Author's response to reviews

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

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Author’s response to reviews:

Dear Reviewers,

Thanks a lot for your helpful critics. Acknowledging our shortage, we have conducted many changes to cover the points mentioned by your reports. Summarized together, the following changes were made:

- 25 more trails were conducted to achieve equality between the 3 groups. Now we have: 29 trails for group 1, 31 trails for group 2 and 32 trails for group 3. Together they made up 92 trails.

- We have introduced a new concept for measuring the contractile force and the duration of contraction. Instead of measuring values in a specific time point (after 5 minutes, after 10 minutes and after 15 minutes) like in the first review. We measured now the averages over a 5-minute period for the contractile force time of contraction. This “measuring period” was defined clearly to be the 5-minutes period between minute 5 and minute 10 (see Fig 2 for more details). This helped us to generate fewer, but more significant numbers, which should be easier for the readers.

- A new figures were presented.

- The statistic evaluation was completely redone
- Changes in the introduction and discussion were made to address your critics.

- The following declarations were provided in the main manuscript as wanted:
  - Ethics approval and consent to participate
  - Consent to publish
  - Availability of data and materials
  - Competing interests
  - Funding
  - Authors' Contributions
  - Acknowledgements

Respond to Reviewer report:

1. Proofreading was done

2. We acknowledge this. However, myocardial tissue from healthy individuals is difficult to obtain. A section discussing potential differences between the physiology of myocardial tissue in our patients and in healthy people was included in the discussion, paragraph 2.
   
a. This was reviewed in discussion, paragraph 3.

   b. As we aimed at demonstrating that the inotropic effect is neglectable, we have chosen somewhat higher concentrations to be on the safe side. The concentrations we have chosen correspond to plasma concentration levels of caffeine and taurine after high to excessive consumption of coffee/energy drinks but are non-toxic (discussion, paragraph 1). With regard to dose-response effects, we would like to point out that taurine had no effect at all. As for caffeine, we suppose that the effect is dose-dependent but demonstrating dose dependency and investigating the effect of CVD on myocardial response to caffeine exceeded the framework of our current project.

3. We agree and have therefore implemented more experiments (25 trails) to reach equal numbers of samples in each group. Now we have: 29 trails for group1, 31 trails for group 2 and 31 trails for group 3. Together they made up 92 trails.

4. We agree that ingestion of sugar can impact the inotropic state in humans. We did not use sugar in our experiments as we aimed to demonstrate the (separate) effects of taurine and caffeine. We have changed the title of our paper to reflect this.
a. This is correct. We changed our wording to indicate that there was no significant different in heart rate INCREASE between the two groups (introduction, paragraph 5).

b. We have read the recommended review and found that its findings are largely in keeping with our results as far as the effects of caffeine and taurine are concerned.

c. The effects of sugar in energy drinks are undisputable but exceed the limits of our current work, which was very specific in that we applied an in-vitro model to investigate the effects of caffeine and taurine on the contractile properties of human myocardium. Although, this study (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) has simply neglected the substantial effect of giving 20 young people 500ml water (as a volume) after 12 hours of fastening, which would simply improve the hemodynamics, causing increase in SV und cardiac output und decreasing the peripheral resistance, which Grasser self, has proven in another work (Grasser EK, et al. 2014. Cardio- and cerebrovascular responses to the ED Red Bull in young adults: a randomized cross-over study. Eur J Nutr). This leaves fructose as the potential culpable.

5. We changed our wording to render our conclusion more precise and pointed out that our in-vitro experiments showed that one of the two compounds we investigated may exert a positive inotropic effect when ingested as part of an energy drink (discussion, paragraph 10).

6. We are aware of the difference between in-vivo responses to ingestion of energy drinks and our in-vitro experiments. We are not aware, however, of any previous studies using the same or a similar in-vitro Study. Therefore, we discussed our results against the background of in-vivo effects. With regard to the type of myocardial fibers used, we included a “Limitations”.

Minor comments:

1. Corrections have been made.

2. We have added a sentence to this effect.

3. Results have been rounded.

4. We have corrected this shortage and mentioned only the patients, which were used in the successful experiments. The required information has been also added.

5. The statistical analysis part has been once more reviewed by our statistician.

6. We have redone our calculations as recommended and changed the presentation of data accordingly.

Answers to Reviewer 2:
Further experiments have been conducted (25 more experiments) to achieve groups of similar size. Now we have: 29 trails for group 1, 31 trails for group 2 and 31 trails for group 3. Together they made up 92 trails.

- The required information has been provided.
- The required figure has been prepared.
- Corrections were made.
- Figures were revised.

Sincerely yours,

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