

# Analysis of macronutrient composition of binge-eating episodes in a nonclinical normal weight sample

Romina Müller, Friederike Barthels, Frank Meyer, Reinhard Pietrowsky

# Abstract

**Purpose:** In a sample of normal weight persons with binge eating behavior this study assessed the macronutrient composition of binges and additionally investigated food intake and psychopathological aspects compared to a sample of non-bingeing normal weight controls.

**Method:** 17 participants with a subclinical binge eating disorder (BED; binge group, BG), and 19 non-bingeing participants (control group, CG) recorded their eating behavior for seven days, including any binge eating episodes. The BG additionally recorded their binge episodes for subsequently another two months.

**Results:** Both groups consumed an equal amount of energy per day (2220 vs. 2230 kcal). Overall eating behavior of BG contained equal amounts of fat (35% vs. 34%), carbohydrates (48% vs. 48%) and proteins (16% vs. 17%) when compared to the CG. Macronutrient composition of binge episodes was characterized by a high intake of fat (45%) and carbohydrates (44%) and less proteins (10%). Participants of the BG had a significantly higher pathological eating behavior, were more depressed and felt less vital than the controls. **Conclusions:** Overall, macronutrient composition of binges in normal weight subjects with subclinical BED is in line with the macronutrient composition of binges displayed by bulimic and overweight BED individuals. Therefore, the binge-associated clinical symptoms in these patient groups seem to be caused by factors other than binge composition, which might be psychological determinants as well as differences in metabolism and hormone levels.

**Keywords:** binge eating disorder, macronutrients, normal weight, binge quality, eating disorder

#### Citation

Müller R, Barthels F, Meyer F, Pietrowsky R: Analysis of macronutrient composition of binge eating episodes in a non-clinical normal weight sample. Ernahrungs Umschau 2021; 68(1): 18–25. This article is available online: DOI: 10.4455/eu.2021.004

#### **Peer-Reviewed**

Manuscript (original contribution) received: May 12, 2020 Revision accepted: September 14, 2020

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# Introduction

Atypical eating habits, especially stress-induced eating, has become more common even in the normal population. Lifetime prevalence rates of people suffering from binge eating disorder (BED) have been, according to DSM-5 criteria, proposed to rise enormously (2.9% in women; 3.0% in men; [1). Binge eating is primarily characterized by frequent binge eating episodes defined as "(...) eating, in a discrete period of time, an amount of food that is definitely larger than what most people would eat during a similar period of time under similar circumstances (...)" as well as "(...) a sense of lack of control over eating during the episode" [2]. Although atypical eating behavior is the key symptom of the disorder, there are only a few studies concerning quality, quantity and especially the macronutrient composition of binge episodes. In contrast to bulimia nervosa, there are only a few and mainly laboratory studies focusing on food consumption during a binge episode in BED. Fitzgibbon and Blackman [3] compared the composition of binges of binge eaters with those of bulimic individuals. They found a higher consumption of snacks and desserts in subjects with BED as well as a higher intake of fat, less consumption of proteins and a comparable intake of carbohydrates.

Concerning quality and quantity of binge episodes in non-purging subjects, Rossiter et al. [4] reported the mean duration of a binge to be about 38 minutes. Total energy intake on binge days was about 2400 kilocalories compared to 1500 kilocalories per day on non-binge days. Beyond that, a significantly lower consumption of proteins on binge days could be observed [4]. Other findings suggest a significantly higher energy intake of obese individuals on binge days than of the non-binge obese control



group. Food was mainly ingested in the evening [5]. Moreover, there are hints of a reduced overall energy intake of subjects with BED [5].

Allison and Timmerman [6] investigated the components of binges in obese or at least overweight females. Reported food items during a binge predominantly consisted of carbohydrates (bread or pasta; 64.6%), food rich with sugar (56.2%) as well as high fat items (45.3%) and salty snacks (39.6%). Bingeing sweets was related to more binge days than other binge preferences. Concerning body weight, the authors stated that females who binge on high fat food during meal times had a significantly higher ody ass ndex (BMI) in contrast to females who mainly binge while having a snack. This finding is in line with results by Guss et al. [7] who found a positive correlation between food intake and BMI in BED participants classified into the more-obese group in comparison to the less-obese BED group in which this correlation could not be found. They pointed out that a greater BMI is associated with a greater meal size. Furthermore, meals of obese samples consisted of a higher percentage of fat (38.5%) than those of normal weight controls (30.8%; [7]). Another comparison between an overweight female binge eating sample and overweight controls revealed a higher overall energy and fat intake per day in females with BED, but there were no significant differences in the consumption of carbohydrates and proteins [8]. Raymond et al. [5] compared binge days with non-binge days of participants with BED and found a significantly higher consumption of all macronutrients in binge days at which they pointed out a less preferred intake of carbohydrates in comparison to high fat nutrients on binge days.

Most of the published data on binge macronutrient composition is based on overweight or obese samples and includes analyses of food records in laboratory settings, which seem to increase pathological eating behaviors [9]. A main advantage of food records in the natural environment is the possibility to gain a more realistic insight into the eating habits of investigated samples at home, because individuals have access to all kind of foods. This is the reason why we decided to assess eating behavior under natural conditions. Furthermore, eating behavior is quite often assessed retrospectively in the form of the food frequency questionnaire. This method is available in different forms (e. g. non-quantitative, semi-quantitative and quantitative), which in turn can influence the results. This depends, among other things, on the items selected and the response format. In addition, the focus here is on the foodstuff and not so much the consumed amount [10, 11]. Thus, food consumption is assessed prospectively in a natural setting in the present study.

While binge eating behavior has so far been mainly examined in the context of bulimia nervosa, BED, and obesity, there are only few studies focusing on the occurrence of bingeing in (non-clinical) normal weight individuals in natural conditions, although this could help to gain a better understanding of the binge phenomenon itself.

Hence, the aim of the present study was to analyze the macronutrient composition of binge episodes on the basis of food records in a subclinical BED normal weight sample (BG). We anticipated that participants classified as at least subclinical binge eaters would have a smaller or even comparable amount of energy intake per day to the control group (CG) as was shown for clinical BED [5]. Concerning binges, we hypothesized the macronutrient composition of binge eating episodes to be mainly made up of a high intake of fat and carbohydrates and less of proteins. Furthermore, we expected significant differences of psychopathological aspects of eating behavior, e. g. eating concerns, weight concerns as well as differences in body images in the BG compared to the CG. Apart from that, we expected participants with binge behavior to have more depressive symptoms than healthy controls.

# Methods

# Sample

Participants were recruited via advertisements at the Heinrich Heine University Düsseldorf and on social media. Normal weight individuals who reported to either have binge episodes at least once a week or are without any eating pathologies were invited to participate in the study. A total of 68 normal weight women and men between 18 to 35 years of age were recruited. A majority of them were students. Criteria for exclusion were as follows: current dieting, intake of appetite-suppressing or appetite-stimulating medication, symptoms or diagnosis of anorexia nervosa or bulimia nervosa and intake of specific medications which have a sustainable impact on hunger or repletion. 32 individuals had to be excluded because they did not fulfill the criteria, leaving a sample of 36 participants. According to the screening data and a short interview addressing binge habits, participants were either assigned to a (subclinical) binge eating group (BG; intervention group) or to a control group (CG), where the participants had no binges. The BG counted an overall of 17 participants, and consisted of 13 females and 4 males. 19 participants, 12 females and 7 males, were assigned to the CG.

Mean age was 22 years (standard deviation [SD] = 3.3; range 19–31; BG: M = 21.94, SD = 3.03; range 19–30; CG: M = 21.42, SD = 3.61; range 19–31). According to BMI, all participants were normal weight (M = 22.14, SD = 2.56 kg/m<sup>2</sup>). Nevertheless, individuals in the BG had a slightly, but not significantly higher BMI (M = 22.76, SD = 3.06 kg/m<sup>2</sup>) than the CG (M = 21.59, SD = 1.95 kg/m<sup>2</sup>; t = 1.39, p = .17). There were no vegans in the total sample and only one vegetarian in the CG. All participants gave written informed consent.

#### Measures

First, socio-demographic status (e. g. gender, age) and eating behavior (e. g. general eating habits, satisfaction with eating habits) was assessed. Subsequently, both groups received the following instruments: the Eating Disorder Examination-Questionnaire 6.0 (EDE-Q; [12]; German version: [13]), the Beck-Depression-Inventory (BDI-V; [14]), the Body Image Questionnaire (*Fragebogen zum Körperbild*, FKB-20; [15]) and the Düsseldorf Orthorexie Scale (DOS; [16]). After group assignment, the same food diaries were administered to both groups.

Additionally, participants were asked to rate their main problems regarding their eating behavior (Three Factor Eating Questionnaire; TFEQ, [17]).

The EDE-Q [13] assesses participants' eating behavior for the past 28 days. Participants should rate on a 7-point scale which methods (e.g. restrictions, weight control) they use concerning their eating behavior and weight control. The four subscales restraint, eating concern, weight concern and shape concern reflect the extent of a potentially disordered eating behavior, which may be classified as anorectic or bulimic. Furthermore, binge eating behavior is assessed via items 13, 14 and 15. Item 13 addresses the number of days in which participants ate an amount of food that others would describe as unusual under similar conditions. Item 14 deals with the number of days in which participants experienced a loss of control over their eating habits. Item 15 combines Items 13 and 14 and therefore measures the amount of eaten food that is accompanied by a loss of control. These items were critical for the group allocation in the present study. Scores for all subscales as well as the total EDE-Q score were computed. Psychometric properties of the questionnaire can be regarded as satisfactory [13].

The DOS [16] measures orthorectic eating behavior, which is defined as a fixation on only eating food considered to be healthy according to subjective criteria and to avoid food which is perceived as unhealthy [18]. The DOS score ranges from 10 to 40 points. Cronbachs alpha of .83 [16] and first indications of convergent validity suggest good psychometric properties.

Depressive symptoms were assessed with the BDI-V [14]. Participants rate their answers on a 6-point Likert scale. A total score with a maximum of 100 points can be reached. A score of 35 is used as the cut-off for depressive symptomatology of clinical relevance. The BDI-V is a valid questionnaire to assess depressive symptoms [19].



To assess body image, two subscales on cognitive and affective body image from the German version of the Body Image Questionnaire [15] were used. The subscale Rejecting body image is used to measure dissatisfaction with one's body. Energetic and physical related aspects are measured with the subscale Vital body dynamics. For both scales, a total sum ranging from 10 to 50 points can be computed. Psychometric properties of the questionnaire are good [20, 21].

Daily eating behavior was assessed via food diaries in which participants recorded their eating behavior for seven days. They were instructed not to change their eating behavior and to report their food intake throughout the day as precisely as possible. Besides date and type of meal (breakfast, lunch, dinner or snacks), the participants were also asked to specify the situation in which each meal was consumed (e.g. alone, in haste). Subjects of the BG were asked to make a note if the meal was classified as a binge according to their subjective criteria.

#### Procedure

First, inclusion criteria were assessed using an online screening questionnaire. Afterwards, participants of both groups were briefly interviewed. In this interview participants were asked the following questions, addressing DSM-5 Criteria to ensure that those of the BG suffer from binge episodes at least once a week to guarantee that the criteria for at least a subclinical BED are fulfilled:

- 1. How often do you have binge episodes on average per week?
- 2. Would you describe the quantity of the food consumed during a binge as above average?
- 3. Have you ever lost control during a binge episode?
- 4. Can you enjoy the food?
- 5. Do you eat faster than usual during a binge episode?
- 6. Are you suffering from the binge episodes?

The questions listed here were all part of the screening and served to verify the screening data in order to be able to make an adequate group allocation.

Next, eligible participants received another questionnaire to investigate their eating



habits as well as their emotional state, which was assessed via the BDI-V [14].

Afterwards, participants of both groups received a food diary, which they were asked to fill out in the upcoming seven days. This first food diary was given to both groups to compare the eating behavior of participants with binge eating tendencies and healthy controls. It was emphasized in the instructions that participants should not change their eating habits. Furthermore, they were asked to document their daily eating behavior as accurately and as detailed as possible and to take note if a binge episode occurred. There were no predefined criteria for the classification of meals as a binge, so participants had to rate themselves which meals count as a binge according to their own subjective evaluation. As a general guideline to help participants rate their eating behavior, a binge was described as a condition in which one eats without control and with the feeling of not being able to stop or to influence what and how much one eats.

Participants of both groups received as compensation an attendance allowance or in the case of psychology students, research credits. Participants of the BG were given an additional food diary in which they were asked to only document binge episodes for two months. If a binge occurred, they were required to further retrospectively record other food consumed on this day. After two months, the follow-up diaries were returned and participants received another attendance allowance or research credits as compensation. The two-monthdiary on binge episodes was established to verify and receive more detailed information about the macronutrient compositions of binge episodes of the participants with binge eating behavior.

#### **Design and Analysis**

Overall eating behavior, the quantity as well as the macronutrient composition of the binges were analyzed using Fddb Internetportale GmbH [22], which is an online database consisting of more than 333 000 food items including information on micro- and macronutrients. Before running the statistical analyses, mean energy intake and macronutrient composition in grams and percentages per day for both groups were calculated. Furthermore, energy intake and the macronutrient composition for each meal subjectively classified as binges were calculated. The same calculations were made for the two-month follow up of the BG group. Following a between-subject design, analyses were conducted using IBM SPSS Statistics 24 for Windows. For descriptive data, means (M) and standard deviations (SD) are reported. t-Tests for independent samples were used with a p value of .05 for significance to assess differences in the overall energy intake as well as the macronutrient compositions between the two groups by using group (BG vs. CG) as the independent variable and the overall energy, fat, carbohydrates and proteins for seven days as dependent variables. In addition, binges of the BG group for the seven day food diary and the subsequent two-month diary were examined separately by calculating means for energy, fat, carbohydrates and protein intake for each reported binge. Eating disorder symptomatology, body image, depressive symptoms and BMI were analyzed by using independent t-Tests with the factor group as the independent variable. The assumptions underlying the parametric procedures performed have been verified and can be considered as given.

# Results

## Psychopathology

Results of group comparisons for clinical data can be seen in • Table 1. Participants with binge eating behavior were significantly more depressed (M = 28.9, SD = 18.4) than non-binge subjects (M = 18.1, SD = 9.7; t[23.66] = 2.17, p < .05). Results of the EDE-Q total score indicated significantly higher pathological eating behavior for individuals of the BG than for those of the CG (t[28.96] = 2.34, p < .05). Regarding the subscales of the EDE-Q, participants of the BG scored significantly higher on the subscales eating concern (t[34] = 2.61, p < .05) and weight concern (t[34] = 2.12, p < .05) than controls. Furthermore, differences in body image between the two groups can be noted. Descriptively, but not significantly (t[34] =1.02, p > .05), subjects of the BG reported more rejection of their body than controls. Nevertheless, a significant difference can be found between the groups concerning vital body dynamics. The CG scored significantly higher on the vitality index than participants with binge eating behavior (t[34] = -2.66, p < .05). Furthermore, the mean score on the Düsseldorf Orthorexie Scale (t[34] = -.39, p = .70) was for both groups within normal range, stressing the fact that the sample does not seem to be affected by self-selection according to interest in healthy nutrition.

In the TFEQ rating, the BG reported stress and eating in society as their main problems. In contrast, individuals of the CG saw themselves faced with the problem of not daring to eat until they are full.

#### **Overall food intake**

The overall nutrient intake as well as the macronutrient compositions of both groups are presented in  $\bullet$  Table 2. In the seven day food records, binge episodes occurred on average two times (SD = 2) in the BG group with a minimum of zero and a maximum of six



binges. There are no significant differences between the groups concerning the total energy per day (including binge episodes if they occurred) as well as for all macronutrient compositions (• Table 2).

Nevertheless, descriptive data reveal differences in the consumption of macronutrients. While both groups ate nearly the same amount of carbohydrates and fat, the CG consumed more proteins than the BG group.

## **Binge episodes**

The macronutrient composition of the binges in the twomonth food record was as follows: Participants reported a mean number of 5 (SD = 3; min = 1, max = 9) binge episodes which occurred in the eight week period. The average energy intake of a binge was 1726 (SD = 666) kcal, with a maximum of 2881 kcal and a minimum of 809 kcal. For fat, a minimum of 46 g and a maximum of 260 g was reported per binge (M = 101 g, SD = 62). The binge with the highest amount of carbohydrates included 335 g and the smallest was 86 g (M = 184, SD = 78). Comparatively few proteins were eaten with a maximum of 70 g and a minimum of 11 g (M = 39, SD = 19).

|  | BG<br>(N = 17)            | CG<br>(N = 19)           | BG vs. CG     |                |
|--|---------------------------|--------------------------|---------------|----------------|
|  | M (SD)                    | M (SD)                   | Т             | р              |
| BMI (kg/m²)  | 22.8 (3.1)                | 21.6 (2.0)               | 1.35          | 0.17           |
| BDI-V  | 28.9 (18.4)               | 18.1 (9.7)               | 2.17          | 0.04*          |
| FKB-20<br>Rejecting body image<br>Vital body dynamics* | 24.4 (10.8)<br>32.8 (6.5) | 21.0 (9.1)<br>38.5 (6.5) | 1.02<br>-2.66 | 0.31<br>0.01** |
| DOS  | 15.1 (3.0)                | 15.5 (4.1)               | -0.39         | 0.70           |
| EDE-Q*   | 1.7 (1.0)                 | 1.0 (0.7)                | 2.34          | 0.03*          |
| Restrained Eating                                      | 1.3 (1.5)                 | 0.8 (0.9)                | 1.22          | 0.24           |
| Eating concern*  | 1.5 (0.9)                 | 0.8 (0.6)                | 2.61          | 0.01**         |
| Weight concern*  | 1.9 (1.4)                 | 1.1 (1.0)                | 2.12          | 0.04*          |
| Shape concern  | 2.2 (1.5)                 | 1.4 (1.1)                | 1.76          | 0.09           |

Tab. 1: Demographic and clinical data for participants with binge eating behavior (BG) and control group (CG)

BDI-V = Beck-Depression-Inventory; FKB 20 = Body Image Questionnaire (*Fragebogen zum Körperbild*); DOS = Düsseldorf Orthorexie Scale; EDE-Q = Eating Disorder Examination-Questionnaire; BG = binge group; CG = control group.

M = arithmetic mean; N = sample size; SD = standard deviation; \*p <.05, \*\*p < .01

Binges recorded in the two-month period were characterized by a high percentage of fat (M = 45 %, SD = 5) and carbohydrates (M = 44 %, SD = 7) and a small percentage of proteins (M = 10 %, SD = 4) of the total amount of energy.

# Discussion

Aim of the present study was to analyze the macronutrient composition of binges in normal weight subjects with subclinical binge eating behavior. Results indicate an equal energy amount eaten over the day between the BG and CG. Binges of the BG were marked by a high intake of carbohydrates and fat and comparatively less proteins. Moreover, the BG had more concerns about their eating behavior and weight, which can be seen in the higher scores of the subscales eating concern and weight concern. This group also seemed to experience less vitality of their body image and significantly higher depressive symptoms in comparison to the CG. Consequently, the present results allow for the analysis of possible risk factors in view of psychopathologies of BED, as well as for comparisons of the macronutrient composition of binges to obese binge eaters and their consequences for the development of BED.

Since participants in this study recorded their food intake in their natural environment at home, this study reveals everyday eating behavior of mild binge eaters, without restrictions in food selection and consumption. The use of food intake recordings at home offers the advantage of observing binges appearing in natural conditions and not artificially induced, which allows a deep insight into food consumed in realistic conditions. Of course, these food records might lack of precision, as participants might not record every meal and their estimations of food intake might be biased [23]. In this respect, mistakes in the recordings must be taken into account. This could be, among others, the wrong evaluations of quantities, source-monitoring errors or rather participants might forget to note down everything they consumed. Yet, the advantages justify the effort of food recordings in the form of food diaries.

Binges mainly consisted of fat (40 %) and carbohydrates (49 %) with only few proteins included (11 %). These results are comparable to previously found macronutrient compositions of obese binge eaters [4], which indicates other factors to be involved in the development and maintenance of BED.

Regarding overall food consumption and especially the macronutrient composi-



tion, no significant differences between the groups were found. However, in line with the findings of Guss et al. [7], it is remarkable that the participants in our sample with binge eating behavior consume more fat in grams per day. However, the difference failed to reach statistical significance. Furthermore, consumption of carbohydrates was higher in bingeing participants. While Raymond et al. [8] report an equal amount of consumed carbohydrates and proteins of BED and non-BED participants, our results indicate that more fat was consumed by BED-participants, which may resulted in a slightly but not significantly higher total energy intake, since there was no difference in the quantity of food consumed. Therefore, the quantity of consumed food might be less relevant and instead an emphasis on the macronutrient composition of the consumed food should be pursued. This assumption can be seen in the work by Latner and Schwartz [24], who examined the energy intake of normal weight and obese subjects after having either a high-carbohydrate, high-protein or a mixed meal. After the high-protein meal, participants had a lower energy intake compared to the other two options.

In view of the lack of significant differences in the quantity and quality of food consumed between the two normal weight groups, other factors may play a role in the development and maintenance of BED in normal weight individuals. This is, amongst others, particularly true for psychopathological determinants. Participants of the BG were significantly more depressed than those of the CG, which is in line with previously reported findings of primarily obese binge eaters [25]. Apart from that the present findings indicate that changes in metabolism which may lead to obesity in the long term must be involved. Thus, further research is needed to investigate potential risk factors which may contribute to the symptomatology and maintenance of binge eating disorder in normal weight subjects who might be at risk to develop overweight.

|                | BG<br>(N = 17)           | CG<br>(N = 19) | BG vs. CG |      |
|----------------|--------------------------|----------------|-----------|------|
|                | M (SD)                   | M (SD)         | Т         | р    |
| Binge-episodes | 2 (2) (Min = 0, Max = 6) | -              | -         | -    |
| Total kcal     | 2220 (706)               | 2230 (614)     | -0.05     | 0.96 |
| CHO, g         | 259 (88)                 | 251 (69)       | 0.30      | 0.77 |
| (%)            | 48 (6)                   | 48 (6)         | 0.35      | 0.73 |
| Pro, g         | 82 (23)                  | 94 (43)        | -1.06     | 0.29 |
| (%)            | 16 (4)                   | 17 (6)         | -0.71     | 0.48 |
| Fat, g         | 91 (36)                  | 87 (32)        | 0.29      | 0.77 |
| (%)            | 35 (5)                   | 34 (6)         | 0.77      | 0.45 |

Tab. 2: Macronutrient composition of recorded eating behavior in participants with and without binge eating behavior

BG = binge group; CG = control group; N = sample size;

kcal = kilocalories, CHO = carbohydrate, Pro = protein, g = grams

# Limitations

Group assignment can be considered as a limitation of this study. Assignment was primarily based on self-reported eating behavior assessed via questionnaires which might have been influenced by social desirability. It is important to note that the sample does not include diagnosed binge eaters, but participants with binge eating behavior fulfilling at least a subclinical criterion for BED. Therefore, our results cannot be generalized to samples who fully complete the criteria for BED, but instead provide insights into the eating behavior of subclinical binge eaters with normal weight.

Although food records via food diaries provide some advantages, they also have some disadvantages that must be taken into account. Besides errors in recalling and willingness to record all food consumed throughout the day, there might also be limitations in the precision of the documented quantity of consumed food. Not every event allows precise information about food intake in grams, especially when eating in restaurants. Therefore, an analysis of food intake must be interpreted with caution. However, there is evidence that food records in the form of a 7-day-diet diary is a proven instrument especially in case of nutrition analyses [e. g. 26]. Also, the use of the online diary Fddb.info to analyze the energy intake and macronutrient composition of consumed food supports the assumption of reliable estimates due to its extensive amount of listed foods and notably its listed brand products.

Another limitation refers to the small number of included male participants. Even though more females are affected by this type of disordered eating, it should be kept in mind that our results do not allow conclusions concerning male binge eaters.

Moreover, there is no consistent definition of a binge or the condition of loss of control while bingeing [see 5]. Therefore, meals analyzed and classified as binges rely on subjective experiences and may strongly vary in their composition.



# Conclusion

In general, our results reveal that the quantity and the macronutrient composition of food in normal weight subjects with binge-eating behavior is comparable to those of normal weight healthy persons. In binge episode, normal weight subclinical BED persons consume food quantities of macronutrient composition as reported for obese persons with BED [4]. Carbohydrates as well as fat represent the largest part of consumed macronutrients in a binge. Proteins seem to play only a minor role in selected food during a binge. This special macronutrient composition seems to have impact on the overall energy intake [24] and raises the question which nutritional as well as psychopathological or physical factors might be involved in the creation and maintenance of BED.

## **Ethical Approval**

Local ethics committee approval was not required for the study that only included surveys. Surveys are common used research tools and the technique itself received ethical approval several times. Participants were asked about their eating behavior, and questions like these are not supposed to cause any harm to adult human beings. Even in the very unlikely case that participants felt uneasy while answering the questions, they could easily cancel the survey at any time without any disadvantages. Participants were informed that their participation is voluntary and anonymous, and that their data is handled according to privacy policy. Furthermore, after they were instructed about study conditions they gave their informed consent and were enlightened to be free to cancel the study at any time without disadvantages (see above). All procedures performed in the study involving human participants were in accordance with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.



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#### Conflict of interest

The authors declare no conflict of interest.

#### Acknowledgements

The authors like to thank Sarina Pelkmann, Jana Willemssen and Svenja Witte for their great help in collecting the data and Hannah Kiesow-Berger for her valuable assistance with language revision.

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## DOI: 10.4455/eu.2021.004