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**Title page****Title:**

Weight loss decreases self-reported appetite and alters food preferences in overweight and obese adults: observational data from the DiOGenes study

**Author Names:** Charlotte Andriessen <sup>a,b</sup>, Pia Christensen <sup>a</sup>, Lone Vestergaard Nielsen <sup>a</sup>, Christian Ritz <sup>a</sup>, Arne Astrup <sup>a</sup>, Thomas Meinert Larsen <sup>a</sup>, J. Alfredo Martinez <sup>c</sup>, Wim H.M. Saris <sup>d</sup>, Marleen A. van Baak <sup>d</sup>, Angeliki Papadaki <sup>e,f</sup>, Marie Kunesova <sup>g</sup>, Susan Jebb <sup>h</sup>, John Blundell <sup>i</sup>, Clare Lawton <sup>i</sup>, Anne Raben <sup>a</sup>

**Author affiliations:**

<sup>a</sup> Department of Nutrition, Exercise and Sports, University of Copenhagen, Denmark (CA, PC, LVN, CR, AA, TML, AR)

<sup>b</sup> Department of Human Nutrition, Wageningen University, The Netherlands (CA)

<sup>c</sup> Center for Nutrition Research, University of Navarra, Pamplona, CIBERObn, Fisiopatología de la Obesidad y Nutrición, Madrid, Spain (JAM)

<sup>d</sup> Department of Human Biology and Movement Sciences, NUTRIM School of Nutrition and Translational Research in Metabolism, Maastricht University, The Netherlands (WHS, MAvB)

<sup>e</sup> Centre for Exercise, Nutrition and Health Sciences, School for Policy Studies, University of Bristol, Bristol, United Kingdom (AP)

<sup>f</sup> Department of Social Medicine, Preventive Medicine & Nutrition Clinic, University of Crete, Heraklion, Crete, Greece (AP)

<sup>g</sup> Institute of Endocrinology, Obesity Management Centre, Prague, CR (MK)

<sup>h</sup> Nuffield Department of Primary Care Health Sciences, University of Oxford, UK (SJ)

<sup>i</sup> School of Psychology, University of Leeds, Leeds, United Kingdom (JB, CL)

**Corresponding Author:**

Dr. Pia Christensen,

Department of Nutrition, Exercise and Sports,

Rolighedsvej 26, 1; 1958 Frederiksberg, Denmark

Email: [piach@nexs.ku.dk](mailto:piach@nexs.ku.dk); Telephone +45 3533 2614

**Authors' last names and e-mail addresses:** Andriessen ([c.andriessen@alumni.maastrichtuniversity.nl](mailto:c.andriessen@alumni.maastrichtuniversity.nl)), Christensen ([piach@nexs.ku.dk](mailto:piach@nexs.ku.dk)), Vestergaard Nielsen ([lvn@nexs.ku.dk](mailto:lvn@nexs.ku.dk)), Ritz ([ritz@nexs.ku.dk](mailto:ritz@nexs.ku.dk)), Astrup ([ast@nexs.ku.dk](mailto:ast@nexs.ku.dk)), Meinert Larsen ([tml@nexs.ku.dk](mailto:tml@nexs.ku.dk)), Martinez ([jalfmtz@unav.es](mailto:jalfmtz@unav.es)), Saris ([w.saris@maastrichtuniversity.nl](mailto:w.saris@maastrichtuniversity.nl)), van Baak ([m.vanbaak.maastrichtuniversity.nl](mailto:m.vanbaak.maastrichtuniversity.nl)), Papadaki ([Angeliki.Papadaki@bristol.ac.uk](mailto:Angeliki.Papadaki@bristol.ac.uk)), Kunesova ([mkunesova@endo.cz](mailto:mkunesova@endo.cz)), Jebb ([susan.jebb@phc.ox.ac.uk](mailto:susan.jebb@phc.ox.ac.uk)), Blundell ([J.E.Blundell@leeds.ac.uk](mailto:J.E.Blundell@leeds.ac.uk)), Lawton ([C.L.Lawton@leeds.ac.uk](mailto:C.L.Lawton@leeds.ac.uk)), Raben ([ara@nexs.ku.dk](mailto:ara@nexs.ku.dk))

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**Running title:** Weight loss and eating behaviour

**Abbreviations:** AUC, Area Under the Curve; CCK, cholecystokinin; CID, Clinical Investigation Day; d-AUC, delta Area Under the Curve; DiOGenes, Diet, Obesity and Genes; E%, Percent of Energy; FCPQ, Forced Choice Photographic Questionnaire; FCQ, Food Choice Questionnaire; FPC, Food Preference Checklist; LCD, Low Calorie Diet; LMM, Linear Mixed Model; VAS, Visual Analogue Scale; WHO, World Health Organization

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125  
126

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128

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## 1 **Abstract**

2 People with obesity often struggle to maintain their weight loss after a weight loss period.  
3 Furthermore, the effect of weight loss on appetite and food preferences remains unclear.  
4 Hence this study investigated the effect of weight loss on subjective appetite and food  
5 preferences in healthy, overweight and obese volunteers. A subgroup of adult participants (n  
6 = 123) from the Diet Obesity and Genes (DiOGenes) study (subgroup A) was recruited from  
7 across six European countries. Participants lost  $\geq 8\%$  of initial body weight during an 8-week  
8 low calorie diet (LCD). Subjective appetite and food preferences were measured before and  
9 after the LCD, in response to a standardized meal test, using visual analogue rating scales  
10 (VAS) and the Leeds Food Choice Questionnaire (FCQ). After the LCD, participants reported  
11 increased fullness ( $p < 0.05$ ), decreased desire to eat ( $p < 0.05$ ) and decreased prospective  
12 consumption ( $p < 0.05$ ) after consuming the test meal. An interaction effect (visit x time) was  
13 found for hunger ratings ( $p < 0.05$ ). Area under the curve (AUC) for hunger, desire to eat and  
14 prospective consumption was decreased by 18.1%, 20.2% and 21.1% respectively whereas  
15 AUC for fullness increased by 13.9%. Preference for low-energy products measured by the  
16 Food Preference Checklist (FPC) decreased by 1.9% before the test meal and by 13.5% after  
17 the test meal ( $p < 0.05$ ). High-carbohydrate and high-fat preference decreased by 11.4% and  
18 16.2% before the test meal and by 17.4% and 22.7% after the meal ( $p < 0.05$ ). No other  
19 effects were observed. These results suggest that LCD induced weight loss decreases the  
20 appetite perceptions of overweight volunteers whilst decreasing their preference for high-fat-,  
21 high-carbohydrate-, and low-energy products.

22 **Keywords:** LCD; weight loss; body weight maintenance; hunger; Leeds Food Choice  
23 Questionnaire; Visual Analogue Scale

## 24 **Introduction**

25 According to the World Health Organization, the prevalence of obesity more than doubled  
26 between 1980 and 2014, rising to over 600 billion adults with obesity worldwide (1). This  
27 increase is often attributed to an increasingly obesogenic environment, characterized by a  
28 sedentary lifestyle and by easily available, energy-dense foods (2). Losing weight seems to be  
29 a logical solution for the obesity epidemic. However, losing weight and, in particular,  
30 maintaining this weight loss proves difficult. A study conducted in the US showed that less  
31 than 20% of people who attempted to lose weight could maintain a 10% weight reduction for  
32 over a year (3). Since diet plays an important role in weight regain, a better understanding of  
33 the effect of weight loss on subjective appetite and food choice is required.

34 Subjective appetite is generated, in part, by physiological mechanisms occurring before and  
35 after a meal. In turn, these mechanisms respond to, and are modulated by, long-term energy  
36 intake and expenditure (4, 5). Weight loss is typically caused by a long-term energy deficit  
37 and might, therefore, influence physiological appetite mechanisms (6). Indeed, previous  
38 studies have shown that a period of weight loss increases self-reported perceptions of hunger  
39 and the drive to eat (7).

40 The hedonic value of food also plays a role in eating behaviours. In essence, people are able  
41 to eat foods they find palatable in the absence of hunger (8). Obese people generally report a  
42 higher preference for high-fat and high-sugar products than lean people, which has been  
43 hypothesized to stem from a decreased sensitivity to sweet and fatty tastes (9). However, to  
44 date it is unclear if obesity is the cause or the consequence of this decreased sensitivity (9).  
45 Studies in obese participants who have undergone Roux-en-y gastric bypass surgery have  
46 shown that high-fat and high-sugar products are preferred before surgery whereas fruit and  
47 vegetable products are preferred after surgery (10). It is currently unknown if this shift in food  
48 preference is caused by physiological changes due to the surgery or by the weight loss

296  
297  
298 49 resulting from the surgery (11). Weight loss has, however, been shown to alter food  
299  
300 50 preferences in previous studies (12, 13).  
301  
302 51 The findings of previous studies examining changes in post weight loss appetite and food  
303  
304 52 preferences are inconsistent (7, 12-17). Understanding the factors that influence eating  
305  
306 53 behaviours at the end of a weight loss period is, however, essential to develop strategies to  
307  
308 54 prevent subsequent weight regain. Hence, this study focuses on the effect of substantial  
309  
310 55 weight loss induced by an 8-week LCD on self-reported appetite and food preferences in  
311  
312 56 overweight and obese adults (18).  
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## 318 **Participants and Methods**

### 320 321 322 **Study design**

323  
324 61 Participants included in the reported study were a sub-group of participants (subgroup A)  
325  
326 62 from the overarching Diet Obesity and Genes (DiOGenes) study ([http://www.diogenes-](http://www.diogenes-eu.org/)  
327  
328 63 [eu.org/](http://www.diogenes-eu.org/)) (18). The DiOGenes study was a pan-European, randomized intervention study that  
329  
330 64 examined the long-term effect of five different intervention diets on body weight maintenance  
331  
332 65 after a weight loss period (18, 19). Enrolment onto one of the intervention diets, was  
333  
334 66 dependent upon participants losing at least 8% of their initial body weight by means of an 8-  
335  
336 67 week low-calorie diet (LCD) (Modifast; Nutrition et Santé, Revel, France, **Table 1**). The diet  
337  
338 68 provided participants with 3.6 MJ energy per day, which they could supplement with up to  
339  
340 69 400 g of raw vegetables, resulting in a maximal energy intake of 4.5 MJ per day. This sub-  
341  
342 70 study reports on the effect of substantial weight loss induced by a LCD on subjective appetite  
343  
344 71 and food preferences in response to a standardized meal test administered before and on the  
345  
346 72 last day of the weight loss period, with participants still in negative energy balance. These  
347  
348 73 outcome n variables represent secondary outcomes of the overarching DiOGenes study upon  
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355  
356  
357 74 which a priori analyses were performed to identify psychological predictors of weight regain.  
358  
359 75 The DiOGenes study has previously been described in more detail by Larsen et al. and Moore  
360  
361 76 et al. (18, 19). The present results have not previously been published.  
362  
363  
364 77

## 365 366 78 **Participants**

367  
368 79 Both male and female participants were recruited from November 2005 to April 2007.  
369  
370 80 Participants were either overweight or obese (body mass index [BMI] between 27-45 kg/m<sup>2</sup>)  
371  
372 81 and were between 18 and 65 years old. Only participants who completed the meal test before  
373  
374 82 and at the end of the weight loss period are included in the statistical analyses of this sub-  
375  
376 83 study. An extensive overview of the inclusion and exclusion criteria for the DiOGenes study  
377  
378 84 is provided by Larsen et al. (18). Procedures followed in the DiOGenes study were in  
379  
380 85 accordance with the Declaration of Helsinki and approved by local ethics committees in all  
381  
382 86 participating countries. Written informed consent was obtained from all participants.  
383  
384  
385 87

## 386 387 88 **Standard meal test**

388  
389 89 A homogeneous test meal consisting of 220 g pasta served with 75 g of an oven roasted  
390  
391 90 vegetables sauce (Dolmio express! Fusili Pasta and Dolmio ‘Stir-in’ sauce - Oven Roasted  
392  
393 91 Vegetables, MarsFoods, Dublin, Ireland) was provided to all participants before (Clinical  
394  
395 92 Investigation Day [CID] 1) and at the end of the LCD (CID2) at lunchtime. The test meal  
396  
397 93 provided a total weight of 295 g (total energy: 1.6 MJ, macronutrient content: 13 g, 13 percent  
398  
399 94 of energy (E %) protein, 11 g, 26 E% fat and 63.7 g, 61 E% carbohydrates).  
400  
401 95 Participants were requested to fast overnight before each test meal and were allowed to drink  
402  
403 96 a maximum of 1 dl water before the test. Participants were instructed to consume all of the  
404  
405 97 test meal and were free to drink as much water as they wanted during the test. Visual  
406  
407 98 analogue rating scales (VAS) and the Leeds Food Choice Questionnaire (FCQ) were used to  
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414  
415  
416 99 assess appetite perceptions and food preferences, respectively (15, 20-24). The FCQ was  
417  
418 100 completed 15 minutes before and after consumption of the test meal. The VAS appetite  
419  
420 101 ratings were obtained at 15 minutes before and then at 15, 30, 60, 90, 120, 150, and 180  
421  
422 102 minutes after the start of the test meal.  
423  
424

425 103

#### 426 104 **Appetite questionnaires**

427  
428 105 The VAS for appetite measurement consisted of a series of 100 mm horizontal lines anchored  
429  
430 106 with extreme appetite perceptions on both ends of each line (e.g. not at all hungry – very  
431  
432 107 hungry). They were used to answer each of the following 4 questions: How hungry are you?  
433  
434 108 (not at all hungry – very hungry), How full do you feel? (not at all full – very full), How  
435  
436 109 strong is your desire to eat? (not at all strong – very strong), How much food do you think you  
437  
438 110 can eat? (none at all – a large amount). Participants from all research centres received the  
439  
440 111 same instructions on how to fill out the VAS (15). VAS were digitally presented to the  
441  
442 112 participants and were available in the languages of all participating countries. In case of  
443  
444 113 computer problems, paper and pencil VAS were provided.  
445  
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449 115

#### 450 116 **Leeds Food Choice Questionnaire**

451  
452 117 Food preferences were measured using the FCQ. The FCQ consisted of the Food Preference  
453  
454 118 Checklist (FPC) and the Forced Choice Photographic Questionnaire (FCPQ) which were  
455  
456 119 adapted to fit with the eating habits of the participating countries as necessary (20-24). These  
457  
458 120 questionnaires were digitally presented to the participants and were available in the languages  
459  
460 121 of all participating countries. Paper and pencil questionnaires were provided to participants in  
461  
462 122 case of computer problems.  
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465 123

#### 466 124 *Food Preference Checklist*

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474  
475 124 The FPC is composed of a list of written descriptions of 32 common food items. Participants  
476  
477 125 were asked to examine each individual food item (e.g. a roast chicken breast) in turn and to  
478  
479 126 make an assessment as to whether or not they would like to eat it at that particular moment in  
480  
481 127 time by responding “yes” or “no”. Participants were instructed to consider food items  
482  
483  
484 128 independently from each other, and to limit their thinking time for any one of the food items.  
485  
486 129 The foods that were described could be divided into one of four different categories: high-fat,  
487  
488 130 high-carbohydrate, high-protein (each food contained at least 50 % of total energy as the  
489  
490 131 macronutrient by which it was categorised and foods were presented in portions  
491  
492 132 corresponding to approximately equal energy content [180-220 kcal]), with the exception of  
493  
494 133 low-energy foods (averaging 25 kcal per portion). There were 8 food descriptions per  
495  
496 134 category. Within the high-fat, high-carbohydrate and low energy categories, 4 foods were  
497  
498 135 savoury and 4 were sweet. In the high-protein category all foods were savoury. Hence, the  
499  
500 136 minimal score for each category was 0 (“no” for each question) and the maximal score was 8  
501  
502 137 (“yes” for each question). In addition, the total frequency of chosen food items was calculated  
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505 138 (score ranged between 0 and 32).

507 139

#### 509 140 *Forced Choice Photographic Questionnaire*

510  
511 141 During the FCPQ, participants were presented with photographs of two different foods and  
512  
513 142 instructed to indicate which one they preferred more. Participants were instructed to imagine  
514  
515 143 they could eat as little or as much of the chosen food as desired. In total, 30 pairs of food  
516  
517 144 photographs were shown and the foods (20 in total) could be divided into one of the following  
518  
519 145 categories: high-fat/savoury, high-fat/sweet, low-fat/savoury, and low-fat/sweet. For example,  
520  
521 146 participants could choose between a picture of a doughnut (high-fat/sweet) or a jelly pudding  
522  
523  
524 147 (low-fat/sweet). Each preference category was presented 5 times in 3 different combinations,  
525  
526 148 providing 15 presentations in total. Thus, the total score for each category ranged from 0 to  
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532  
533  
534 149 15. From these scores, separate scores for the sub-categories high-fat (sweet plus savoury),  
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536 150 low-fat (sweet plus savoury), savoury (low-fat plus high-fat), and sweet (low-fat plus high-  
537  
538 151 fat) were calculated. The minimal score for the sub-categories was 5, since there was always  
539  
540 152 one combination in which a sub-category was inevitably chosen (e.g. high-fat/sweet vs high-  
541  
542 153 fat/savoury would always give a high-fat preference), and this combination occurred 5 times  
543  
544 154 in the questionnaire. There were 4 combinations in which a sub-category could be chosen  
545  
546 155 over the other sub-categories, and these combinations occurred 5 times. Including the minimal  
547  
548 156 score of 5 (above), the maximal score was, therefore, 25 for each sub-category.  
549  
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## 552 553 158 **Statistical methods**

554  
555 159 Linear mixed models (LMM) were used to assess differences in subjective appetite and food  
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557 160 preferences before and at the end of the weight loss period. All LMMs included visit (before  
558  
559 161 [CID1] or at the end of the weight loss period [CID2]), time (time points at which the  
560  
561 162 questionnaires were filled out) and their interaction as well as adjustment for age and sex.  
562  
563 163 Random effects for centre and subjects were also included in all models. For appetite ratings,  
564  
565 164 the baseline value (CID1, at 15 minutes before the meal test) of the appetite rating was also  
566  
567 165 included as a covariate. If a significant time-visit interaction effect was found, pairwise  
568  
569 166 comparisons between CID1 and CID2 were performed for each time point. Tukey's test was  
570  
571 167 used to correct for multiple comparisons. In an additional analysis, centres were treated as  
572  
573 168 fixed effects to allow comparisons between centres. In case a centre effect was present,  
574  
575 169 pairwise comparisons with a Tukey correction were performed to identify differences between  
576  
577 170 centres.  
578  
579 171 To assess if the change in appetite perception from CID1 to CID2 was affected by the weight  
580  
581 172 loss period, a linear regression model was fitted with delta area under the curve ([d-AUC],  
582  
583 173 defined as AUC at CID1 minus CID2) of the VAS appetite rating as the outcome variable,  
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591  
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593 174 and absolute weight loss as the independent variable. Total AUC was calculated using the  
594  
595 175 trapezoid method (25). Age and sex were included in this model as covariates. Furthermore,  
596  
597 176 Pearson's correlation coefficients between weight loss percentage and d-AUC of appetite  
598  
599 177 were calculated. Statistical analyses were performed with the software programme R (26).  
600  
601 178 Data were not transformed prior to analysis. Results were considered significant when  $p <$   
602  
603 179 0.05. Graphs were constructed using GraphPad Prism 7.  
604  
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606 180

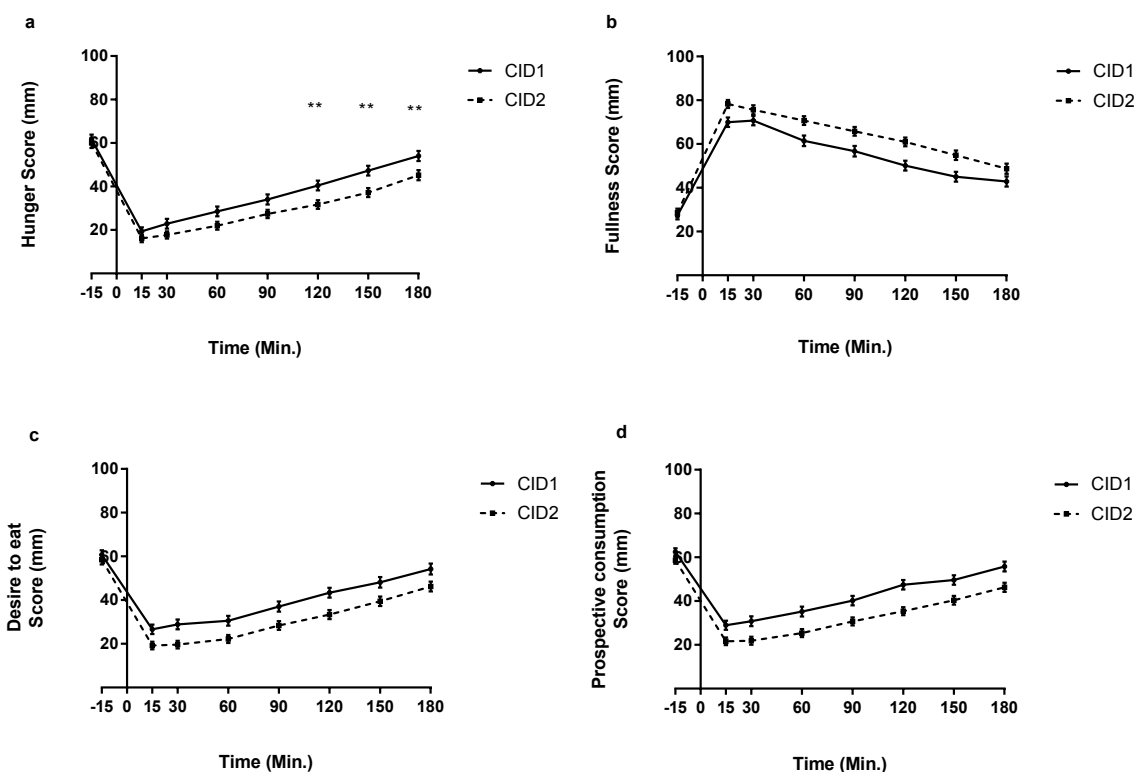
## 607 608 181 **Results**

609  
610 182 In total, 151 participants from across 6 European intervention sites (i.e. the Netherlands,  
611  
612 183 Denmark, United Kingdom, Germany, Spain, and Czech Republic) were included in this sub-  
613  
614 184 study of which 123 (48 males and 75 females) participants participated in the meal test both  
615  
616 185 before and at the end of the LCD. Drop-out rates were highest in Spain (32.3 %) and lowest in  
617  
618 186 Denmark (6.3 %).  
619  
620 187 Participants were on average (mean  $\pm$  SD)  $41.2 \pm 5.2$  years of age with an average (mean  $\pm$   
621  
622 188 SD) body weight of  $100.0 \pm 16.8$  kg at baseline. They had a mean weight loss of  $11.1$  kg ( $\pm$   
623  
624 189 0.2) during the LCD. Due to missing values, data from 11 participants could not be included  
625  
626 190 in any of the AUC analysis of appetite ratings. For 1 additional participant, only AUC for  
627  
628 191 prospective consumption could not be calculated because of missing values.  
629  
630  
631 192

## 632 633 193 **Appetite**

634  
635 194 Mean VAS rating scores for the different appetite perceptions are presented in **Figure 1**. The  
636  
637 195 before meal rating of prospective consumption was significantly lower ( $\chi^2(1) = 4.20$ ,  $p <$   
638  
639 196 0.05) at the end of the LCD period than before this period ( $62.5 \pm 1.7$  before the LCD  
640  
641 197 compared with  $58.6 \pm 1.6$  at the end of the LCD). The before meal ratings of hunger, fullness  
642  
643 198 and desire to eat were not significantly altered by the LCD period ( $p > 0.05$ ). For hunger there  
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199 was a significant time-visit interaction ( $\chi^2(1) = 6.26, p < 0.05$ ), corresponding to significant  
 200 decreases in hunger at CID2 relative to CID1 at 120, 150, and 180 minutes after consumption  
 201 of the test meal. Fullness was generally increased at the end of the weight loss period ( $\chi^2(1) =$   
 202  $91.93, p < 0.05$ ), whilst desire to eat ( $\chi^2(1) = 111.93, p < 0.05$ ) and prospective consumption  
 203 ( $\chi^2(1) = 153.63, p < 0.05$ ) were decreased. There were a number of differences in appetite  
 204 ratings between the countries involved in the research, but no systematic differences were  
 205 observed.



208 **FIGURE 1**

209 Mean  $\pm$  SEM visual analogue scale appetite scores (0 – 100 mm) before (t = -15) and after a test meal (at t = 0)  
 210 before (CID1) and at the end of (CID2) a weight loss period. Appetite measurements included (a) hunger, (b)  
 211 fullness, (c) desire to eat, and (d) prospective consumption. Appetite scores over time were analysed using a  
 212 linear mixed model procedure. \*\* Significantly differences in appetite after weight loss: \*\* P < 0.01.

214 CID, Clinical Investigation Day.

709  
710  
711 215  
712  
713 216 There was an 18 % decrease in AUC for hunger at the end of the LCD period compared to  
714  
715 217 before this period. AUC for desire to eat decreased by 20 %, AUC for prospective  
716  
717  
718 218 consumption decreased by 21 %, and AUC for fullness increased by 14 % at the end of the  
719  
720 219 LCD. Regression analysis of the d-AUC appetite scores showed significant correlations  
721  
722 220 between absolute weight loss (kg) and hunger [F (1, 217) = 9.20, p < 0.05], fullness [F (1,  
723  
724 221 217) = 3.89, p < 0.05], desire to eat [F (1, 217) = 9.95, p < 0.05], and prospective  
725  
726 222 consumption [F (1, 215) = 4.89p < 0.05]. No significant correlations were found between  
727  
728 223 weight loss percentage and delta AUC of hunger, fullness, desire to eat, and prospective  
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730 224 consumption (p > 0.05).  
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## 734 226 **Leeds Food Choice Questionnaire**

### 735 227 736 228 *Food Preference Checklist*

739 229 Significant decreases in preference were found for low-energy foods ( $\chi^2$  (1) = 4.82, p < 0.05),  
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741 230 high-carbohydrate foods ( $\chi^2$  (1) = 11.52, p < 0.05) and high-fat foods ( $\chi^2$  (1) = 22.46, p <  
742  
743 231 0.05) at the end of the LCD period (**Table 2**). Before the test meal, a decrease in preference  
744  
745 232 for low-energy foods (1.9 %), high-carbohydrate foods (11.4 %), and high fat foods (16.2 %)  
746  
747 233 was observed after the LCD compared to before this period. After the test meal, decreases in  
748  
749 234 preference were found for low-energy foods (13.5 %), high-carbohydrate foods (17.4 %), and  
750  
751 235 high-fat foods (22.7 %) in response to the LCD. The total frequency of chosen foods  
752  
753 236 decreased both before (6.3 %) and after the test meal (15.2 %), as a result from the LCD (p <  
754  
755 237 0.05). Preference for high-protein foods remained unaltered at the end of the weight loss  
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757 238 period (p > 0.05).  
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770 239 In response to the test meal, preference for low-energy foods ( $\chi^2(1) = 116.45, p < 0.05$ ), high-  
771  
772 240 protein foods ( $\chi^2(1) = 232.74, p < 0.05$ ), high-fat foods ( $\chi^2(1) = 107.72, p < 0.05$ ), and high-  
773  
774 241 carbohydrate foods ( $\chi^2(1) = 187.08, p < 0.05$ ) was decreased (table 2). Hence, the total  
775  
776 242 frequency of chosen foods was also significantly lower after the test meal compared to before  
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778 243 this meal ( $\chi^2(1) = 239.09, p < 0.05$ ). There were no differences between centres in food  
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781 244 choice measured by the FPC ( $p > 0.05$ ).

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#### 785 246 *Forced Choice Photographic Questionnaire*

787 247 No significant differences in food preferences assessed before and after the weight loss period  
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789 248 were found (**Table 3**,  $p > 0.05$ ). There were, however, significant differences in preferences  
790  
791 249 assessed before and after the test meal. After the test meal, participants reported a higher  
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793 250 preference for sweet ( $\chi^2(1) = 184.34, p < 0.05$ ) and high-fat products ( $\chi^2(1) = 17.64, p <$   
794  
795 251  $0.05$ ) compared to before the meal. Preference for low-fat ( $\chi^2(1) = 17.64, p < 0.05$ ) and  
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797 252 savoury products ( $\chi^2(1) = 184.34, p < 0.05$ ) decreased after the test meal. Differences in  
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799 253 preferences between the centres were also observed, but these differences were not  
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801 254 systematic.

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#### 806 256 **Discussion**

807 257 Our study showed that substantial LCD-induced weight loss generally decreased postprandial  
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809 258 appetite perceptions, when measured while the participants were still in negative energy  
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811 259 balance. Fullness was increased, whilst hunger, desire to eat and prospective consumption  
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813 260 were all decreased in response to weight loss. Furthermore, the overall number of foods  
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815 261 selected from the Food Preference Checklist (FPC) was also decreased in response to the  
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817 262 LCD. Hence, the FPC showed a decreased preference for low-energy-, high-carbohydrate-,  
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819 263 and high-fat foods. In the fasted state, prospective consumption was significantly lower after  
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821 264 the LCD compared to before the LCD. No other significant findings were observed.

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829 265 Both the changes found in subjective appetite and food preferences support a reduced interest  
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831 266 in food after weight loss. Therefore, our findings cannot explain the weight regain people with  
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833 267 obesity often experience after a weight loss period (3). From our results, it appears that  
834  
835 268 subjective appetite is influenced by body weight, which has also been shown in previous  
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837 269 studies (16, 27). However, a cohort study by Gregersen et al. did not show a relationship  
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839 270 between BMI and subjective appetite (28). These investigators compared lean with obese  
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841 271 participants, without a weight loss intervention. In contrast, we assessed subjective appetite in  
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843 272 overweight and obese participants before and immediately after a weight loss period. At the  
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845 273 end of this period, participants were still in negative energy balance. It might, therefore, be  
846  
847 274 that the state of negative energy balance influenced the appetite sensations we measured.  
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849 275 Indeed, the study of Sumithran et al. showed that different physiological processes occur  
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851 276 during an energy depleted state and during refeeding. This study found the VAS appetite  
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853 277 ratings from their overweight and obese male and female participants remained unchanged  
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855 278 after an 8-week very low-calorie diet (17). They hypothesized that this response might have  
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857 279 resulted from the ketogenic state participants were experiencing due to the low carbohydrate  
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859 280 content of the weight loss diet. This hypothesis was also supported by the observation that,  
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861 281 after 2 weeks of refeeding, appetite ratings were significantly increased compared with those  
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863 282 assessed immediately after the weight loss period. This increase in appetite after 2 weeks  
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865 283 might also indicate that different physiological processes occur immediately after a weight  
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867 284 loss period compared to those occurring during a subsequent period of weight maintenance. It  
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869 285 is likely that the LCD used in our study did not lead to a ketogenic state in all our participants.  
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871 286 However, it is possible that the LCD induced a ketogenic state in men, since men generally  
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873 287 have a higher energy expenditure than women. Unfortunately, we do not have measurements  
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875 288 to support this theory.  
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888 289 In contrast to our study, a study by Seimon et al. found an increase in desire to eat in both lean  
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890 290 and obese men after consuming a 30 % energy restricted diet for 4 days (29). However, the  
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892 291 hormonal response in this study differed from the response normally associated with an  
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894 292 increase in appetite after a period of fasting. Specifically, the anorexic hormones peptide YY  
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896 293 (PYY) and cholecystinin (CCK) were increased in both lean and obese male participants.  
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898 294 Normally, decreases in PYY and CCK, together with an increase in hunger, are reported after  
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900 295 a period of fasting (30). This finding reflects the complexity of the physiological processes  
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902 296 occurring during energy restriction. A possible explanation for this discrepancy could be that  
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904 297 physiological processes regulating appetite in the body are influenced by the duration of the  
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906 298 energy restriction. Also, it has been hypothesized that the relative fat content of the diet can  
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908 299 alter gastrointestinal transit time, and thereby modulate appetite response (14, 31, 32). The  
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910 300 genetic profile of obese people may also modulate the effect of dietary fat on the consequent  
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912 301 appetite response (33). Moreover, the study by Seimon et al. measured subjective appetite in  
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914 302 response to an intraduodenal lipid infusion, whereas our study measured appetite in response  
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916 303 to consumption of a test meal (29). Hence, the lipid infusion in the study by Seimon et al. did  
917  
918 304 not pass the stomach. Bypassing of the stomach has major impact on subjective appetite (5).  
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920 305 Therefore, it is highly likely that the differences in appetite found in the study by Seimon et  
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922 306 al. (increased desire to eat) and our study (decreased appetite) can be attributed to processes in  
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924 307 the stomach that influence appetite.

928 308 Similarly, patients that undergo Roux-en-Y gastric bypass (RYGB) surgery frequently report  
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930 309 increased postprandial satiety after surgery (34-37). The surgery is accompanied by a  
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932 310 substantial amount of weight loss (approximately 35 % of initial body weight), which seems  
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934 311 to be sustained long term (at least two years) (38, 39). Surgery increases the postprandial  
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936 312 levels of the orexigenic hormones glucagon-like peptide 1 (GLP-1) and PYY, which might  
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938 313 cause the increased satiety and subsequent weight loss (34-37). However, it is also possible  
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947 314 that the weight loss itself results in altered hormone levels and increased satiety, since the  
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949 315 RYGB studies have not been able to draw inferences on causality due to their observational  
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951 316 nature (34-37). Our study cannot support the hypothesis that weight loss increases  
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953 317 postprandial satiety through increased levels of PYY and GLP-1 response, as we did not  
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955 318 measure any biological parameters related to satiety. Previous studies did show an increased  
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957 319 level of postprandial GLP-1 and PYY after dietary weight loss, indicating that weight loss  
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959 320 might be accountable for the observed changes after RYGB (40, 41). Both GLP-1 and PYY  
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961 321 are related to decreased appetite and seem to inhibit gastric emptying, which in turn also  
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963 322 affects appetite (5). At present, it is unclear how gastric emptying is affected by RYGB and  
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965 323 studies investigating the effect of dietary weight loss on gastric emptying are scarce (42, 43).  
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967 324 Interestingly, the decrease in food preference (measured with the FPC) for high-carbohydrate  
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969 325 and high-fat foods that we observed at the end of the LCD also corresponds to the decreased  
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971 326 food preference for high-energy foods reported after RYGB surgery (10). This finding seems  
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973 327 to strengthen the hypothesis that the observed changes in food intake after RYGB can, at least  
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975 328 partially, be attributed to weight loss.  
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977 329 However, it is also possible that other mechanisms than the weight loss itself influenced our  
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979 330 results. For example, the negative energy balance of the participants might have affected the  
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981 331 sympathetic nervous system (SNS). The SNS has a profound role in gastric intestinal  
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983 332 processes. In lean people, fasting suppresses the SNS and increases appetite (5, 44). However,  
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985 333 obese people appear to have an over-activity of the SNS and therefore their appetite response  
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987 334 to a decreased SNS activity might be different (44). It might be that a suppressed SNS in obese  
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989 335 people leads to a more sensitive response to incoming nutrients which results in decreased  
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991 336 postprandial appetite.  
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1006 338 Furthermore, appetite is not only influenced by physiological mechanisms but also by  
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1008 339 psychological mechanisms (45). Habituation to the LCD is a potential psychological  
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1010 340 mechanism that could have influenced our results. Before the LCD, participants could have  
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1012 341 been habituated to portion sizes that were bigger than our test meal and thus reported lower  
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1014 342 feelings of satiety after eating the test meal. Unfortunately, we did not measure eating habits  
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1016 343 before the LCD. A study by Berg et al. supports habituation to bigger meals before the LCD.  
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1018 344 This study showed that obese men and women generally choose bigger sized portions than  
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1020 345 lean people. In contrast, this same study also presented an association between being obese  
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1022 346 and omitting lunch (46). This association might suggest that most of our (overweight and  
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1024 347 obese) participants were not habituated to having lunch. Thus, comparing the habitual lunch  
1025  
1026 348 (nothing) of our participants with the test meal (which was served at lunch time) does not  
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1028 349 favour a decrease in post-prandial satiety after the test meal (before the LCD). However, it  
1029  
1030 350 could be possible that participants became habituated to the LCD. The portions during the  
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1032 351 LCD were smaller compared to the test meal and therefore participants might have  
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1034 352 experienced a higher postprandial satiety at the end of the weight loss period.  
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1036 353 In addition, our results might have been affected by the motivation to lose weight. Only  
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1038 354 participants that lost 8% of their body weight were included in this sub-study, which includes  
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1040 355 only participants that were very motivated to lose weight. In addition, the 8% weight loss  
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1042 356 might have served as an additional motivational factor by improving the body image of our  
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1044 357 participants. This motivation might have resulted in lower appetitive standards. Furthermore,  
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1046 358 participants were subjected to a 6- or a 12-month weight maintenance diet after the LCD. In  
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1048 359 anticipation of the weight maintenance period it would be undesirable for participants to feel  
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1050 360 hungrier, which might have also added to the lower appetitive standards. Results from the  
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1052 361 FPC showed that participants appeared to have a lower preference for most food types.  
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1054 362 Hence, the total frequency of chosen products was decreased after the LCD induced weight  
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1065 363 loss, reflecting a lower preference for low-energy-, high-carbohydrate-, and high-fat foods.  
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1067 364 Our findings contradict the majority of studies reporting that the reward value of food is  
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1069 365 increased after food deprivation (8). The changes in food preferences observed in our study  
1070  
1071 366 may be partially explained by the study by Anton et al. (12). In this two-year intervention  
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1073 367 study, overweight participants were assigned to one of four different weight loss diets. Each  
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1075 368 diet provided participants with a 750 kcal deficit in daily energy intake calculated from the  
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1077 369 participants' baseline energy expenditure. Regardless of the diet, participants reported  
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1079 370 reductions in food cravings for high-fat foods, fast-food fats, sweets, and  
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1081 371 carbohydrates/starches after 6 months, 12 months and 24 months of dieting. It was  
1082  
1083 372 hypothesized that the participants' association between consumption of the typically  
1084  
1085 373 unhealthy foods and the feeling of emotional relief became lower after a prolonged period of  
1086  
1087 374 limited intake of these products. This decreased association was thought to decrease the  
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1089 375 preference for unhealthy foods (12).  
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1091 376 A previous study also showed that a 3-month weight loss period induced an earlier satiation to  
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1093 377 a sweet stimulus. In this study, participants repeatedly ingested a sweet stimulus until they felt  
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1095 378 displeased with the stimulus. The time leading up to displeasure was significantly shorter after  
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1097 379 the weight loss period than before. It was hypothesized that the earlier satiation experienced  
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1099 380 with the sweet stimulus was indicative of a lowered body weight set-point (47). Hence,  
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1101 381 homeostatic mechanisms would favour a lower food intake after weight loss to accommodate  
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1103 382 the decreased body weight. Our study supports this theory since food preference in our study  
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1105 383 resulted in a decreased preference for food in general. However, the frequently observed  
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1107 384 regain of body weight after a weight loss period suggests that there are mechanisms that can  
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1109 385 override the body weight set-point (3).  
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1124 387 The Forced Choice Photographic Questionnaire (FCPQ) did not reveal any alterations in food  
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1126 388 preference. A possible explanation for the discrepancy between the results of the FPC and the  
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1128 389 FCPQ is that the FCPQ forces the participant to choose between two target food stimuli and  
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1130  
1131 390 thereby measures the relative behavioural preference (48). Furthermore, although the FCPQ  
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1133 391 has been validated in a wide range of research, the FCPQ is highly dependent on the quality  
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1135 392 of the pictures (48-50). It is generally assumed that the way food is presented visually can  
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1137 393 influence people's flavour perception and modify their food choices (51). Despite the fact that  
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1139 394 each food in the photographs was presented in a standardized fashion (i.e. on a white plate or  
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1141 395 in a glass bowl), it is possible that the appearance of the food itself might have influenced  
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1143 396 participants' choices.  
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### 1148 398 **Strengths and limitations**

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1150 399 Our study has several limitations. Firstly, we examined subjective appetite and food choice in  
1151  
1152 400 response to a fixed test meal with use of VAS rating scales and food choice questionnaires,  
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1154 401 rather than assessing objective satiety and food choice via an ad libitum test meal. Although  
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1156 402 VAS ratings are a validated tool to measure subjective appetite, in some studies subjective  
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1158 403 appetite does not reflect actual food intake (52, 53). In our study, we found significant  
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1160 404 reductions in subjective appetite following weight loss. However, the changes observed were  
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1162 405 rather small. Hence, it is not clear if the differences in appetite would translate to a lower food  
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1164 406 intake. Often, an ad libitum test meal is offered to participants after a preload to objectively  
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1166 407 assess the effects of the preload on food choice and energy intake (4). However, an ad libitum  
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1168 408 test meal is not itself infallible since the variety of foods offered to participants is typically  
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1171 409 different from their usual eating pattern. This, therefore, acts to stimulate interest in the  
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1173 410 different foods provided and thus promotes increased food intake from the ad libitum meal  
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1175 411 (4).  
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1183 412 Another limitation of our study is the possibility that the interpretation of the end points of the  
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1185 413 VAS scales (e.g. not at all hungry – very hungry) was different before compared to after the  
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1187 414 weight loss period. Hence, this potential difference in interpretation could have influenced the  
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1189 415 resulting appetite ratings. A method that circumvents the problem of interpretation of the  
1190  
1191 416 endpoints is the general Labeled Magnitude Scale (gLMS). This method uses a scale with  
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1193 417 endpoints that are external to the perception measured (i.e. no perception – strongest  
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1195 418 imaginable perception of any kind). Since these endpoints refer to any kind of perception  
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1197 419 experienced, the gLMS eliminates difficulties with interpretation of the endpoints (54, 55). In  
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1199 420 contrast, it has been found that the VAS and gLMS give comparable results when assessing  
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1201 421 within-subject differences, while they differ when comparing across different subject groups.  
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1203 422 Since our study only examined within-subject differences, it appears that both methods  
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1205 423 would have been suitable tools to measure appetite (54, 55). Nevertheless, a validation study  
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1207 424 that compares appetite perceptions before and after a weight loss period using both the VAS  
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1209 425 and the gLMS seems to be warranted.  
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1215 427 Finally, we did not control for individual differences in food preferences when using the  
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1217 428 FCPQ (4). However, these individual differences could be compensated for by the large  
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1219 429 sample size of our study. This large sample size, in combination with the multi-centred nature  
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1221 430 of our study, increased the external validity of our study and allowed us to observe small but  
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1223 431 potentially important changes in food choice and appetite.  
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### 1227 433 **Future studies**

1230 434 Future studies are necessary to gain more insight into the mechanisms responsible for the  
1231  
1232 435 changes in appetite and food preferences observed in our study. Indeed, it might be interesting  
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1234 436 to examine the effect of weight loss combined with increased exercise on appetite, since fat  
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1242 437 free mass has been positively associated with energy intake and self-determined meal size in  
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1244 438 obese participants (56). Additionally, exercise has been shown to affect food preference (57).  
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1246 439 Currently, the PREVIEW study investigates how intensity of exercise (and type of diet) might  
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1248 440 help to improve weight loss maintenance, amongst other study outcomes (58). It might also be  
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1250  
1251 441 interesting for future studies to examine eating behaviour throughout a period of weight  
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1253 442 maintenance. Measuring appetite in an energy homeostatic state eliminates interference of the  
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1255 443 effects of a negative energy balance on appetite, and therefore permits conclusions about the  
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1257 444 effect of weight loss on appetite. Also, the period of weight loss maintenance could provide  
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1259 445 information on the time it takes for participants to habituate to a diet.  
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#### 1262 1263 447 **Conclusion**

1264  
1265 448 In conclusion, our study showed that postprandial appetite and food preferences were altered  
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1267 449 in favour of a decreased food intake after the substantial weight loss induced by a LCD.  
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1269 450 Results from our study show that eating behaviour immediately after a period of LCD-  
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1271 451 induced weight loss does not seem to explain the weight regain frequently reported in other  
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1273 452 studies (3). Hence, it is likely that appetite and food preferences observed after a weight loss  
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1275 453 period are altered during the phase of weight loss maintenance.  
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1304  
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1307  
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1309  
1310 460 A., P. C., L. V. N., C. R., A. M., M. A. B., A. P., M. K. and A. R. analysed data; C. A., P. C.,  
1311  
1312 461 C.L., and A. R. wrote the paper; A. R. had the primary responsibility for the final content; All  
1313  
1314 462 authors read and approved the final manuscript..  
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**TABLES****TABLE 1**

Nutritional composition of the LCD per kg of diet

	<b>LCD</b>
<b>Energy, MJ / kg</b>	16.4
<b>Protein, g / kg</b>	254.6
<b>Carbohydrates, g / kg</b>	545.5
<b>Fat, g / kg</b>	90.9

LCD, Low Calorie Diet

**TABLE 2**

The frequency of each food type chosen on the Food Preference Checklist before and after test meal consumption at each visit

	Frequency				P-values		
	CID1		CID2		Time	Visit	Visit x Time
	Before meal (n=117)	After meal (n=119)	Before meal (n=117)	After meal (n=116)			
<b>High-fat</b>	3.7 ± 2.2	2.2 ± 0.2	3.1 ± 2.3	1.7 ± 0.2	< 0.001	< 0.001	0.88
<b>High-carbohydrate</b>	4.4 ± 1.9	2.3 ± 0.2	3.9 ± 2.0	1.9 ± 0.2	< 0.001	< 0.001	0.71
<b>High-protein</b>	5.2 ± 2.1	2.3 ± 0.3	5.2 ± 2.1	2.0 ± 0.2	< 0.001	0.86	0.24
<b>Low-energy</b>	5.2 ± 1.7	3.7 ± 0.2	5.1 ± 1.9	3.2 ± 0.2	< 0.001	< 0.05	0.25
<b>Total frequency</b>	18.4 ± 5.6	10.5 ± 0.7	17.3 ± 6.2	8.9 ± 0.7	< 0.001	< 0.001	0.46

Before meal values are represented as mean ± SD; After meal values are presented as mean ± SE. The values indicate how often a certain food type was chosen on the Food Preference Checklist. Time represents before and after the test meal. Visit represents the visits before (CID1) and after (CID2) the 8-week low calorie diet. Results were analysed using a linear mixed model procedure.

CID, Clinical Investigation Day

**TABLE 3**

The frequency of each food type chosen on the Forced Choice Photographic Questionnaire before and after test meal consumption at each visit

	Frequency				P-values		
	CID1		CID2		Time	Visit	Visit x Time
	Before meal (n=114)	After meal (n=109)	Before meal (n=115)	After meal (n=112)			
<b>High-fat</b>	13.3 ± 3.5	14.1 ± 0.3	13.2 ± 3.4	14.1 ± 0.3	< 0.001	0.26	0.16
<b>Low-fat</b>	16.1 ± 3.5	15.9 ± 0.3	16.8 ± 3.4	15.9 ± 0.3	< 0.001	0.26	0.16
<b>Savoury</b>	18.9 ± 3.6	14.3 ± 0.5	19.6 ± 3.8	14.5 ± 0.5	< 0.001	0.61	0.45
<b>Sweet</b>	11.1 ± 3.6	15.7 ± 0.5	10.4 ± 3.8	15.5 ± 0.5	< 0.001	0.61	0.45

Before meal values are represented as mean ± SD; After meal values are presented as mean ± SE. The values indicate how often a certain food type was chosen on the Forced Choice Photographic Questionnaire. Time represents before and after the test meal. Visit represents the 8-week low calorie diet. Results were analysed using a linear mixed model procedure.

CID, Clinical Investigation Day.









































































