Reviewer’s report

Title: In-vitro examination of the positive inotropic effect of caffeine and taurine, the two most frequent active ingredients of energy drinks

Version: 0 Date: 16 Dec 2016

Reviewer: Erik Konrad Grasser

Reviewer’s report:

The study authors investigated the impact of caffeine and taurine on myocardial tissue derived from patients suffering from cardiovascular diseases. Applying an electrophysiological test design, maximal isometric contractile force and contractile duration was measured in-vitro for a control period over 10 minutes and was followed by a post-treatment period of 20 minutes. To distinguish between the effects for caffeine and taurine alone and in combination three groups were ascertained where group 1 consisted of 15 "specimens (number of myocardial tissue)" , group 2 of 21, and group 3 of 32. The applied dosages for caffeine and taurine have been chosen equivalent to twofold of the maximal plasma concentration found after excessive usage of energy drinks. The study authors concluded that caffeine, caffeine and taurine combined, but not taurine alone, increased contractile forces in human right-atrium myocardium derived from older patients suffering from cardiovascular diseases.

Although this study provides some interesting new findings regarding vasoactive ingredients found in energy drinks, there are a number of criticism based on the style, the presentation and the study design, which should be answered in a revised version of this manuscript.

Major comments:

1. This manuscript is suggested, from my point-of-view, having a critical reading from a native English speaking person, which would improve the readability significantly.

2. The findings here are derived from myocardial tissue from patients suffering from CVD where the majority of literature discussed here is derived from a healthy collective.
   a. It would better for the general reader to discuss in detail potential differences between the physiology of myocardial tissue in this patients and in healthy people in order to understand better differences between your findings and findings from other groups mentioned in this manuscript.
   b. What was your initial assumption/idea using substantially higher concentrations for caffeine and taurine than found in energy drink users? Could there be a dose-response characteristic for caffeine and taurine when it comes to myocardial tissue and could the condition of the tissue (older people with CVD) make a difference in the response?
3. Your stratification process (i.e. how you generated your groups => one group consists of 15 samples and one other consists of 32 samples with one in the middle) is not clear and leaves room for speculation that this heterogeneity (n=15, 21, 32) could have a statistical impact on your results.

4. You missed in your study design a very important and powerful contributor to changes in hemodynamic parameters in response to a commercial energy drink: "Sugar"

a. For example, you mentioned in your introduction that inconsistent study outcomes exit when it comes to human studies with regard to energy drink intake. In this context, you mentioned that Svatikova et al could not find an increase in heart rate, which was in opposition to the findings of Grasser et al. This is not correct because in the study by Svatikova et al both, placebo and energy drink (delta +4.3 bpm and +3.1, respectively), increased heart rate with NO difference between the groups!

b. I really would advise you to read the following review where potential mechanisms in response to energy drink consumption were discussed in detail: Grasser EK et al 2016. Energy Drinks and Their Impact on the Cardiovascular System: Potential Mechanisms. Advances in Nutrition.

c. Moreover, it has been shown that ingestion of glucose and sucrose (contained in a commercial available energy drink), but not fructose, increased inotropic cardiovascular parameters, therefore it seems that not only sugar can impact on the inotropic state in healthy humans it could also important which kind of mono-saccharide is used (Grasser EK et al. 2014. Cardiovascular responses to the ingestion of sugary drinks using a randomised cross-over study design: does glucose attenuate the blood pressure-elevating effect of fructose? British Journal of Nutrition).

5. You mentioned in your conclusions, at the end of the discussion, that energy drinks do exhibit a relevant inotropic effect. This statement is neither justified by your results nor your design because you investigated merely two compounds found in energy drinks but generalized it to overall energy drinks, which is a far-fetched generalization.

6. I believe that your experimental study design cannot be directly compared to study designs where energy drinks were ingested and cardiovascular responses measured afterwards in a "whole" organism. Therefore, it is not surprising to me that differences were found between your group and Doerner et al. Perhaps it would be a better discussion strategy to compare your findings with others using the same or a similar study design (tissue derived from the myocardium => by the way, could it be that myocardial cells from the atrium responds differently compared to myocardial cells from the ventricle in response to caffeine and taurine? There is a substantial pressure difference between the two compartments and the atrial contraction is not really substantially contributing to the filling of the ventricle (just ~20%) ….).

Minor comments:
1. Findings where no significant differences (e.g. everything related to taurine alone) have been found should not be presented in detail in the abstract and result part. => the whole paragraph for contractile duration can be summarized in one sentence. If you mention "subtle reduction" without a p-value, then this information is not really understandable to the general reader => if p > 0.05, then there is no reduction, it is simply no difference between the groups.

2. Please indicate clearly that your myocardial tissue was derived from patients suffering from CVD in order to avoid misunderstandings when it comes to comparisons with findings from other study groups.

3. Please round results everywhere in the manuscript ("p=0.00105" should be avoided).

4. In your table you should put the actual patients used but not all of them, and all the relevant information related to this patient cohort should go in the table, including medication.

5. In the statistical analysis part there is no mention of your chosen level of significance and if SEM or SD was applied. Moreover, why did you use SPSS for comparison and R for ANOVA analysis? SPSS and R can do both.

6. Please change your figures and present averages over 5 minute periods, starting with baseline and throughout your post-treatment period. Moreover, please provide information for your axes directly on the figure (e.g. Y-Axis: Percentage changes relative to …; X-Axis: time line in minutes) and consider presenting absolute instead of relative values (and explain it in the figure legend).

Are the methods appropriate and well described?
If not, please specify what is required in your comments to the authors.

No

Does the work include the necessary controls?
If not, please specify which controls are required in your comments to the authors.

Unable to assess

Are the conclusions drawn adequately supported by the data shown?
If not, please explain in your comments to the authors.

No

Are you able to assess any statistics in the manuscript or would you recommend an additional statistical review?
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I recommend additional statistical review
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Please indicate the quality of language in the manuscript:

Not suitable for publication unless extensively edited

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