direct contamination of the oral cavity with glucose contained in the diet.
2 The overall correlations were calculated by ANOVA for repeated measures (SAS PROC MIXED; SAS Institute, Cary, NC).
3 Because of a delayed insulin response in whole saliva, blood insulin concentrations 15, 45, 105, 165, and 225 min after the meal were correlated with the insulin concentration in saliva at 75, 105, 165, 225, and 285 min after the meal.
4 Because the correlations between leptin concentrations in blood and saliva were positive and negative in the BN and control groups, respectively, no correlations for the total group were calculated.

The peak insulin concentration was delayed ~60 min compared with the meal-induced insulin response in serum (Figure 3). The saliva insulin concentration in the BN group was significantly higher (P = 0.016) than that in the control group during the postprandial period. However, the salivary insulin output did not differ significantly between groups.

Correlations between blood and saliva insulin. The insulin concentrations in blood and saliva did not correlate significantly (Table 4). However, the concentration of insulin in blood correlated with the output of insulin in whole saliva (P = 0.018) (data not shown) when adjusted for the 60-min time difference in the response described above (Figure 3). Generally, insulin concentrations in blood and saliva were within the same range.

Leptin

Plasma. There were no significant differences between the groups with regard to the effect of the standardized breakfast on plasma leptin concentration (Figure 4).

Saliva. In saliva, the concentration of leptin did not differ significantly between groups after the standardized breakfast. In both the BN group and the control group the saliva leptin concentration increased slightly just after the meal, after which time the leptin concentration followed a pattern similar to the leptin response seen in plasma (Figure 4). Leptin output in saliva did not differ significantly between the groups.
Correlations between blood and saliva leptin. Leptin concentrations in blood and saliva correlated mainly due to a positive correlation between the concentration of leptin in blood and saliva in the BN group (Table 4); on average, the concentration of leptin in blood was 5 times the concentration in saliva (Figure 4). No significant correlation between blood leptin concentration and leptin output in saliva was shown.

Ghrelin

Plasma. The effect of the breakfast on ghrelin concentrations in plasma was not significantly different between the 2 groups. Thus, the ghrelin concentration 15 min after the meal was significantly higher than the concentration in the following 2.5 h and lower than the concentration 285 min after the meal ended (Figure 4).

Saliva. In saliva the ghrelin concentration did not differ significantly between the groups after the standardized breakfast (Figure 4).

Correlations between blood and saliva ghrelin. The ghrelin concentration in blood did not correlate with the ghrelin concentration in saliva or the output of salivary ghrelin (Table 4). Generally, the ghrelin concentrations in blood and saliva were within the same range (Figure 4).

Hunger and satiety and relations to hormone response

At baseline, hunger and satiety sensations did not differ significantly between the groups, regardless of smoking (Figure 5). Intake of the standardized breakfast resulted in a decrease in scores of hunger and an increase in scores of satiety compared with baseline values in both groups. Later than 15 min after the meal ended, hunger scores were significantly lower in the BN group during the postprandial period than in the control group (P = 0.004), whereas no significance differences in satiety scores were observed between groups.
and the strongest relation was observed in the control group. Differences in satiety scores between the groups increased during the post-prandial period; the lowest scores of hunger were in the BN group. The sensations of hunger between the 2 groups increased during the post-meal, the hunger scores increased and the satiety scores decreased. The scores for sensation of hunger differed significantly ($P = 0.004$) between the 2 groups. Thus, the difference in sensation of hunger between the 2 groups increased during the post-prandial period; the lowest scores of hunger were in the BN group during the entire period (Figure 5). There were no significant differences in satiety scores between the groups.

The sensations of hunger and satiety were inversely related, and the strongest relation was observed in the control group (Table 5). The sensations of hunger and satiety were not related with consistency to any of the included blood variables (data not shown).

### DISCUSSION

#### Whole saliva flow rate

Preprandially and postprandially, women in the BN group had a lower whole saliva flow rate than did the controls. This finding was primarily explained by differences in medication use between the groups, which agrees with the literature describing potentially xenogenic effects in persons taking antidepressants and other drugs (17).

#### Glucose and hormones in blood

Preprandially, concentrations of insulin and glucose in blood were not significantly different between the 2 groups, which agrees with previous findings in control subjects and in persons with bulimia nervosa (19, 20). The present finding of similar preprandial blood leptin and ghrelin concentrations in the BN group and the control group agrees with the findings of other studies (20–22). Meanwhile, others have shown lower concentrations of leptin in persons with bulimia nervosa (19, 23–26) and higher concentrations of ghrelin under fasting conditions (27–29). It has been shown that persons with bulimia nervosa with low plasma concentrations of leptin had higher frequencies of binge/vomiting episodes (2.1 episodes/d) than did persons with bulimia nervosa with normal leptin concentrations (1.0 episodes/d) (30), and ghrelin concentration have been reported to be positively correlated with frequencies of binge/purging episodes (29). In our study population, only 2 subjects in the BN group had more than one binge/vomiting episode per day, which might explain why we found no alterations in leptin and ghrelin concentrations.

Postprandial blood glucose and insulin responses in both groups were normal (10, 31–34). Thus, judged from the resemblance between the groups preprandially and the postprandial blood glucose and insulin responses, one would expect that all participants had a normal response to food intake in that respect. This suggestion was supported by the blood responses of leptin concentrations, which tended to increase above the fasting level about 3 $h$ after intake of the meal; this finding agrees with that of other studies conducted in healthy persons (7, 10, 35, 36) and in persons with bulimia nervosa (19). Similarly, ghrelin responses in our study were in agreement with previous studies conducted in healthy persons (5–7, 31, 37). Thus, we showed a decrease in the ghrelin concentration induced by the meal with the lowest concentration approximately 1–2 $h$ after the meal and a slow recovery to baseline values during the following hours. The notion that persons with purging type bulimia nervosa have a blunted suppression of circulating ghrelin by food intake (20, 27) could not be verified in the present study.

Apparently, the subjects in the BN group in the present study responded normally to a carbohydrate-rich meal with regard to blood glucose, insulin, leptin and ghrelin. However, it cannot be ruled out that different meal compositions (both quantitatively and qualitatively) may result in different responses of these substances. The fact that our BN group had a frequency of binge/purge cycles of less than once a day might explain why we observed a normal diet-induced response in contrast with other studies that included persons with bulimia nervosa with a higher frequency of binge/purge cycles (29, 30).

#### Glucose and hormones in saliva

Preprandially, there were only minor differences in saliva concentrations between the groups. Calculations of saliva output were carried out to reduce the influence of differences in saliva flow rate (mL/min) between the groups. Thus, although there was a difference in glucose concentration (mg/L) in saliva between the BN group and the control group, the salivary glucose outputs ($\mu$g/min) did not differ. Because the salivary leptin concentration (ng/mL) was not significantly different between the 2 groups, the significantly decreased salivary leptin output (ng/min) seen in the BN group likely reflected the differences in salivary flow rate between the groups.

Postprandially, the 2 groups responded similarly, except for the response of salivary glucose concentration. Glucose measured in whole saliva reflects mainly glucose originating directly from foods eaten and to a minor degree from glucose transported from blood to the saliva. Thus, the peak glucose concentration seen in saliva just after intake of the breakfast was due to direct contamination of glucose from the foods eaten rather than from glucose originating from the circulation. The oral clearance of glucose is highly dependent on saliva flow rate (38, 39). Thus, subjects from the BN group with a lower flow rate eliminated oral glucose from the food more slowly than did the controls; as a result, the oral tissues were exposed to higher salivary glucose concentrations for a longer period of time. Accordingly, the glucose concentration in the BN group was approximately twice that of the controls 15 $h$ after the meal. After approximately

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**TABLE 5**

Correlations between scores of hunger and satiety in the bulimia nervosa (BN) and control groups determined with a visual analogue scale (VAS)

<table>
<thead>
<tr>
<th>VAS satiety</th>
<th>BN group (n = 19)</th>
<th>Control group (n = 20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>-45 min</td>
<td>$r = -0.59, P = 0.007$</td>
<td>$r = -0.59, P = 0.006$</td>
</tr>
<tr>
<td>15 min</td>
<td>$r = -0.51, P = 0.026$</td>
<td>$r = -0.54, P = 0.013$</td>
</tr>
<tr>
<td>45 min</td>
<td>$r = -0.46, P = 0.049$</td>
<td>$r = -0.62, P = 0.003$</td>
</tr>
<tr>
<td>75 min</td>
<td>$r = -0.59, P = 0.008$</td>
<td>$r = -0.81, P &lt; 0.0001$</td>
</tr>
<tr>
<td>105 min</td>
<td>$r = -0.61, P = 0.005$</td>
<td>$r = -0.85, P &lt; 0.0001$</td>
</tr>
<tr>
<td>165 min</td>
<td>$r = -0.51, P = 0.025$</td>
<td>$r = -0.82, P &lt; 0.0001$</td>
</tr>
<tr>
<td>225 min</td>
<td>$r = -0.42, P = 0.071$</td>
<td>$r = -0.75, P = 0.0001$</td>
</tr>
<tr>
<td>285 min</td>
<td>$r = -0.58, P = 0.009$</td>
<td>$r = -0.61, P = 0.0041$</td>
</tr>
<tr>
<td>Total$^1$</td>
<td>$P &lt; 0.0001^2$</td>
<td></td>
</tr>
</tbody>
</table>

$^1$ The overall correlation was calculated by ANOVA for repeated measures (SAS PROC MIXED; SAS Institute, Cary, NC).

$^2$ Additionally, the correlation between hunger and satiety was significantly different in the 2 groups ($P = 0.005$). Thus, the strongest correlations between hunger and satiety were seen in the control group.
1 h, the salivary glucose concentration had returned to the baseline level in both groups.

Hunger and satiety

Although all measured physiologic variables suggested that the BN group was not significantly different from the controls in response to a meal, the subjective feeling of hunger differed between the groups. Thus, the BN group rated their subjective feeling of hunger to be lower than that of the controls, whereas no significant differences in ratings of satiety were found. Several studies suggest that persons with BN have altered sensations of fullness, hunger, and satiety (2–4, 40), but other studies found no differences (41–43). In our study, most of the subjects in the BN group felt that the served breakfast was fairly substantial, and this was not the case for control persons. This might explain the lower ratings of hunger in the BN group. In a previous study conducted along these lines, it was reported that some persons with bulimia nervosa excessively restrict their dietary intakes when they are unable to vomit (44).

Meal-induced changes in blood mirrored by the saliva?

Generally, the meal-induced compositional changes in blood were not reflected directly in saliva composition. Thus, there were no correlations between glucose, insulin, and ghrelin concentrations in blood and saliva. However, leptin concentrations in blood and saliva were correlated, which is supported by other studies that describe a strong correlation between leptin in blood and stimulated whole saliva (45, 46).

Furthermore, the salivary output of glucose correlated with the blood glucose concentration, when the measurements for whole saliva contaminated with glucose originating directly from the food ingested were excluded. Because of both contamination with and clearance of glucose in whole saliva after intake of glucose or carbohydrate-containing foodstuffs, it would be more appropriate to carry out glucose measurements in saliva collected selectively from the parotid gland in preference to whole saliva, if the aim is to study the relation between glucose in blood and saliva. Nevertheless, our finding of a whole saliva glucose concentration that, on average, was 94–144 times lower than in blood agrees with results from a study conducted on stimulated parotid saliva that showed 120 times lower glucose concentrations in stimulated parotid saliva than in the glucose concentration in blood after oral administration of a glucose (47).

Insulin concentrations in blood correlated with salivary insulin output. Previous studies have described similar (38) or somewhat smaller insulin concentrations in saliva than in blood (32, 33, 48). Also, the postprandial delay in peak insulin in saliva as compared with plasma was shown previously (32–34, 48). However, additional studies are needed to cast light on the exact transport routes of insulin from blood across the glandular tissue to saliva to understand why a delay in the peak concentration of insulin between blood and saliva was observed.

No correlations between blood ghrelin concentrations and whole saliva ghrelin concentrations or outputs was shown. Previous studies have suggested that the salivary gland produces its own ghrelin (49, 50), and a direct correlation between ghrelin concentration in blood and saliva is therefore not expected. However, a previous study showed a correlation between blood and saliva ghrelin concentrations (50).

In conclusion, meal-induced changes in blood composition are not reflected directly by saliva. However, when the present methodologic considerations were taken into account, correlations for some substances were shown. Thus, saliva sampling is suitable as a noninvasive alternative to blood sampling.

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The authors’ responsibilities were as follows—AWD, AB, AA, BP, and BN: designed the study; BP: recruited the participants; AWD: conducted the study and carried out the glucose, insulin, and leptin analyses; JH: measured ghrelin concentrations; AWD and BN: analyzed the data and wrote the first draft of the manuscript; and all authors: provided advice and consultation and reviewed the final draft of the manuscript. None of the authors had any personal or financial conflict of interest.

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