Author's response to reviews

Title: Lack of effect of high-protein vs. high-carbohydrate meal intake on stress-related mood and eating behavior

Authors:

Sofie Lemmens (s.lemmens@maastrichtuniversity.nl)
Eveline Martens (eap.martens@maastrichtuniversity.nl)
Jurriaan Born (j.born@maastrichtuniversity.nl)
mieke Martens (mieke.martens@maastrichtuniversity.nl)
Margriet Westerterp s.lemmens@maastrichtuniversity.nl
m.westerterp@maastrichtuniversity.nl

Version: 2 Date: 14 November 2011

Author's response to reviews: see over
Rebuttal MS: 3166034355038183

Title: Eating in the absence of hunger: interaction between stress, macronutrient intake and disinhibition

Reviewer(s)' Comments to Author:

Reviewer 1

Title: Eating in the absence of hunger: interaction between stress, macronutrient intake and disinhibition

Version: 1 Date: 14 June 2011

Reviewer: Marian Tanofsky-Kraff

Reviewer's report:

This study reports the results from a randomized crossover 2 x 2 design in which the wanting/liking, eating in the absence of hunger, and mood of university students was measured in a stress (vs. rest) condition and after a protein (vs. carbohydrate) meal. The stated purpose of the study was to investigate whether macronutrients affect wanting/liking, eating in the absence of hunger, and mood in stressful or restful states. The design of the study is interesting and the objective methodology used for eating in the absence of hunger and wanting/liking is commendable. However, there are a few significant issues with the manuscript, which require attention.

Major:

My biggest concern is that the manuscript is poorly organized and difficult to follow in multiple places:

- The abstract and introduction is confusing and hard to follow and should be re-worked for greater clarity

We tried to re-work the abstract and introduction for greater clarity.

- The method section requires some major re-organization. For example, the wanting/liking test session is mentioned early without any description, but then later on, it is described in a bit too much detail. This makes it very hard to follow what has been done.

The method sections regarding the study design and ‘liking’ and ‘wanting’ computer test) were re-organized.

- In the method section, line 153, the test meal procedures are discussed, but there is no mention of a screening session referred to earlier in the participants section. Also,
the placement/order of this section seems strange with some measures/procedures discussed in the participants section.

The remark was taken into account. The order of the section was changed.

- The meal had to be consumed in entirety. It should be mentioned earlier on in the methods section how the researchers adjusted for different energy needs of men and women of different body sizes.

This was changed in the manuscript: “After completing the ‘liking’ and ‘wanting’ computer test participants were offered the test meal (lunch), which was either a high-protein meal or a high-carbohydrate meal, and which had to be consumed entirely. Participants received an amount corresponding to 30% of their daily energy requirements.”

- Lines 289-300 should be specified in the statistical plan rather than the results section itself. A rationale for this hypothesis should be clear from the Introduction. Also, a “role for disinhibition” needs to be clarified – main effect? Interaction (if so, with what)?

This was changed in the manuscript (see Statistics and Participant characteristics, and Introduction).

A second major concern is the choice or lack of covariates. What covariates if any did the authors account for? In the results section, it is unclear what is meant by: “Since there were neither gender differences, nor differences according to weight status concerning possible conditional effects of stress vs. rest and of high-protein vs. high-carbohydrate on data of the questionnaires (POMS, STAI, VAS), on data of the ‘liking’ and ‘wanting’ computer test, and on energy intake, data were analyzed for all participants together.” It seems very odd not to control for BMI, as we know BMI has been related to liking/wanting and strongly related energy intake.

We investigated whether consumption of high-protein vs. high-carbohydrate meals would affect in particular visceral overweight individuals and individuals characterized by disinhibited eating behavior, as it has been shown that those individuals are more vulnerable to stress-induced eating (Lemmens et al. 2011, Rutters et al. 2009). However, neither differences according to weight status (visceral overweight vs. normal weight) nor gender differences (men vs. women) were detected.
Other:

The hypotheses stated on page 5 should be clarified. The authors hypothesize that a high-carbohydrate meal would increase subsequent eating in the absence of hunger during stress relative to what? Relative to protein under stress? Carbohydrate and protein under no stress? Ditto for protein.

This was changed in the manuscript Introduction: “Based upon this latter study, and the endocrinological studies by Lacroix et al., Martens et al., and Vicenatti et al. [10-12] showing that a high-protein meal prevents increases in stress cortisol levels, we hypothesized that a high-protein meal, in contrast to a high-carbohydrate meal, may reduce post-meal energy intake during stress.”

What was the rationale of the BMI inclusion range? The criterion for “only visceral overweight participants” is completely unclear. How is “overweight” defined? Does this mean if someone had a BMI of 26 but was not viscerally fat by the authors’ definition, they would be excluded?

The following was mentioned in the manuscript: “Inclusion criteria comprised BMI 20-30 kg/m$^2$, … Regarding overweight participants only participants with abdominal adiposity were included, as chronic stress has been associated with visceral fat accumulation and obesity [1, 33, 34]. Abdominal adiposity was defined as having a waist circumference of ≥ 80 cm in women and ≥ 94 cm in men [35].”

We chose this BMI range to include normal weight as well as overweight subjects. Visceral overweight subjects, compared with normal weight subjects, might be more vulnerable to stress-induced eating and consequently the development of obesity, as shown in several studies (Lemmens et al. 2011, Block et al. 2009, Davis et al. 2004, Saelens and Epstein. 1996)

How was food intake measured?

All the food items chosen post test meal by means of the ‘wanting’ part of the computer test, were offered to the participants at a fixed amount, which was described to the participants beforehand, and food items were eaten completely (Figure 1, ‘wanted meal’). Total energy content (post-meal energy intake) and macronutrient composition of the consumed wanted food items was calculated.
Was statistical power calculated? How did the authors decide upon the number of participants included in the study?

The main objective of this study was to investigate possible effects of consumption of a high-protein vs. high-carbohydrate meal on the stress-induced psychological mood response, the rewarding value of food, and the stress-induced food choice and food intake in the absence of hunger. The rewarding value of the foods is expressed as ‘liking’ and ‘wanting’, which is measured by means of a computer test. We calculated the number of participants needed based on the results reported in a previous study (Lemmens et al., Physiology & Behavior 103 (2011) 157–163).

Mean of \( \mu_1 \) = 35 %; Mean of \( \mu_2 \) = 10 %

Standard deviation of the population (\( \sigma \)) = 35 %

Significance level for each test (alpha = 0.05)

Taken into account the Bonferroni correction (multiple-comparison correction) for multiple testing: alpha = 0.05/4 = 0.0125

\( \beta = 0.10 \)

Power = 1 - \( \beta = 1 – 0.10 = 0.90 \)

Two sided = \( H_0 - H_a \)

\( n = \text{number of participants needed} \)

\( n = 2 + \left[ 15 \times (\sigma)^2 / (\mu_1 - \mu_2)^2 \right] \)

\( n = 2 + \left[ 15 \times (35)^2 / (35-10)^2 \right] = 31 \)

This leads to a sample size of 31 participants. Taking into account a dropout of 7 subjects, this results in a total of 38 subjects.

The following was added to the manuscript (‘Participants’): “Based upon the study by Lemmens et al., power analysis showed that with an \( \alpha \) of 0.0125 (taking into account the Bonferroni correction for multiple testing) and \( \beta \) of 0.10 (power=1-\( \beta \)=0.90), at least 31 participants were needed.”

There are a number of stylistic issues with the manuscript throughout, including the use of abbreviations that are unclear such as “En% P/C/F” or the use of “i.e.” not within parentheses in multiple places throughout the text. There are no indentations at the start of paragraphs. The manuscript would benefit from the review of a native English-speaker.
The abbreviation “En% P/C/F” (= energy percentage protein/carbohydrate/fat) was clarified in the text as well as in the abbreviation section, and is a common used abbreviation.

Indentations were added to the start of paragraphs.

Minor:
Regarding the TFEQ page 6, line 145, “unrestraint” and “restraint” are awkward – perhaps “unrestrained” and “restrained”?
Regarding the spelling of the terms “unrestraint” and “restraint”, they are written correctly as such as they are used as a noun. The spelling of “unrestrained” and “restrained” should be used in the case of an adjective, such as ‘restrained subjects’.
The statistical plan requires a bit more detail. For instance, specify the levels within “time.”
The statistical plan was changed in the manuscript.

Reviewer 2
Title: Eating in the absence of hunger: interaction between stress, macronutrient intake and disinhibition
Version: 1 Date: 24 July 2011
Reviewer: David Levitsky
Reviewer's report:
The manuscript by Lemmens et al. describes a well-designed, well-controlled, and well-executed study of the relationship between stress and food intake in humans. The literature on the effect of stress on human food intake, and especially as a cause of human obesity, is confusing at best due to the ambiguity of terms and non-standardized methodologies used.
The authors’ examined the effect of inducing a stress (high or low stress) and the role of the protein/carbohydrate content (high protein vs. high carbohydrate) of a meal on mood, “liking” and “wanting” of food, and energy intake. Despite all the possible effects and interactions that could have resulted from observing these variables, only obvious effects (e.g., satiety increases following eating) and only two interactions were observed that directly related to the question posed by the investigators: an interaction between diet and stress and “wanting” and an interaction between diet and
stress and energy consumed. However, neither effect was statistically demonstrated by an interaction.

One of the difficulties in appreciating these results lies in the description of the statistics used to analyze the results. The statistics were described as being a “Factorial ANOVA with repeated measures” and Student t tests. It is not clear whether all the factors were subjected to a single ANOVA or the analysis was broken up according to which dependent variable was measured. Moreover, it was not stated whether the t tests were corrected for repeated testing. No explanation was provided as to why standard post-hoc tests were not used?

Per dependent variable (e.g. ‘wanting’, or post-meal energy intake) all the factors were subjected to a single ANOVA to check for significant interactions. Next, paired and unpaired Student’s t-tests were used as Post hoc analyses for significant interactions.

Unpaired Student’s t-tests were used to analyze differences in participant characteristics between men and women. Factorial ANOVA with repeated measures was used to study the effects between subject groups (men vs. women, overweight vs. normal weight, high vs. low disinhibition) of the conditions of stress vs. rest and of high-protein vs. high-carbohydrate, and of time (pre and post test meal), on data of the questionnaires (VAS, POMS, STAI), on data of the ‘liking’ and ‘wanting’ computer test, and on energy intake.

Neither gender differences (men vs. women), nor differences according to weight status (overweight vs. normal weight) were detected concerning possible conditional effects of stress vs. rest and of high-protein vs. high-carbohydrate on data of the questionnaires (POMS, STAI, VAS), on data of the ‘liking’ and ‘wanting’ computer test, and on post-meal energy intake. However, an effect of disinhibited eating behavior was detected for some of the measured variables. Therefore, results were presented firstly for all participants together and secondly for individuals characterized by high vs. low disinhibited eating behavior (high F2 score: n=16, 6 men and 10 women; low F2 score: n=22, 13 men and 9 women).

It is interesting that two subject variables, that were not part of the hypothesis being tested, were reported as significant: dietary restraint and feelings of hunger. However, a question that was not asked was the correlation between these two variables? Does one still observe differences in the feeling of hunger when dietary restraint is
controlled? The answer would be of interest to many involved in human research as well as clinicians working on human eating disorders. Unfortunately, no information concerning the correlation between these two factors was provided.

Significant differences regarding dietary restraint and the feeling of hunger were only detected between men and women, which is a commonly found result. There was no effect of dietary restraint and the feeling of hunger on the measured variables (VAS, POMS, STAI, ‘liking’ and ‘wanting’, energy intake) in the different conditions. The correlation between dietary restraint and the feeling of hunger goes beyond the scope of this manuscript, however it has been described by others (e.g. Stunkard and Messick, 1985).

In line 293, it was stated, “Since there were neither gender differences, nor differences according to weight status concerning possible conditions effects of stress vs. rest and of high-protein vs. high-carbohydrate on data of the questionnaires (POMS, STAI, VAS), on data of the ‘liking’ and ‘wanting’ computer test, and on energy intake, data were analyzed for all participants together.” On what basis were no “differences” made. Were there no interactions between any of the psychological variables and gender observed?

Indeed, neither interactions were observed between any of the measured variables and gender, nor between any of the variables and weight status (normal weight vs. visceral overweight).

The description of the relationship between stress and disinhibition and eating behavior described between lines 298 to 309 is quite confusing. The first sentence (Butters et al. showed that acute psychological stress leads to eating in the absence of hunger, especially in vulnerable individuals characterized by disinhibited eating behavior.) should be in the discussion section, not in the results. The rest of the paragraph discusses “wanting”, not food intake and the relationship between disinhibition and hunger, two subject variables, neither of which was related to the hypothesis discussed in the introduction. Moreover, although it is stated that “An effect of disinhibited eating behavior was detected for post-meal average food ‘wanting’ (‘wanting’ for food items from all the five food categories taken together) and energy intake”. Unfortunately, no data are presented, not allowing the reader to get a grasp of the magnitude of the effect. The text goes on to say “Hence, data were
analyzed firstly for all participants together and secondly for individuals characterized by high vs. low disinhibited eating behavior.” But the data presented in the text was the relationship between disinhibition and feelings of hunger, not disinhibition and intake.

The remark was taken into account and changes were made in the manuscript (Introduction, and Statistics; see also remark Reviewer 1).

The data presented in the text was the relationship between disinhibition and food ‘wanting’, and disinhibition and post-meal energy intake. We did not present the relationship between disinhibition and feelings of hunger.

The data central to the hypothesis being tested, that composition of the meal (high or low protein) affects in interaction between stress and intake, is shown in figures 4 and 5. But the data are not complete. Figure 4 shows the overall effect of meal composition and the administration of the stressor on meal consumption. There was no overall effect of either stress or meal composition, a finding that gets lost in the discussion. Figure 5 shows the data only for the subjects with high disinhibition scores. The critical figure would be a comparison in intake of the subjects with high disinhibition to those with low disinhibition as a function of stress administration and an accompanying significant triple interaction between disinhibition, stress, and dietary composition. The same criticism applies the figures 2 and 3, which argues for the effect of meal composition on “wanting”. Indeed, it appears that the only difference that emerged from this work occurred on the test day where the participants were tested under non-stressful conditions following the ingestion of the high protein meal. Could something have happened when that particular condition was being tested that was different from the other three testing conditions?

The figures 3 and 5 were changed: a comparison was made between the subjects with high disinhibition to those with low disinhibition.

The 4 testing conditions were all executed in the same way, except for the meals (high-protein vs. high-carbohydrate) and the stress/rest condition. Nothing exceptional happened during the course of the test days.

Eating in the absence of hunger was used in the title and throughout the manuscript, but there was no discussion of why this is an important variable. Eating in the absence of hunger generally refers to eating of snacks, not of a succeeding meal. More
discussion of the definition of eating in the absence of hunger should be devoted in the discussion section, since it nothing about its significance is mentioned. Indeed, eating in the absence of hunger generally refers to eating of snacks, not of a succeeding meal. In our study, participants still had the chance to compose a meal in the satiated state (including bread, filling, drinks, desserts, and snacks) by means of the ‘wanting’ part of the computer test. The use of ‘eating in the absence of hunger’ was changed throughout the manuscript.

The study is important not for what it found, but what it didn’t find. The discussion should be re-written to reflect this observation. The study basically did not find an effect of overall stress on eating or wanting or needing, except under a very specific condition: the non-stressful condition when the test meal was high in protein. How does this finding relate to the subject of the introductory sentence – stress might be the cause of increasing obesity? The study did not find intake of a meal was related to gender or body weight. They did not find an overall effect of protein composition on food consumption or psychological indicators of stress. They did not find an overall effect of protein composition of the meal on measure hunger of satiety, an observation that appears to contradict other findings by this group. In fact, they observed a dissociation between measures of hunger and satiety and protein content of the test meal because of the decrease in intake observed in the high disinhibition participants, but this condition was not reflected in hunger of satiety measures.

The remark was taken into account. We tried to re-work the Discussion.

Finally, I believe the title should be changed for two reasons. First, the study does not examine the issue of eating in the absence of hunger as an independent variable. Second, the lack of effects of stress should be emphasized in the title rather than the existence of effects.

The title was changed to: ‘Lack of effect of high-protein vs. high-carbohydrate meal intake on stress-related mood and eating behavior.’

Level of interest: An article whose findings are important to those with closely related research interests
Quality of written English: Acceptable
Statistical review: Yes, and I have assessed the statistics in my report.
Reviewer 3

Title: Eating in the absence of hunger: interaction between stress, macronutrient intake and disinhibition

Version: 1 Date: 25 July 2011
Reviewer: Kathleen Melanson

Reviewer's report:

This manuscript describes a 4-visit randomized crossover design in 38 adults, to test the effects of an acute laboratory stressor and macronutrients (protein vs. CHO) on wanting, liking, and energy intake (EI). While hypothesized differences did not show for the group as a whole, subjects characterized by disinhibition showed lower wanting and EI after the protein-rich meal as compared to the CHO-rich meal during rest conditions. This macronutrient effect was not apparent during stress conditions, suggesting that stress might override the satiating effects of protein in such individuals.

This work is well-written, and justified based on the scientific literature. The research questions and methods are clearly described, although this reviewer requests a few more methodological details. Appropriate controls were used in procedures, and test meals were nicely matched. The results can be easily followed, and the discussion is based on them. Findings are relevant for both researchers and clinician.

MAJOR COMMENTS:

1. In the Methods section, last paragraph describing test meals, please indicate the types of carbohydrates comprising the shakes and/or test meals as a whole. For example, it is known that different sugars, starches, and fibers can have differential impacts on appetite, so it is important for readers to understand carbohydrate types comprising the test meals. If available, it would be helpful to know a bit more about the protein sources in the shakes too. Such information could be presented as text or a small table.

The type of carbohydrate and the protein source from the shakes was added to the manuscript: protein source: milk protein; type of carbohydrate: dextrin maltose.
2. Were any power calculations run? If so, on what were they based?

Power calculations were run (see also remark of Reviewer 1).

We calculated the number of participants needed based on the results reported in a previous study (Lemmens et al, Physiology & Behavior 103 (2011) 157–163).

Mean of H₀ (µ₁) = 35 %; Mean of Hₐ (µ₂) = 10 %

Standard deviation of the population (σ) = 35 %

Significance level for each test (alpha = 0.05)

Taken into account the Bonferroni correction (multiple-comparison correction) for multiple testing: alpha = 0.05/4 = 0.0125

β = 0.10

Power = 1 - β = 1 – 0.10 = 0.90

Two sided = H₀ - Hₐ

n = number of participants needed

n = 2 + [15 x (σ)² / (µ₁–µ₂)² ]

n = 2 + [15 x (35)² / (35-10)² ] = 31

This leads to a sample size of 31 participants. Taking into account a dropout of 7 subjects, this results in a total of 38 subjects.

The following was added to the manuscript (‘Participants’): “Based upon the study by Lemmens et al., power analysis showed that with an α of 0.0125 (taking into account the Bonferroni correction for multiple testing) and β of 0.10 (power=1-β=0.90), at least 31 participants were needed.”

MINOR COMMENTS:

1. In the first paragraph of the Methods section (lines 132-135), recruitment based on visceral overweight is described, but waist circumference is used as the criteria. Since waist circumference cannot discern between visceral and subcutaneous adipose, the term “abdominal adiposity” is suggested instead.

The remark was taken into account, the term ‘abdominal adiposity’ is used now.

2. In the first paragraph of the Methods section (lines 137-140), please provide the VAS values on which “liking” of food items was based.

The following was added to the manuscript (Methods > Test meals): “All food items scored more than 60 mm on a 100-mm VAS.”

3. In the Methods section, please specify the duration of time between test visits to the lab. What instructions were provided to subjects during these interims?
The duration of time between the test visits was at least one week (see Methods > Study design). Subjects did not get any specific instructions during these interims.

4. In the Results section, the second paragraph, first sentence under “Appetite Profile”, in the parentheses with data, please provide the p-value for the comparison between pre- and post-meal liking.

The p-value was <0.001 and was added to the Results.

DISCRETIONARY REVISIONS:

1. Spell the word “minute” instead of abbreviating it to “min”; sometimes this abbreviation is used for “minimum”.

This was changed in the manuscript.

2. In the second paragraph of the Methods section, second sentence, “unrestraint” should be changed to “unrestrained”.

Regarding the spelling of the terms “unrestraint” and “restraint”, they are written correctly as such as they are used as a noun. The spelling of “unrestrained” and “restrained” should be used in the case of an adjective, such as restrained subjects.

3. In the Methods section, under Study Design, please clarify how long the fast prior to testing was.

The fast prior to testing was at least 8 h and was added to the manuscript.

4. In the Abstract and/or Methods, the term ‘ad lib’ could be used to describe the self-selection of the second meal.

The wanted food items were self-selected, however they were not offered in an ad libitum amount, they were offered to the participants at a fixed amount, which was described to the participants beforehand, and food items were eaten completely. This was mentioned in the manuscript.

5. In the last paragraph of the Results, first sentence (line 383), do you mean, ‘showed no OVERALL differences between macronutrients’?

The following was changed in the manuscript: “Analyzing the amount of carbohydrates, fat, and proteins consumed for the ‘wanted meal’, showed that consumption of the high-protein meal, vs. high-carbohydrate meal, induced lower subsequent intake of carbohydrates, fat, as well as proteins in individuals with high disinhibition, during the rest condition (P<0.05), but not during the stress condition.”

6. In the first figure legend, change “was” to “were”.

The remark was taken into account.
ACCEPT AFTER REVISIONS