Increased meal-induced neurotensin response predicts successful maintenance of weight loss

Data from a randomized controlled trial

Brethvad, Annemette Overgaard; Zakariassen, Hannah Louise; Holt, Joachim; Lundgren, Julie Rehné; Jakobsen, Alexander; Hartmann, Bolette; Lehmann, Eva Winning; Kissow, Hannelouise; Holst, Jens Juul; Madsbad, Sten; Torekov, Signe Sørensen; Holst, Birgitte

Published in:
Metabolism: Clinical and Experimental

DOI:
10.1016/j.metabol.2023.155534

Publication date:
2023

Document version
Publisher's PDF, also known as Version of record

Document license:
CC BY-NC-ND

Citation for published version (APA):

Download date: 27. sep., 2023
Increased meal-induced neurotensin response predicts successful maintenance of weight loss – Data from a randomized controlled trial

Annemette Overgaard Brethvad a,1, Hannah Louise Zakariassen a,b,*, Joachim Holt a,1, Julie Rehné Lundgren b, Alexander Jakobsen b, Bolette Hartmann a,b, Eva Winning Lehmann a, Hannelouise Kissow a,b, Jens Juul Holst a,b, Sten Madsbad c, Signe Sørensen Torekov a,c,2, Birgitte Holst a,2

1 These authors contributed equally to this work.
2 These authors share co-last authorship.

ARTICLE INFO

Keywords:
Neurotensin
NT
Obesity
Diet-induced weight loss
Weight loss maintenance
Weight regain
Radioimmunoassay

ABSTRACT

Background: The gut derived anorexigenic hormone neurotensin (NT) is upregulated after bariatric surgery which may contribute to the sustained weight loss. In contrast, diet-induced weight loss is most often followed by weight regain. We therefore investigated whether diet-induced weight loss impacts levels of circulating NT in mice and humans and whether NT levels predicts body weight change after weight loss in humans.

Methods: In vivo mice study: Obese mice were fed ad-libitum or a restricted diet (40–60 % of average food intake) for 9 days to obtain similar weight loss as observed in the human study. At termination, intestinal segments, the hypothalamus and plasma were collected for histological, real time PCR, and radioimmunoassay (RIA) analysis.

Clinical trial: Plasma samples from 42 participants with obesity, completing an 8-week low-calorie diet in a randomized controlled trial, were analyzed. Plasma NT was measured by RIA at fasting and during a meal test before and after diet-induced weight loss and after one year of intended weight maintenance.

Results: In obese mice, food restriction-induced body weight loss of 14 % was associated with a 64 % reduction in fasting plasma NT (p < 0.0001). In the mouse duodenum (p = 0.07) and jejunum (p < 0.05), NT tissue concentration was decreased without tissue atrophy indicative of a physiological downregulation. In the mouse hypothalamus a downregulation of Pomc (p < 0.01) along with upregulation of Npy (p < 0.001) and Agrp (p < 0.0001) expression was found after restricted feeding in support of increased hunger after diet-induced weight loss. Therefore, we investigated the NT response in humans undergoing weight loss maintenance. In humans, similar to the mice, the low-calorie diet induced weight loss of 13 % body weight was associated with 40 % reduction in fasting plasma NT levels (p < 0.0001). Meal-induced NT peak responses were greater in humans who lost additional weight during the 1 year maintenance phase compared to participants who regained weight (p < 0.05).

Conclusion: Diet-induced weight loss decreased fasting plasma NT levels in both humans and mice with obesity, and regulated hunger-associated hypothalamic gene expression in mice. Meal-induced NT responses were greater in humans who lost additional weight during the 1 year maintenance phase compared to participants who regained weight. This indicates that increased peak secretion of NT after weight loss may contribute to successful maintenance of weight loss.

Clinical trial registration number: NCT02094183.

Abbreviations: GLP-1, glucagon-like peptide-1; NTSR1, neurotensin receptor 1; RIA, radioimmunoassay; NT, neurotensin.

* Corresponding authors.
E-mail addresses: hlas@sund.ku.dk (H.L. Zakariassen), torekov@sund.ku.dk (S.S. Torekov).

https://doi.org/10.1016/j.metabol.2023.155534
Received 21 December 2022; Accepted 10 March 2023
Available online 16 March 2023
0026-0495/© 2023 The Authors. Published by Elsevier Inc. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).
1. Introduction

Global obesity rates and complications to obesity represent an urgent and overwhelming socio-economic challenge [1]. Weight losses of 5 to 10 % have been shown to prevent or delay the development of many obesity-related co-morbidities, including type 2 diabetes [2]. While clinical trials show that weight loss can be achieved through lifestyle interventions involving diet-restriction, the majority of participants gradually regain weight if no weight loss maintenance treatment is initiated [2–9]. As of now, bariatric surgery, although highly invasive, provides the most efficient weight loss maintenance [10]. However, incretin-based pharmacotherapy, leading to almost similar weight loss, has recently been approved [11, 12]. Furthermore, we have recently shown that combination of a GLP-1 receptor agonist and exercise further enhances diet-induced weight loss during a 1 year follow up period [7].

Human studies have suggested that diet-induced weight loss causes alterations in circulating levels of the neuroendocrine hormones ghrelin, peptide YY (PYY), leptin, insulin and GLP-1 targeting the arcuate nucleus [13–18]. Thus, in response to acute weight loss, increased levels of the orexigenic gut hormone, ghrelin, have been observed [13, 16, 17]. Interestingly, with gastric bypass decreased levels of ghrelin along with consistent increases in anorexigenic hormones have been reported [19–22]. Thus, surgical and diet-induced weight reductions seem to result in opposite physiological responses with respect to secretion of neuroendocrine hormones, which may be responsible for a stronger tendency to weight regain after diet-induced weight loss compared to surgery-induced weight loss.

The neuropeptide gut hormone neuropeptide Y (NT), primarily secreted from cells in the brain and in the gastrointestinal tract [23–25], has not previously been investigated in the context of diet-induced weight loss. NT from the enteroendocrine N-cells in the gut is released in response to food, particularly fat, and gastrointestinal tract fluids [26]. Peripherally administered NT in pharmacological doses inhibits food intake in rodents, inducing weight loss upon sustained exposure [27]. Further, plasma NT levels are significantly increased in patients after Roux-en-Y gastric bypass surgery [28–33], and antagonism of the NT receptor 1 (NTSR1) increases food intake in a rat model of gastric bypass surgery [20]. Additionally, NTSR1 knock out mice are less prone for gastric sleeve surgery induced anorexia [34]. This suggests that increased NT may contribute to the appetite lowering effects observed after bariatric surgery.

The hypothalamus is an important region for homeostatic appetite regulation, integrating signals related to nutritional state sent from the periphery. Neurons of the hypothalamic arcuate nucleus co-expressing neuropeptides neuropeptide Y (NPY) and agouti-related protein (AgRP) relay signals related to hunger, whereas pro-opiomelanocortin (POMC) induces satiety signaling when activated. The levels of expression of these peptides relay information that thus impacts on the intrinsic drive to eat [35].

We therefore investigated whether diet-induced weight loss impairs NT secretion along with the associated physiological gut and hypothalamic responses in mice undergoing food-restricted weight loss. In a previous study, we investigated whether diet-induced weight loss impacts levels of circulating NT in mice and humans and whether NT predicts weight change after weight loss in humans.

2. Materials and methods

2.1. In vivo study in mice

2.1.1. Animal husbandry and diet

Sixteen male C57BL/6J mice (Janvier, Le Genest-Saint-Ile, France) were fed a high-fat, high-sucrose diet (Surwit diet, 58 % kcal fat, D12331i, Research diets, New Brunswick, NJ, USA) from the age of 6 weeks, and were maintained on the diet for 9 months until they reached a body weight of ~48 g and the study was initiated. Animals had unlimited access to food and tap water during the entire study period, unless otherwise stated, and were housed in temperature- (22 ± 2 °C) and humidity-controlled rooms with a 12:12 h light-dark cycle. Experiments were conducted in accordance with bioethical guidelines and approved by the Animal Experimentation Inspectorate, Ministry of Environment and Food, Denmark (license no. 2014-15-0201-00181).

2.1.2. Study design

Mice were single housed for 2 weeks before study initiation. Eight days before study start, mice were weighed and MR scanned (Ecco MRI, Huston, TX, USA) before being allocated into two study groups according to body weight (primary) and fat mass (secondary) as follows: 1) food ad-libitum fed controls (n = 8) and 2) food restricted to 60 % (day 0–5) or 40 % (day 6–8) of their baseline food intake (average of 4 days) (n = 9). Food restriction to 40 to 60 % of average intake was chosen to induce a body weight loss (in per cent) similar to that observed in the clinical trial participants after 8 weeks on low calorie diet. During the study mice were weighed and their food intake was registered. On day 8, mice were MR scanned in the early light phase of the day.

2.1.3. Termination and tissue sampling

Mice were fasted 4 h prior termination in the early light phase of day 9. Blood samples were collected in EDTA tubes from the orbital venous plexus before euthanasia by cervical dislocation. The entire small intestine and colon were isolated from each mouse. Fecal matter was thoroughly removed using isotonic saline solution before recording of small intestine and colon weights. Samples were subsequently collected from the duodenum, jejunum, ileum and proximal colon for protein extraction and histological evaluation of the different segments. From the ventral side of the brain, the whole hypothalamus was collected by carefully isolating it using a microspatula from immediately posterior to the optic chiasma to the border of the mammillary bodies [36].

2.2. Tissue extraction and plasma analysis

Blood samples were centrifuged at 8000g for 10 min at 4 °C and plasma was stored at −20 °C until analysis. Intestinal tissue samples were stored at −80 °C until extraction. For hormone extraction, tissue was homogenized in 1 % trifluoroacetic acid and purified using Sep-Pak as described in [36]. Plasma and tissue extract concentrations of total NT, including intact active NT plus C-terminally truncated forms, were measured using our in-house-developed radioimmunoassay (RIA) (antibody code: 3D97) targeting the N-terminal of the NT molecule as previously described [37].

2.3. Histological evaluation of intestinal segments

Tissue from the intestinal segments was fixed in 4 % paraformaldehyde for 24 h at room temperature, dehydrated using 70 % ethanol and paraffin embedded. 4 μm transverse sections of the embedded tissues were cut and stained with hematoxylin. Sections were subsequently evaluated by an observer blinded for the protocol. The area of the mucosa, the crypt depth and the villus height were measured in each section using a light microscope connected to a camera (Zeiss Axio Lab.A1, Brock & Michelsen, Birkeroed, Denmark) and Zeiss Zen lite software (Carl Zeiss Microscopy GmbH, Göttingen, Germany). Results were related to body weight to account for differences in body weight between groups [38].

2.4. Real time PCR

Hypothalamus samples were snap frozen in liquid nitrogen and stored at ~ −80 °C before qPCR analysis. Extraction of RNA from the hypothalamus was performed using the RNeasy Lipid Tissue Mini kit (Qiagen, Hilden, Germany) with DNase digestion according to the manufacturers’ instructions. SuperScript III Reverse Transcriptase kit
(Thermo Fisher Scientific, Waltham, MA, USA) was utilized for synthesizing cDNA from RNA matching samples according to the manufacturers’ instructions. Real time PCR was performed using the PrecisionPLUS Master Mix on a LightCycler 480 (Roche Applied Science, Penzberg, Germany). Gene expression levels were calculated using the \( \Delta \Delta C_t \) method with gene expression levels normalized to the geometric mean of the housekeeping genes TATA-box binding protein (Tbp) and 14-3-3 protein zeta/delta (Ywhaz). Genes of interests evaluated were Pomp, Agrp and Npy (primer sequences, Supplementary Table A.1).

### 2.5. Randomized controlled clinical trial: weight loss maintenance after diet-induced weight loss in humans with obesity

We analyzed data from a randomized controlled trial that was carried out at the Department of Endocrinology, Hvidovre. A detailed description of the study outcome has been published previously [17].

#### 2.5.1. Study participants

Participants with obesity and without diabetes were included according to the following inclusion criteria; BMI > 30 and <40 kg/m\(^2\), age > 18 and <65 years. Exclusion criteria included acute or chronic illness (including diabetes) or participants taking pharmacological medication with known effects on glucose- and lipid metabolism. The trial followed the principles of the Declaration of Helsinki and Good Clinical Practice guidelines and was approved by the local ethics committee (reference number: H-4-2010-134). Participants provided written informed consent prior to inclusion. Participants were recruited from September 2011 to March 2012. The intervention period ran from October 2011 to June 2013. Baseline general and metabolic characteristics of participants are presented in Table 1.

#### 2.5.2. Study design

**2.5.2.1. Weight loss phase.** All participants were individually instructed by a clinical dietician on how to follow an 8-week low-calorie diet (800 kcal per day) from Cambridge Diet (Cambridge Weight Plan, Corby, UK).

**2.5.2.2. Weight loss maintenance phase.** After the acute 8-week weight loss, participants were randomized 1:1 into two groups, one group receiving the GLP-1 receptor agonist liraglutide 1.2 mg per day and the second group served as controls. In the following 52 weeks, both groups were instructed to follow a calorie-restricted diet (600 kcal less than their daily energy need). The liraglutide and control groups equally maintained the initial weight loss [17]. The participants were divided into subgroups regardless of the intervention described above: 1) “Weight Reduction” - participants that lost >3 % weight from “after weight loss” to week 52, and 2) “Weight Regain” - participants that gained ≥5 % weight from “after weight loss” to week 52 (Supplementary Figs. A.1 and A.2).

#### 2.5.3. Measurements and outcomes

Participants underwent a 3-hour liquid mixed meal test before and after the 8 weeks low-calorie diet. The participants met in the morning after an overnight fast and a cannula was inserted into a cubital vein for blood sampling. Fasting samples were drawn before the participants consumed a Fresubin Energy Drink (Fresenius Kabi Deutschland GmbH, Bad Homburg, Germany) of 600 kcal (35 % fat, 20 % protein and 50 % carbohydrate) followed by blood sampling at 15, 30, 45, 60, 90, 120, 150 and 180 min after meal consumption.

Plasma samples from a total of 42 participants who completed both test days and followed the weight maintenance phase were analyzed for concentrations of total NT (i.e. intact active NT plus C-terminally truncated forms) using an in-house-developed radioimmunoassay (RIA) (antibody code: 3D97) targeting the N-terminal of the NT molecule as previously described [37]. Plasma leptin, plasma PYY\(_{3-36}\) and plasma ghrelin were measured by radioimmunoassay (Millipore, Billerica, MA, USA). Concentrations of serum insulin was measured with Immulite 2000 solid-phase chemiluminescent immunometric assays (Immulite 2000; Siemens, Erlangen, Germany). Radioimmunological determinations of total plasma GLP-1 were performed as described [39].

### 2.6. Statistical analysis

Data from diet-induced obese mice were analyzed using GraphPad Prism version 8.3.1 (GraphPad Software). Differences between groups were examined by unpaired t-test. Food intake and body weight development over time was examined by two-way repeated measures ANOVA followed by Tukey’s post hoc test. All figures were generated using GraphPad Prism and show mean +/- SEM unless otherwise stated. One mouse was excluded from all data analysis of tissue concentrations of NT, while one colon sample from another mouse was excluded, due to obvious analytic errors.

Results from clinical trial study participants were analyzed using SAS enterprise guide version 7.15 (SAS institute, Cary, NC, USA). The primary outcome for the present analysis was change in fasting and meal-induced change (0-30 min) in peak NT concentrations. Analyses of before and after weight loss within-participant differences from all study participants were evaluated using paired student’s t-test. For comparison

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Participant characteristics from clinical trial.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before weight loss</td>
</tr>
<tr>
<td>Bodyweight (kg)</td>
<td>97.5 ± 1.7</td>
</tr>
<tr>
<td>BMI (kg/m(^2))</td>
<td>34.4 ± 0.5</td>
</tr>
<tr>
<td>Fat mass (%)</td>
<td>42.1 ± 0.9</td>
</tr>
<tr>
<td>Lean mass (%)</td>
<td>55.7 ± 0.8</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>124 ± 2</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>79 ± 2</td>
</tr>
<tr>
<td>HbA1c (mmol/mol)</td>
<td>44 ± 0.9</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>5.1 ± 0.1</td>
</tr>
<tr>
<td>Fasting values</td>
<td></td>
</tr>
<tr>
<td>Insulin (pmol/l)</td>
<td>77 ± 6</td>
</tr>
<tr>
<td>Leptin (ng/ml)</td>
<td>32 ± 2</td>
</tr>
<tr>
<td>Neurotensin (pmol/l)</td>
<td>7.9 ± 0.8</td>
</tr>
<tr>
<td>PYY(_{3-36}) (pg/ml)</td>
<td>76 ± 4</td>
</tr>
<tr>
<td>Ghrelin (pg/ml)</td>
<td>790 ± 55</td>
</tr>
<tr>
<td>GLP-1 (pmol/l)</td>
<td>7 ± 6</td>
</tr>
</tbody>
</table>
of the subgroups of “Weight reduction” and “Weight regain”, within-participant variances and simple differences between subgroups were examined by paired or unpaired student’s t-test, respectively. Differences in total area under the curve (AUC) covering time points 0–180 min were computed by a linear mixed model with an unstructured covariance pattern. Missing data were implicitly handled by maximum likelihood estimation in the model. Forty-three (43) participants with obesity were included in the analysis. Results from 1 participant was excluded in the analysis of NT due to analytical error. A flow chart of study participants with available data and reasons for missing data is shown in Supplementary Fig. A.2.

3. Results

3.1. Food restriction-induced weight loss in diet-induced obese mice decreases fasting plasma NT levels

A cohort of diet-induced obese mice was exposed to 40 to 60 % food restriction for 9 days and compared to ad libitum fed mice (control group). The food restriction resulted in a significant weight loss of approximately 14 % (p < 0.01) similar to the weight loss in the human cohort described above. Also, fat mass was significantly reduced on day 8 in the food restricted group relative to the control group (p < 0.01) (Fig. 1a–d). Leptin decreased by 28 % (control group average 1.98 ± 0.09 ng/ml; food restricted group average 1.42 ± 0.11 ng/ml; p < 0.01) and insulin by 47 % (control group average 0.62 ± 0.06 ng/ml; food restricted group average 0.33 ± 0.03 ng/ml; p < 0.001) in the food restricted group compared to mice in the control group (Fig. 1f–g). Diet-induced weight loss led to a significant reduction in fasting plasma NT levels by 64 % in food restricted mice at day 9 (control group average 98.6 ± 10.9 pmol/l; food restricted group average 35.8 ± 3.8 pmol/l; p < 0.0001) (Fig. 1h).

3.2. Food restriction induces increased Npy and Agrp expression along with reduction in Pomp expression

When assessing alterations in hypothalamic gene expression levels of mice in response to diet induced weight loss, we found a significant downregulation of the expression of Pomp (p < 0.01). Moreover, levels of Agrp (p < 0.0001) and Npy (p < 0.001) expression were both significantly upregulated in the food restricted group relative to ad libitum fed control mice (Fig. 1e). The gene expression is consistent with an orexigenic response in the food restricted mice.

3.3. Weight loss induced a reduction in duodenal and jejunal NT concentrations without inducing intestinal tissue atrophy in mice

The observed decrease in fasting NT levels in plasma could be linked to changes in the secretion of NT from the intestines and/or atrophy of the intestinal tissue. To investigate the source of the reduced plasma levels, different intestinal segments from the mice were weighed and measured for NT tissue concentration as well as evaluated histologically for signs of atrophy. The restricted feeding and body weight loss did not affect the small intestinal or the colon weight relative to body weight in the food restricted group (Fig. 2a–b). Moreover, histological evaluation showed no differences in overall area of mucosa, villus height or crypt depth between groups in any of the tissue segments sampled (Supplementary Figs. A.3 and A.4). Overall, these results show that restricted feeding did not induce atrophy of the intestine presumably reflected in an unchanged number of NT-producing enteroendocrine cells.

Evaluation of tissue concentrations of NT in intestinal segments revealed significantly decreased NT levels in the jejunum, amounting to a decrease of 34 % in mice from the food restricted group compared to controls (control group average 81.6 ± 6.1 pmol/g; food restricted group average 53.8 ± 9.5 pmol/g; p < 0.05). No significant changes, but numerically reductions in NT concentrations was found in other intestinal segments. NT decreased by 43 % in the duodenum (p = 0.07), 21 % in the ileum (p = 0.33) and 11 % in the colon (p = 0.6) of food restricted mice (Fig. 2c–f).

Fig. 1. Food-restriction prompted weight loss in obese mice, induced altered expression levels of energy balance related genes in the hypothalamus and decreased fasting plasma NT levels. Diet-induced obese mice subjected to ad libitum (Food ad-lib) (n = 8) or restricted feeding for 9 days (n = 9). a) Cumulative food intake day 0–9 (g), b) body weight development day 0–8 (ΔBody weight (%)), c) absolute body weight day 8 (g), d) fat mass day 8 (g), e) Quantitative qPCR analysis of expression levels of the hypothalamic genes Pomp, Agrp and Npy (arbitrary units representing % of Food ad-lib group), f) fasting plasma leptin at termination day 9 (ng/ml), g) fasting plasma insulin at termination day 9 (ng/ml) and h) fasting plasma NT at termination day 9 (pmol/l). Data are shown as mean ± SEM. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001.
3.4. Diet-induced weight loss induces reduction in fasting plasma NT levels in humans with obesity

To assess whether NT secretion was altered after weight loss in participants with obesity, plasma NT levels was analyzed at fasting and during a meal test before and after the participants completed 8 weeks on a low-calorie diet. Participants lost a total of 12.3 kg weight (95 % CI: 13.2 to 11.3) equivalent to 12.6 % (p < 0.0001) after the low-calorie diet. BMI and body fat percentage were reduced by 4.2 kg/m$^2$ (95% CI: 4.6 to 3.9, p < 0.0001) and 2.2 % (95 % CI: −2.8 to −1.7, p < 0.0001), respectively. Total lean mass percentage increased by 2.7 % (95 % CI: 2.1 to 3.3, p < 0.0001) (Table 1). After weight loss, fasting plasma NT levels decreased by approximately 40 % upon diet-induced weight loss, (mean difference: 3.2 pmol/l, 95 % CI: 4.9 to 1.4, p < 0.001) (Fig. 3). The meal responses of peak NT (change from 0 to 30 min) and total AUC$^{0-180}$ were unchanged by diet-induced weight loss (mean difference: 1.95 pmol/l, 95 % CI: 3.85 to 7.75 and 205 pmol/l, 95 % CI: 647 to 237, respectively, Supplementary Fig. A.5a).

3.5. Increased meal-induced NT response predicts successful maintenance of weight loss

To investigate whether the plasma concentration of NT after diet-induced weight loss could predict weight-loss maintenance success during a subsequent 52-week follow up period, we analyzed samples from a sub-population of trial participants that regained >5 % weight (n = 15) or lost >3 % additional weight (n = 9) in this period (Fig. 4a). The meal response of peak NT (change from 0 to 30 min) immediately after the weight loss was significantly higher in the subgroup that lost additional weight compared to the group that regained weight (difference in mean change 10.1 pmol/l, 95 % CI: 1.0 to 19.3, p < 0.05, Table 2, Fig. 4 and Supplementary Fig. A5.b). Excluding the four men did not change the results. Fasting plasma NT levels after weight loss did not vary between the two subgroups. The peak meal response of NT was similar in the group treated with liraglutide compared to the control group after 52 weeks of weight maintenance (difference between means 3.6 (95 % CI -5.5 to 12.7), p = 0.43).

To further evaluate pathophysiological aspects of the complex neurohormonal circuitry that regulates changes in body weight the neuroendocrine hormones leptin, insulin, PYY$_{3-36}$, ghrelin and GLP-1 were also analyzed.

Concentrations of leptin, insulin, PYY$_{3-36}$, ghrelin and GLP-1 before and after weight loss are shown in Table 1. Comparisons of the neuroendocrine hormones in the weight reduction and weight regain groups immediately after weight loss are shown in Table 2. Interestingly, only NT concentrations were significantly higher in participants who reduced weight compared to the participants who regained weight (Table 2).
Fig. 4. Baseline meal-induced peak NT response is significantly increased in participants who lost additional weight after a 52 week follow up period. Participants were subdivided into a “weight regain” group that regained >5 % weight (n = 15) or “weight reduction” group who lost an additional >3 % weight (n = 9), during the 52-weeks weight maintenance period. a) Body weight development during the clinical trial period from after diet induced weight loss (baseline (week 0)) and to after the weight maintenance period (week 52). b) Meal-induced plasma NT response (pmol/l) from fasting to 30 min post-prandially (∆ NT 30 min–0 min) in the weight regain group (squares) and weight reduction group (circles). Data are shown as mean ± SEM, *p < 0.05.

### 4. Discussion

The underlying mechanisms responsible for weight regain after diet-induced weight loss remain unclear [40,41], and very little is known about the hormonal regulation following diet-induced weight loss [42–44]. Here we show that fasting levels of NT in plasma decrease after diet-induced weight loss in both humans and mice with obesity. Interestingly, participants that experienced an additional weight loss of >3 % in the course of a 52-week maintenance period had an immediately increased meal-induced NT peak response compared with the group of participants who regained >5 % in body weight after initial weight loss of 12.3 kg. This suggests that an initial increase in meal-induced NT response could predict successful maintenance of weight loss.

Given the evidence of anorexigenic action of circulating NT in the interstitial space of the gut predominantly into the NT molecule (13 amino acids) along with a larger molecule (125 amino acids) containing the remaining part of the precursor (pro-NT) [26] (Supplementary Fig. A.6). Both molecules are subsequently released into the circulation [26], however, pro-NT displays an improved stability in plasma samples indicative of prolonged half-life compared to the 1.5 min reported for NT in human studies [46–48]. Previous evaluations of plasma pro-NT in human cohorts from prospective and cross-sectional studies have identified associations between fasting plasma pro-NT levels and several metabolic conditions such as obesity, diabetes mellitus, cardiovascular disease and non-alcoholic fatty liver disease [49–53]. In contrast, our analyses were performed utilizing a RIA measuring total NT, i.e. the sum of the intact, active NT plus C-terminal truncated forms from the gastrointestinal tract [37] (see Supplementary Fig. A.6). The primary (inactive) metabolite displays a relatively short half-life in humans [47]. Thus, our measurements

### Table 2

Baseline characteristics of participants who reduced weight or regained weight after diet-induced weight loss. Absolute values are shown as mean ± SEM. Differences between means are shown with 95 % confidence intervals.

<table>
<thead>
<tr>
<th></th>
<th>Weight Reduction (n = 9 women)</th>
<th>Weight Regain (n = 11 women, 4 men)</th>
<th>Mean difference (95 % CI)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>47 ± 4</td>
<td>49 ± 2</td>
<td>−2 (−10 to 6)</td>
<td>0.58</td>
</tr>
<tr>
<td>Weight (kg) after LCD</td>
<td>84.8 ± 2.4</td>
<td>86.4 ± 2.5</td>
<td>−1.6 (−9.4 to 6.2)</td>
<td>0.68</td>
</tr>
<tr>
<td>BMI (kg/m²) after LCD</td>
<td>31.3 ± 0.7</td>
<td>30.4 ± 0.7</td>
<td>0.8 (−1.4 to 3.1)</td>
<td>0.45</td>
</tr>
<tr>
<td>Fasting values</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Insulin (pmol/l)</td>
<td>41 ± 4</td>
<td>46 ± 5</td>
<td>−5 (−20 to 10)</td>
<td>0.49</td>
</tr>
<tr>
<td>Plasma leptin (ng/ml)</td>
<td>14 ± 2</td>
<td>13 ± 3</td>
<td>1 (−8 to 10)</td>
<td>0.81</td>
</tr>
<tr>
<td>Meal induced change (0–30 min)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neurotensin (pmol/l)</td>
<td>31 ± 3</td>
<td>21 ± 3</td>
<td>10.1 (1.0 to 19.3)</td>
<td>0.03</td>
</tr>
<tr>
<td>GLP-1 (pmol/l)</td>
<td>17 ± 4</td>
<td>10 ± 2</td>
<td>7 (−1 to 16)</td>
<td>0.10</td>
</tr>
<tr>
<td>PYY3-36 (pg/ml)</td>
<td>35 ± 9</td>
<td>22 ± 6</td>
<td>12 (−11 to 36)</td>
<td>0.29</td>
</tr>
<tr>
<td>Ghrelin (pg/ml)</td>
<td>−85 ± 32</td>
<td>−101 ± 44</td>
<td>16 (−127 to 158)</td>
<td>0.82</td>
</tr>
</tbody>
</table>
reflects the biological active NT better than measurements of pro-NT.

Our investigations of how weight loss affected plasma NT levels in an obese mouse model showed that mice subjected to restricted feeding displayed similar decreases in fasting plasma NT levels after weight loss as those observed in humans. When we evaluated the intestine of the mice, the results suggested that weight loss induces a physiological decrease in NT secretion rather than a pathological decrease due to intestinal cell atrophy. As such, our examinations points to an active regulation of NT synthesis and secretion from the gut in response to weight loss. This is in agreement with the role of NT as an anorexigenic hormone serving a compensatory function to maintain energy balance. In support of the increased hunger sensation upon diet-induced weight loss, we evaluated the neuroendocrine hormones, leptin, insulin, ghrelin, PYY, NPY and AGRP gene expression levels in hypothalamic biopsies from mice subjected to restricted feeding.

To further define the human pathophysiological aspects of the complex neurohormonal circuitry that regulates body weight we also evaluated the neuroendocrine hormones, leptin, insulin, ghrelin, PYY, NPY and AGRP in controls and obese individuals. Interestingly, only meal-induced NT concentrations were significantly higher in the group of participants who lost additional weight compared to the group who regained weight. This underlines the potential of NT as a predictor of weight maintenance success.

The strengths of this study include the use of a translational approach to investigate the impact of diet-induced weight loss on levels of circulating NT in mice and humans, in prediction of weight change after weight loss in humans. The findings were based on observational results from a randomized controlled clinical trial. Furthermore, NT was measured using a RIA which measures total NT thereby reflecting the biological active NT. A limitation of the study was the relatively low number of subjects in each subgroup, with mostly women included in the study, which is a normal feature of human weight loss studies [7].

5. Conclusion

Maintenance of weight loss imposes an unsolved challenge in obesity treatment and limited molecular and cellular understanding is currently available to explain the mechanism underlying the almost invariable weight regain. We aimed to investigate whether weight loss impact levels of NT in the circulation at fasting and peak response to a meal. Moreover, we wanted to examine whether changes in circulating NT levels could predict successful weight loss maintenance. Utilizing a novel approach directly targeting the total secretion of NT (i.e. the sum of the intact molecule and its degradation products), we found that diet-induced weight loss results in a marked decrease in fasting levels of NT in both humans and mice with obesity. Participants that lost additional weight in a 52-week maintenance period had an immediate higher NT secretory response after a meal compared to participants that regained weight. Based on these finding we suggest that an efficient NT response to a meal challenge may predict success of weight loss maintenance in humans. Thereby highlighting the importance of future targeting methods for increased neurotensin for successful weight loss maintenance in humans.

CRediT authorship contribution statement

A.O.B., H.L.Z.; contributed to the conceptualization of the studies, data collection, analysis and interpretation of pre-clinical and clinical data, wrote and edited the original draft of the manuscript. J.H.; contributed to data collection, analysis and interpretation of the clinical data, edited and revised the manuscript. J.R.L., A.J., B. Hartmann, J.J.H., E.W.L., H.K.; contributed to data collection, edited and revised the manuscript. S.M.: edited and revised the manuscript. S.S.T., B.Holst.: contributed to the conceptualization of the studies, interpretation of pre-clinical and clinical data, edited and revised the manuscript.

Declaration of competing interest

E.W.L. has since January 2022 been employed at Novo Nordisk A/S. B. Holst has since October 2021 been employed at the Novo Nordisk Foundation. S.M. and J.J.H. have performed consulting services for Novo Nordisk. SST have received research grants and lecture fees from Novo Nordisk. The remaining authors have no competing interests to declare.

Acknowledgements

This work was supported by a research grant from the Danish Diabetes Academy, which is funded by the Novo Nordisk Foundation, grant number NNFI7SA0031406U and by an Excellence grant (NNFI6OC0019968, to Dr. Torekov) from the Novo Nordisk Foundation. The clinical trial project was supported by funding from The Danish Research Counsel, Health and Disease (reference number: 11-107683). Cambridge Weight Plan products were donated from Cambridge Weight Plan. The funding sponsors were not involved in study design, conduct of the study, data analysis or approval of manuscript.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.metabol.2023.155534.

References
