Response to reviewer 1

Comments and Suggestions for Authors

In the review article, Drs Merckx and Paepe introduce the roles of taurine on the skeletal muscle functions, its dynamics in Duchenne muscular dystrophy and the effectiveness of its treatment on the diseases. Authors review a lot of information about pathology, physiology, biochemistry, and etc. about the skeletal muscles, however, there are many explanations of basic knowledges as literature levels especially in sessions 2 and 3. Authors need to brush up the explanation of basic knowledges and more focus on the evidence of taurine. Authors introduce many evidence and studies about taurine, particularly in its physiological roles, however, most of them are obtained not only from skeletal muscles but various other tissues and cells. It is unclear whether these identified roles of taurine can be adapted to skeletal muscle. So, authors need to explain which tissue the evidence was obtained from and whether it is the same in skeletal muscle.

It is also advisable to add figures and schemes to make the readers easier to understand.

We thank the reviewer for thorough reading of this manuscript and the well-defined comments made. Indeed, the manuscript recapitulates basic knowledge, yet we consider broader introduction necessary as the readers' interest in this special issue focussing on taurine may not be familiar with muscle function and disease. Therefore, we explained in more detail excitation-contraction coupling etc. We have tried to make a concise summary of these general mechanisms/processes since this review is already lengthy. We have attempted to limit information of basic cellular processes to what we consider necessary to comprehend the review, yet we are open to suggestions to reduce these sections. We agree that some of the literature is less contemporary, yet these concern key-findings still often referred today. Indeed, general mechanisms such as protein stabilization or osmoregulation have at times been explained in a different context than in muscle cells, but only when investigation of these basic concepts is not yet available in muscle cells. To accommodate these shortcomings, we ended each paragraph with discussion of the relevance for the function of muscle cells. We tried to clearly state when experiments were carried out in skeletal muscle cells and when other cells were investigated as clarified in lines 208, 210, 211, 276, 353, 441, and 454. This manuscript reviews the (broad) role of taurine in skeletal muscle, making it challenging to create 1 comprehensive figure that summarizes the manuscript. However, if the reviewers think the graphical abstract would add a surplus value, this will be provided.

Specific comments:

There are coexisted expressions about taurine transporter knockout including TauT KO, TauT-/-, and TauT deficient, and about canticum and Ca2+. Please, make them uniform expressions.

The expressions were uniformised.

Once an abbreviation has been set, the word must use the abbreviation. For example, "taurine transporter" and "knock out" appeared many times after setting of their abbreviations.

The necessary changes were implemented.

Lines 67-69: In the previously published article, Warskulat et al. showed the double value of serum CK level in sedentary state and the elevated lactate release during exercise in the TauT KO mice compared to those in the respective states of age-matched WT mice, and so, suggested that the pathological abnormalities in the skeletal muscle directly induced by taurine deficiency may related to the reduced exercise performance in the treadmill experiment in the TauT KO mice. Accordingly, this previous article did not hypothesize that hampered exercise performance was more likely attributed to lack of taurine rather than structural aberrations.

This sentence was changed to:" This study reported structural changes in morphology of TauT KO muscle however, Warskulat et al. hypothesized that hampered exercise performance was likely

attributed to muscle dysfunction, resulting from taurine deficiency. In evidence, serum creatine kinase levels are increased in TauT KO and serum lactate levels were raised after exercise [2].”
This way, the structural aberrations that are linked to the lack of taurine are not excluded from the hypothesis. Of note, the paragraph in the article of Warskulat et al. (ref.2) referring is: “However, at present it cannot be excluded that the observed changes of morphology may be due to age-dependent structural defects of skeletal muscle found in inbred mice strains (28). Detailed morphological studies are required to settle these issues. However, it is most likely that changes in taut−/− skeletal muscle account for the decreased overall exercise capacity observed. Furthermore, the increased lactate release during exercise and the elevated serum CK levels in taut−/− mice give additional evidence that the reduced performance of these mice is due to a disturbance of normal skeletal muscle function. The impaired integrity of taut−/− skeletal muscle may be directly linked to the loss of taurine, since taurine has been shown to exert its beneficial effects on muscle function via several mechanisms.”

Line 69: “lactate serum level” should be improved to “serum lactate level”.

The necessary adjustments have been made.

Line 73: “taurine muscle content” should be improved to “muscle taurine content”.

The necessary adjustments have been made.

Line 74: Please add reference following “60%”.

The reference was added following “60%”.

Line 79: Please move “respectively” following “686 days” and add “,” between them.

The necessary adjustment has been made.

Lines 82-83: Please add information of “in vitro model” such as cell type and species (origins).

The in vitro model refers to L6 myogenic rat cells. For the sake of clarity, we added that the in vitro model concerns L6 cells.

Line 84: Please add reference following “taurine treatment”.

The reference was added following taurine treatment.

Line 111: Please delete “taurine transporter”.

Taurine Transporter was changed to TauT.

Lines 130: It wonders why “anoxia” is suddenly mentioned.

Initially this was mentioned as additional information, but is removed from the text.

Line 139: Please add reference following “exclusion”.

The reference was added following “exclusion”.

Lines 133-163: Is the explaining about the membrane stabilization by taurine able to be also adapted to skeletal muscle cell (fiber), and are there evidence in skeletal muscles?

Lines 133-163 provide the current 'basic' mechanisms by which taurine is able to exert protein and membrane-stabilization in general. Lines 174-182 focus specific on the skeletal muscle. In TauT KO-models the unfolded protein response was activated in skeletal muscle and could point towards the importance of taurine in protein stabilization specifically in skeletal muscle [15]. Furthermore, muscle struggle to maintain their native conformation during exercise[46].

Line 177: Please add reference following "mouse model".

The reference was added.

Lines 199-200: Reference [63] was study in the liver and kidney. Does ethanol induce oxidative stress in the skeletal muscle, and does taurine have anti-oxidative effect in the ethanol treated skeletal muscle?

This reference was to show that taurine is widely known for its anti-oxidative properties and this goes beyond the skeletal muscle. For the anti-oxidative mechanisms specifically for skeletal muscle, please consult lines 202-203 and sections 3.2 and 4.2.

Lines 200-201: This sentence is unclear what types of exercise were loaded in the animals and what kinds of oxidative stress were induced by the exercises.

More information on the experimental set-up and read-outs was added.

Lines 221-226: Are there any evidence about the effectiveness of taurine treatment on the mitochondrial encephalomyopathies?

Indeed, taurine improved the occurence of stroke-like episodes in MELAS patients(Clinical Study Phase III) "Taurine supplementation for prevention of stroke-like episodes in MELAS: a multicentre, open-label, 52-week phase III trial"; This citation was added to the revised manuscript [68].

Line 245: "SR-derived rat skeletal muscle" is unclear. Is it "SR derived from rat skeletal muscle"?

Yes, this was corrected in the review.

Line 254: "sarcoplasmic reticulum" should be "SR".

The necessary adjustment has been made.

Lines 258-266: The evidences were obtained from cardiomyocytes. They could be adapted to skeletal muscle?

Although cardiac and skeletal muscle are both striated muscle, they do differ. Similar experiments have not been executed in skeletal muscle, therefore it is explicitly mentioned that the study concerns cardiac muscle tissue.

Lines 277-279": The sentences are repetition of the sentence in lines 133-114.

The repetition was removed from the review.

Lines 280-281: "at day 18 and 42" and "at day 28" are the number of days old?

Yes, it refers to the age of the mouse (at the endpoint of the experiments). For the sake of clarity, we added 'postnatal'.

Lines 279-284 and Lines 285-292: It is unclear what difference between these sentences of the first and second paragraphs mentioned.

In the first paragraph the expression of the Taurine Transporter (TauT) is mentioned, whilst in the second paragraph the results refer to muscle taurine content. Although TauT greatly impacts the muscle taurine content, alterations in TauT activity or posttranslational modifications cannot be ruled out and could also impact on muscle taurine content."

For the sake of clarity we added changed the sentence of paragraph 2 into: Similarly to the expression of TauT, differences in muscle taurine content with age were reported as well, yet with considerable differences between studies".

Lines 294-295: Because there is a variety of evidence for increased, unchanged, or decreased taurine content in mdx mice, the meaning of this statement is unclear. Which are the "most these studies", how is taurine differently regulated in the model mice, and when is the "certain time point"? Please, write it more carefully since this is the conclusion of these studies.

The vast majority of studies (ref. 59,74,79,80) reported a difference in either TauT or taurine content in mdx mice compared to controls at a given moment, however the results differ at certain timepoints and at times conflicting results are reported. Therefore, at this time the conclusion can only be that the taurine pathway is deregulated in the mdx mice compared to wild type mice

The time points differs from study to study and is specified in the review itself, however for the sake of clarity I will list it hereunder.

Timepoint of analysis	Muscle TauT content	Muscle Taurine content	Muscle type	reference
18 days	↓ in mdx mice	≈ ctrl	quadriceps	74
22 days	/	≈ ctrl	quadriceps	81
28 days	≈ ctrl	↓ in mdx mice	quadriceps	74
28 days	≈ ctrl	≈ ctrl	tibialis anterior	79
6 weeks	↓ in mdx mice	≈ ctrl	quadriceps	74
6 weeks	/	↓ in mdx mice	quad/gas	59
6 weeks	/	≈ ctrl	tibialis anterior	82
10 weeks	≈ ctrl	↑ in mdx	tibialis anterior	79
6 months	≈ ctrl	↓ in mdx mice (wet weight) ≈ ctrl (dry weight)	EDL (TauT) plantaris (taurine)	80

Lines 259-297: It wonders why this sentence about glucocorticoid treatment and immunosuppressive therapeutic intervention are suddenly mentioned here.

The entry does not fit in the section 4.5 'combinatory use of glucocorticoids. Glucocorticoids are the golden standard for treatment of DMD patients. We have further highlighted the anti-inflammatory effects of glucocorticoid treatment in the introduction, which is relevant in DMD pathology. Interestingly, upon glucocorticoid treatment taurine levels increase, which might strengthen the hypothesis that alterations in taurine and/or its regulation associate with dystrophinopathology [74-76].

Lines 300-302: The words of "alterations" and "abnormal" are unsuitable, because authors explain in this sentence that the regulations of taurine and its transporter expression in the DMD patients are discerned similarly to canine model. Please, write clearly which "increase" or "decrease". In addition, the evidence in the DMD patients mean the regulation of urinary taurine excretion by TauT in kidney, and therefore, it is unclear whether this evidence can mention taurine content increases in the skeletal muscle in the patients.

The words "alterations" and "abnormal" refer to DMD patients versus controls, so the text now clearly states 'to controls ' . In this paragraph it is also stated that there is a difference in taurine regulation between the murine model and both the canine model and DMD patients. However, taurine regulation in GRMD dogs and DMD patients is consistently and significantly different from healthy dogs and healthy control patients. Similarly, DMD patients were shown to exhibit alterations in taurine regulation (similar to the canine model) under the form of abnormal urinary taurine to creatinine excretion and increased upregulation of TauT protein levels in the muscle compared to controls [84]. Since we do not have full access to article 85, we can only state that there is a difference but not whether this refers to an increased or decreased excretion of DMD patients. Hence, this sentence was removed from the manuscript.

Line 305: Please change "becomes" to "become".

The necessary adjustment has been made.

Line 330-331: In the section 3.2., most of sentences explain the oxidative stress management and protein synthesis in the mitochondria, and only last one sentence mentions about the taurine. Please, more focus on the evidence of taurine, since this article should review about taurine. This comment is the same for Section 3.3.

We understand this remark, however we opted to explain 'Taurine and oxidative stress' in general in section 2.4 and 'the effect of taurine on oxidative stress in view of Duchenne Muscular Dystrophy' in section 4.2. Subsection 3 with the title: "Pathophysiological characteristics of taurine in DMD" than specifically focusses on the link between taurine-related muscle functions and DMD pathophysiology. If the reviewer finds this confusing and would prefer these sections to be fused together, we are prepared to re-write accordingly.

Line 372: The first paragraph introduces both positive and negative effects of taurine on muscle force. So, please improve "positively".

The reviewer presumably is referring to the statement that: "Some mice model studies have reported taurine supplements to positively influence muscle force".

Indeed some studies have reported beneficial effects of taurine treatment on muscle force output. However, others observed no effect of taurine treatment and only one study (ref 100) reported a significant negative effect of taurine treatment in mdx mice. Therefore, we have changed the sentence to: "A beneficial effect of taurine on muscle force remains controversial: supported by some studies yet disproved by others".

Line 379: "1g/kg/day" is lower than the dose of 2.5% wt/vol?

Yes, 2.5 % wt/vol corresponds approximately to 5g/kg/day (calculated from ref. 100).

Line 380: "in vivo force" is unclear.

In vivo force refers here to fore limb force measured by grip strength / body weight. This was adjusted in the text.

Line 387: Is "treated mdx animals" the same to "taurine treated mdx mice"?

Yes, for the sake of clarity, this was adjusted in the text.

Line 394: What does (±) mean, particularly in "minus"?

In this study, taurine was added to drinking water (2% wt/v). Based upon variation of intake of water and the body weight of the individual mouse, this dose corresponds approximately to 4g/kg/day. Both units 2% wt/v and 4g/kg/day were specifically mentioned in ref 82. We did not uniformized the units

throughout the review, but decided to report the same units as mentioned in its corresponding article since the conversion factor might variate between studies, bodyweight and water intake of mice.

Lines 395: The sentence “Whereas no……observed [102]” is repetition in lines 383-384.

In order to allow comparison between the different studies as much as possible, ex vivo force measurements were discussed before in vivo force measurements. The phrase: “Similarly, no effect on maximum specific force in 6-week-old mdx mice was observed when high doses of taurine (16g/kg/day) were administered” refers to the lack of effect in an ex vivo set-up (specific force as output measure), whereas the phrase “ Whereas no effect of high taurine treatment (16g/kg/day) was observed” refers to in vivo force measurements. For better comprehension, this was clarified in the text.

Lines 397-400: Based on the fact that the protocols and results are different in each study, it is unclear what the authors want to mention from those evidence.

This remark presumably refers to the sentences: “In summary, benefit of taurine supplementation on muscle force differed considerably between published studies. Although multiple studies have been executed, these used different concentrations, ingestion methods, and treatment protocols, which might explain the obtained conflicting results and which hampers interpretation”.

We want to point out to the readers that the possible reasons why there are so many contradicting results are differences in research design and protocols.

Lines 407-410: This information is also repetition.

The repetition was removed from the review.

Lines 423-426: Reviewers recommend writing this evidence in more detail on the effect of taurine treatment on the expression of E-C coupling protein.

The effect of taurine on calcium homeostasis and therefore also on E-C coupling was discussed in section 2.6. The role of E-C coupling proteins has been discussed in this section and we did not want to repeat ourselves to much. In section 4.2 the effect of taurine on E-C coupling was discussed specifically in view of dystrophinopathology. Unfortunately, not many studies have investigated the effect of taurine on E-C coupling proteins in mdx mice. Therefore little to no information can be added to section 4.2.

Lines 426-427: It is unclear why taurine treatment could increase calsequestrin expression in rats whereas the expression was unchanged in mice. Are there differences in treatment protocol of taurine (dose or duration) or species difference in the regulation of calsequestrin expression between rats and mice? If the reasons were known or unknown, please describe them.

The dose used in mice and rat studies was 2.5% w/v taurine in drinking water. In the rat study, rats received taurine treatment for a period of 2 weeks, whilst in the murine mdx study the drinking water was supplemented with taurine prior to birth of the animals and continued until the age of 4 weeks or 10 weeks. Thus, although they received the same dose, treatment was of different duration. In the murine study, the mean expression of calsequestrin is a little bit higher in taurine treated mdx than in untreated mdx, but this difference is not significant. In addition, in the murine study taurine was supplemented in mdx mice, whereas in the rat study, Sprague-Dawley rats were used that are not deficient for dystrophin. Differences in treatment protocol, age-dependent calsequestrin regulation, changes in calsequestrin regulation upon dystrophin deficiency, species-dependent regulation are all possibilities that could explain these differences, however a specific explanation cannot be pinpointed with the current literature studies available. We have clarified this in the text.

Line 439: Please explain that PDN is the synthetic adrenocortical hormone.

This additional information was added to the text.

Line 441: Please add reference following “Cozzoli et al”.

The reference was added to the sentence following ‘Cozzoli et al.’

Line 448: Please add information what was histopathological markers.

More details were added to the text.

Line 453-455: “synergistic antimicrobial effects” of taurine with dexamethasone is related to the pathology of dystrophy?

This study describes a synergistic effect of taurine and dexamethasone and is not directly related to the pathology of dystrophy. This merely shows that the combination of TauCl and dexamethasone can be beneficial through synergistic actions, however how this synergistic effect is exerted is not known. Therefore we decided to remove this section.

Lines 459-461: It also is wondering how the effect of taurine on glucocorticoid-induced mitochondrial dysfunction of the bone is related to the pathology of dystrophy?

Glucocorticoid treatment can impair bone health and can induce osteonecrosis. Glucocorticoids, used to treat DMD patients, could also interfere with mineral bone density and osteonecrosis in DMD patients. Therefore it is interesting to know that taurine might counteract potential glucocorticoid-induced bone damage in DMD patients. We have clarified this in the introduction, stating that impairment of bone health is a side-effect of glucocorticoid treatment, and in section 4;5 that dysfunction of the bone is conspicuous in glucocorticoid treated DMD patients.

Lines 472-474: This evidence from [102] is in mdx mice?

Indeed, high taurine treatment (16 g/kg) in 6 weeks-old animals significantly reduced the body weight of mdx mice by more than 20% and shortened tibia length by 10%. Of note, the body weight and tibia length of untreated mdx mice were comparable to those of WT animals in this experiment.

Lines 475-477: In this sentence, authors explain that taurine treatment even if in long-term could not increase muscle taurine level. It is unclear which the authors would like to mention that taurine treatment is not effective on the dystrophy or the increment of muscle taurine content is not necessary on the effective of taurine treatment on the dystrophy.

Indeed, there is a lack of studies investigating the relation between taurine content in mdx muscle tissues and severity of dystrophinopathology. Furthermore, differences in experimental set-up make it difficult to draw conclusions. In reference 79 and 100, taurine supplementation did not increase muscle taurine content and this was accompanied by lack of taurine effect on muscle strength. Whereas in a short-term taurine supplementation in mdx-mice aged 4 weeks (also reference 79) taurine supplementation did increase muscle taurine content and taurine increased specific muscle force and ameliorated histopathological features. The latter might suggest that increasing muscle taurine levels in mdx mice are associated with beneficial effects. However, in the study of Terrill (ref 100) inflammatory markers (such as neutrophil elastase and MPO) were significantly reduced upon taurine treatment yet muscle taurine content was not increased, suggesting more complex mechanisms are at play.

Lines 502-515: Section 4.7. is not related to taurine on dystrophy, and so, it is wondering if this section is necessary to this review of taurine on dystrophy or not.

We added a section on the use of nutritional supplements in DMD patients to highlights the quest for supportive treatments in the form of nutritional supplements, including but not limited to taurine. Supportive treatment in DMD is still relevant and continuously under development. A similar sentence was added to section 4.7 to highlight the importance of nutritional supplements. However, if the reviewer suggests to remove this section, we will follow up on this advice accordingly.

In reference.

Authors need to check the style of reference, especially in the journal style including the large character, and bold and italic.

Reference 14: “J or Amino acid” is incorrect. “Amino Acids” is correct.

The necessary adjustment has been made.

Reference 16: “Taurine” should be changed to “Adv Exp Med Biol”.

The necessary adjustment has been made.

Reference 69: Please add information of issue number and pages.

There was no page number available. However, we added the article ID number.

References 86 and 94: Two references may be same.

The necessary adjustment has been made.