Dear Editor, Michalis G. Noklaidis, and reviewers

We are grateful for all the constructive comments on our manuscript. We have done our best to direct all comments and suggestions in the revised manuscript. Attached you will find a clean version of the revised manuscript and a version that highlights (in red, with “track changes”) all changes made during the revision process. We also provide a point-by-point response explaining how we have addressed each comment. Please let us know if something is still unclear.

On behalf of the authors,

Kristian Lian

## Revision comments and answers

#### Reviewer 1

“Lian et al. investigated the effects of glucose ingestion on the ribosome biogenesis responses post resistance exercise. This is a very interesting research question, which was well performed by this research group. I overall enjoyed reading this well-written manuscript and have very little to suggest. Please see my comments below.”

**Authors reply:** Thank you very much, we are glad the reviewer enjoyed the read and found our research question interesting.

*Comment 1: 25,000 dilution for primary seems quite diluted. Is the dilution is correct? Page 9, line 257-258.*

**Authors reply:** Thank you for bringing this to our attention. By mistake, only the dilution volumes for the secondary antibodies were included in the original description. This section is now updated with dilution volumes of both primary and secondary antibodies. Original draft: Antibodies were diluted in blocking buffer to concentrations corresponding to 1:25 000 (UBF, rpS6) and 1:5000 (c-Myc). Revision: Antibodies were diluted in blocking buffer to concentrations corresponding to 1:500 (UBF and rpS6, primary), 1:2000 (c-Myc, primary), 1:5000 (c-Myc, secondary) and 1:25 000 (UBF and rpS6, secondary). Added on page 11, line 302 (page 10, line 279 in the clean manuscript).

*Comment 2: Most of those antibodies have been discontinued by the commercial provided (Santa Cruz). Did the authors try to validate these antibodies?*

**Authors reply:** Thank you for this input. We did several runs of antibody testing with these specific antibodies before analysing the muscle tissue from the present study. We compared the blots from our test runs to the expected molecular sizes provided by the manufacturer as a form of validation. Additionally, no secondary bands were detected. Unfortunately, we are not in possession of any knock-out tissue for complete validation. We believe all of the primary antibodies are still provided by the vendor:

- UBF: <https://www.scbt.com/p/ubf-antibody-f-9>

- rpS6: <https://www.scbt.com/p/ribosomal-protein-s6-antibody-c-8>

- c-Myc: <https://www.thermofisher.com/antibody/product/c-Myc-Antibody-clone-9E10-Monoclonal/MA1-980?imageId=1024462>

*Comment 3: Given that whey protein was also given to the participants following the resistance exercise session, could the increase insulin from whey protein ingestion already be enough to evoke the anabolic effects related to ribosome biogenesis? Did the authors measure plasma insulin levels? It would be interesting to this story to check insulin levels.*

**Authors reply:** Thank you for bringing up this subject. We did assume that protein ingestion could impact plasma insulin levels. Therefore, the participants waited for 2 hours from ingesting the protein supplement before starting the training sessions, thus “washing out” the potential effect of protein on plasma insulin levels before the training session. However, as noted by the reviewer, the participants did not wait for 2hrs after the sessions to ingest the second/last protein supplement. In the glucose condition sessions, c-peptide levels were significantly higher at the onset of training (Figure 1C, 0min) and at the end of training (Figure 1C, 30min) compared to placebo, and returned to baseline values 2hrs after training (Figure 1C, 120min). So, based on these data, glucose ingestion did significantly increase c-peptide compared to placebo during exercise, with no measured effect of protein ingestion on c-peptide levels from 2hrs pre until 2hrs post exercise. However, we cannot exclude that there were peaks/fluctuations in c-peptide as a response to ingesting protein that we did not measure in this time frame, especially after exercise with a 2-hour-long period without measurements. One could argue that if the protein ingestion *per se* stimulated a large insulin secretion, this would trigger increased glucose secretion from the liver to the bloodstream as a response to the insulin, to stabilise the plasma glucose levels. Our 30min post-protein ingestion measurement of plasma glucose showed no such changes at this time. Nevertheless, without having measured c-peptide/insulin levels more frequently following the training sessions, we have no way of knowing what truly occurred in this time frame.

We decided to measure c-peptide from serum samples rather than insulin. Indeed, this provides an indirect measure as opposed to directly measuring plasma insulin. However, c-peptide stays in the blood for a longer time than insulin, making c-peptide easier to accurately measure over several time points (Leighton, Sainsbury, and Jones 2017). Further, c-peptide is widely used as an insulin secretion measure and is demonstrated to provide insight into changes in plasma insulin levels (Leighton, Sainsbury, and Jones 2017).

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#### Reviewer 2

“This manuscript is an original article that performed double-blinded placebo-controlled crossover trial with the main purpose to evaluate if the glucose supplementation before and after five RT sessions over 12 days will potentiate RT-associated accumulation of markers of ribosomal abundance following five RT sessions in healthy moderately trained young adults. The article is well written and the clinical trial well designed. The study presents novelty. Although, this clinical trial has important limitations: not reaching the calculated sample; intramuscular glycogen and circulating insulin levels were not performed.”

**Authors reply:** Thanks for these kind words. We are glad the reviewer appreciates the manuscript.

*Comment 1: Please, also describe in the abstract the second objective of the study (lines 98-99). The abstract does not state, and, therefore is not clear, what are the main outcomes (parameters) measured in the clinical trial.*

**Authors reply:** Thank you for pointing this out, the abstract has now been updated with this information. - Removed “However, this remains largely unexplored.” - Added “This was investigated with total RNA and ribosomal RNA abundances as main outcomes, with relevant transcriptional or translational regulators (c-Myc/UBF/rpS6) as a secondary outcome.” On page 1, line 14-16 The abstract is now approximately 35 words over the limit of 250 words to accommodate these changes, we hope that this does not cause any inconvenience. If necessary, we will adjust this too.

*Comment 2: In the results the data were described in relation to % ou fold of changes. It is important to report the data also in absolute values.*

**Authors reply:** Thank you for your valuable input. The results are now updated with absolute values of change from baseline to post in both conditions:

Glucose results revision: Glucose ingestion before and after RT led to increases in plasma glucose levels compared to baseline by 38% immediately before RT (Figure 1B, 0 min: 2.05 ± 0.73 mmol/L), by 31% during RT (Figure 1B, 15 min: 1.75 ± 1.44 mmol/L) and by 32% immediately after RT (Figure 1B, 30 min: 1.62 ± 1.10 mmol/L, all : p < 0.001), with no changes being observed in the placebo condition (Figure 1B, 0 min: 0.09 ± 0.3 mmol/L, 15 min: 0.16 ± 0.35 mmol/L, 30 min: 0.18 ± 0.39 mmol/L, p > 0.05). Added on page 12, line 344 (page 11, line 319 in the clean manuscript)

C-peptide results revision: Glucose ingestion before and after RT led to increases in levels of c-peptide compared to baseline, by 95% immediately before (Figure 1C, 0 min: 796 ± 376.0 pmol/L) and 87% after RT (Figure 1C, 30 min: 793 ± 581.0 pmol/L, both p < 0.001), with no changes observed with the placebo condition (Figure 1C, 0 min: 63.7 ± 71.0 pmol/L, 30 min: 53.9 ± 134.0 pmol/L, both p > 0.05). Added on page 12 line 356 (page 11, line 328 in the clean manuscript)

Absolute values for knee extension torque are added to Table 3, referenced by “Table 3 shows the mean change in absolute peak torque per condition and angular velocity.” on page 13 line 376 (page 12, line 347 in the clean manuscript). Baseline muscle strength values are now removed from Table 1, since the detailed table of knee extension torque measurements are added (Table 3).

Revision of total RNA and rRNA: (glucose: 263 ± 50 ng/mg-1 , placebo: 210 ± 121 ng/mg-1) and; (47S; 0.253 ± 1.27 and 0.576 ± 0.677, 18S; 0.336 ± 0.460 and 0.271 ± 0.470, 28S; 0.314 ± 0.504 and 0.311 ± 0.582, 5.8S; 0.388 ± 0.576 and 0.322 ± 0.520, 5S; 0.305 ± 0.608 and 0.292 ± 0.432, arbitrary units for glucose and placebo respectively) added on page 13 line 383 (page 12, line 352 in the clean manuscript).

*Comment 3: There is a lack of information regarding the use of supplements by participants. This aspect is important for characterizing the sample and as stated by the authors on page 3, line 57.*

*Authors reply:* Thank you for this important remark. The participants were asked not to use any other form of supplements during the study. However, the authors can see that this was not clearly stated in the methods chapter, but this has been clarified in the revised manuscript. “Apart from this, participants ingested a self-chosen diet during period II, registered in MyFitnessPal or similar applications” has been changed to “Apart from this, participants ingested a self-chosen diet during period II. Further, participants were asked not to use any other supplements such as additional protein and/or creatine, and to register all food/drink consumption in MyFitnessPal or similar applications.” Page 7, line 183 (page 7, line 163 in the clean manuscript).

*Comment 4: Figure 3 shows the result for RPS6. However, only in the discussion section there is some description about this protein (lines 377 - 381). I suggest that this be addressed in the introduction for better understanding of the reader.*

**Authors reply:** Thank you for pointing this out. The following has been added about rpS6 in the introduction: “Furthermore, Nakada et al. (Nakada, Ogasawara, Kawada, Maekawa and Ishii, 2016) found a correlation between rRNA content and rpS6 content in their synergist ablation model on rats.” Page 5, line 123 (page 5, line 106 in the clean manuscript).

*Comment 5: Figure 1d, use only the word “post” or “after”.*

**Authors reply:** Thank you for bringing this to our attention, Figure 1D is now updated with only “post” instead of the mix of “after” and “post”.

*Comment 6: In Table 1, provide body fat percentage data, together with the absolute lean mass data (already shown), they become more informative to the reader.*

**Authors reply:** Thank you for that suggestion, Table 1 is now updated with body fat %.

*Comment 7: Please specify how the “blinded taste test” (described on page 6, line 120) was carried out.*

**Authors reply:** Thank you for this valuable input. We have added the following description of the blinded taste test: - “In this blinded taste test, the participants were given two glasses of glucose mix (75ml per) and two glasses of the placebo mix (75ml per), consumed in a randomised order per participant. The participants were instructed to finish one bolus, note their guess for its content, and move on to the next glass. On average, the participants had a score of 2 points (2.08 ± 1.24) out of 4 possible.” Page 5-6, line 157 (page 6, line 138 in the clean manuscript)

*Comment 8: Mention more characteristics related to the training of the participants at baseline, such as number of weekly or 14-day RT sessions (which was an inclusion criteria).*

**Authors reply:** Thank you for bringing this up. Sadly, we do not have detailed characteristics on individual training sessions per week prior to enrollment in the study, other than that they did indeed confirm to have a weekly resistance training session volume between two and eight sessions per 14 days for the last six months. The authors realise that this leaves room for variation between participants in terms of training status at baseline, however, variations such as these should be controlled for by the within-participants analyses. This is now addressed in “Limitations and strengths” Page 17, line 519 (page 16, line 471 in the clean manuscript).

**Additional changes to the manuscript:** Knut Sindre Mølmen has been added to the author list. He contributed with *conception of idea, performed experiments and edition of manuscript* and was accidentally omitted from the list of authors in the original submission. Bachelor students Max Ullrich and Chris Sylstad were also accidentally omitted from the acknowledgements in the original submission, but have now been added. We have also taken steps to improve the language and flow of the manuscript at multiple points, as highlighted in red and with “track changes”. Lastly, we added suggestions for where tables and figures may be placed.

**References**

Leighton, Emma, Christopher AR Sainsbury, and Gregory C Jones. 2017. “A Practical Review of c-Peptide Testing in Diabetes.” *Diabetes Therapy* 8: 475–87.