

Response to Reviewer 1 Comments

We appreciate the careful and mindful review by this Reviewer. The comments have been helpful in improving our manuscript. We would like to respond to each comment one-by-one as indicated below. Also please see the list of changes which appears following the response to the reviewer. The list included the changes made in response to another reviewer.

Comment 1

The authors address the question whether levocarnitine supplementation has an impact on muscle strength in chronic hemodialysis patients in a post-hoc analysis of an initially prospective, single center observational study.

The question is very, the findings are potentially interesting, as sarcopenia is an important topic for hemodialysis patients and ways to avoid it would be very valuable.

However, the data are not very strong and the groups compared are not "equal" as they differed in therapy indication (only low carnitine patients were treated).

Our reply

We appreciate this reviewer's accurate general comment and a positive rating on our manuscript.

Comment 2

Few minor issues:

As only patients were treated who had low carnitine levels, I would find it appropriate to write that accordingly.

Levocarnitine supplementation in patients with low carnitine levels had the effect on knee extensor strength, not generally – it was not randomized, it was observational and the groups differed in carnitine level as this was how they were assigned to treatment or not.

This is also one further limitation of the study – we do not know if patients with normal carnitine levels would have gained strength. (Baseline differences are ~2fold.)

Our reply

We agree that we showed that the levocarnitine supplementation was associated with the increased knee extensor strength only in patients with low carnitine levels because levocarnitine was not given to patients with normal carnitine levels. This is one of the study limitations as pointed out by this reviewer. In response to this comment, we have added this as the fifth limitation. Please see the change #12 in the list of changes below.

Comment 3

At baseline, Carnitine levels are about twice as high in the non-treated group than in the treatment group (table 1).

I would like to see the carnitine levels measured after one year of levocarnitine supplementation: Do you have these data?

There we would learn 1.) how well supplementation worked and 2.) if the patients without supplementation remained stable or lost carnitine over time.

Our reply

We appreciate this comment. However, we did not have repeated measurements of serum carnitine levels. This has been added as the sixth study limitation in the revised manuscript (Change #12).

Comment 4

Figure 2: could you also include all data points in your graph showing changes in KES?

Maybe as single data points in a grey shade in the background?

While average plus IQR is nice, it would be nicer and also informative to see all data points (plus average/IQR).

Our reply

We agree that, in Figure 2 right panels, it will be more informative to show individual plots in addition to the summary data as a group. We have done so in the revised Figure 2 (Change #7). Similarly, we have revised Figure 3 (Change #9)

Comment 5

Please go over the manuscript with a native speaker or someone close to. (Please excuse this point – if I had to publish in Japanese, my text would be unreadable. Your manuscript is very readable, but some words/some sentences would be easier to understand for an international medical audience if the text was reviewed for English language.) Examples to correct would be “thigh” instead of “thy” (page 9, muscle volume of the ...) among others. “Treated and untreated” in the abstract. Etc.

Our reply

We appreciate the reviewer’s comment on our careless error in the discussion and another point for correction in the abstract. These sentences have been modified to be more readable in this revision (Changes #1 and #10). Because we had only 10 days for revision and because it was hard to find the assistance by a native speaker during the winter holiday season, we have tried our best in order to improve the English by ourselves.

Comment 6

On page 10, the authors speculate about why knee extensor strength is changed but hand grip is not, and then they argue with muscle fibre composition of quadriceps versus biceps. To my knowledge, biceps muscle is not involved in hand grip.

Do you have data on more distal muscle groups that strengthen your point?

Otherwise I would leave this speculation out. While carnitine is important for mitochondria and

thereby Fatty acid oxidation, you do not have any data point on muscle metabolism in either muscle group (involved in knee extension or hand grip) in your observational post-hoc analysis.

Our reply

Regarding the different changes between knee extensor strength and handgrip strength, we do not have our own data on muscle metabolism to support our speculation. Then, based on the mechanism of action of levocarnitine which is closely related to mitochondria and fatty acid metabolism, we searched for previous reports on muscle fiber composition. According to an abstract written in Japanese, handgrip strength showed a stronger correlation with strength of biceps than other muscle strength. This was the reason why we compared the muscle fiber compositions between biceps and quadriceps muscles. However, we agree that biceps muscle is not directly involved in handgrip. Handgrip is more directly linked with flexor digitorum superficialis, flexor digitorum profundus, and flexor pollicis longus. We looked for papers in which muscle fiber composition was compared between these handgrip muscles and the quadriceps muscle, but no such report was found. In response to this comment, we have modified the 4th paragraph of Discussion (Change #11).

Comment 7

I do very much agree with the last point of the authors that further randomized, controlled and prospective studies are needed to address if and how levocarnitine improves muscle strength in hemodialysis patients. Maybe there could be a little more emphasis on this point. This study could be a good starting point for a randomized, controlled, prospective trial.

Our reply

We very much appreciate this comment. To put more emphasis on this point, we have added one more sentence at the end (Change #13).

Again, we thank the reviewer for the precious time and effort of the reviewer used for the evaluation of our manuscript. If the revision is insufficient, please give us another chance for revision.

List of changes

Change #1

Original, line 26-27 (Abstract)

During the 1-year period between 2014 to 2015, 67 and 37 patients were treated and untreated with intravenous levocarnitine (1000 mg per shot, thrice weekly), respectively.

Revised, line 26-27 (Abstract)

During the 1-year period between 2014 to 2015, 67 patients were treated with intravenous levocarnitine (1000 mg per shot, thrice weekly), whereas 37 patients were not.

Change #2

Original, Line 38-48 (First, second, and third paragraphs of Introduction)

A large number of hemodialysis patients suffer from sarcopenia and muscle weakness which are closely related to impaired activity of daily living (ADL) [1] and high mortality rate [2-4]. Effective prevention and treatment for these muscle problems would result in improvement of their ADL and healthy longevity.

Sarcopenia in hemodialysis patients is associated with age, body mass index (BMI), diabetes mellitus, and inflammation [4-6]. In addition to insufficient intake of protein and energy, the mechanisms for sarcopenia in kidney failure may include metabolic acidosis, inflammation, oxidative stress, decreased anabolic hormones, and others [7,8].

Carnitine deficiency may contribute to sarcopenia, decreased cardiac function, anemia, and other complications of hemodialysis patients [9,10].

Revised, Line 38-48 (First, second, and third paragraphs of Introduction)

A large number of hemodialysis patients suffer from sarcopenia and decreased muscle strength which are closely related to impaired activity of daily living (ADL) [1] and high mortality rate [2-4]. Effective prevention and treatment for these muscle problems would result in improvement of their ADL and healthy longevity.

Sarcopenia and lowered muscle strength in hemodialysis patients are associated with age, body mass index (BMI), diabetes mellitus, and inflammation [4-6]. In addition to insufficient intake of protein and energy, the mechanisms for sarcopenia in kidney failure may include metabolic acidosis, inflammation, oxidative stress, decreased anabolic hormones, and others [7,8].

Carnitine deficiency may contribute to sarcopenia, lowered muscle strength, decreased cardiac function, anemia, and other complications of hemodialysis patients [9,10].

Change #3

Original, Line 111-113

Levocarnitine (L-cartin injection 1000 mg syringe®, Otsuka Pharmaceutical Co., Ltd. Tokyo, Japan) was intravenously administered thrice weekly at the end of each hemodialysis session.

Revised, Line 112-120

In previous studies with hemodialysis patients using intravenous levocarnitine, it was given at the end of each dialysis session at a dose of 10–40 mg levocarnitine per kg of body weight [14, 16] or at a fixed dose of 2000 mg per shot [20]. In our practice during 2014 through 2015, one ampoule of levocarnitine solution (L-cartin injection 1000 mg syringe®, Otsuka Pharmaceutical Co., Ltd. Tokyo, Japan) containing 1000 mg of levocarnitine and dilute hydrochloride as a pH adjusting agent was intravenously administered thrice weekly at the end of each hemodialysis session. This corresponded to 10.6–26.3 mg levocarnitine per kg of body weight.

Change #4

Original, Line 123-125 (Measurement of muscle strength, 1st paragraph)

The value in knee extensor strength was corrected for body size by dividing it by 'dry weight' in kilograms of the patient.

Revised, Line 130-133

The value in knee extensor strength was corrected for body **mass** by dividing it by **body weight** in kilograms of the patient. **We used body weight after removing excessive body fluid by dialysis.**

Change #5

Original, Line 126-130 (Measurement of muscle strength, 2nd paragraph)

The handgrip strength was measured by a digital dynamometer (Grip D; Model T.K.K. 5101, Takei Scientific Instruments Co. Ltd, Niigata, Japan) in a standing position with putting participant's arm down and elbow joint extended [27,28]. The higher value of two consecutive measurements was used for analysis.

Revised, Line 134-139

The handgrip strength was measured by a digital dynamometer (Grip D; Model T.K.K. 5101, Takei Scientific Instruments Co. Ltd, Niigata, Japan) in a standing position with putting participant's arm down and elbow joint extended [27,28]. **For each side, two consecutive measurements were done, and the higher value was recorded. The highest value of the four measurements was used for analysis regardless of dominant or non-dominant hand.**

Change #6

Original, Line 139-142 (Statistical analysis, 1st paragraph)

First, the clinical characteristics in 2014 (baseline) were compared between the two groups. We summarized continuous variables as medians (interquartile ranges) and categorical variables as numbers (percentages). Comparison was done by Mann-Whitney U-test and χ^2 test, respectively.

Revised

First, the clinical characteristics in 2014 (baseline) were compared between the two groups. We summarized continuous variables as medians (interquartile ranges) and categorical variables as numbers (percentages). Comparison was done by Mann-Whitney U-test and χ^2 test, respectively.

We used non-parametric statistics because some variables did not follow normal distribution.

Change #7

Figure 2 has been modified so that the right panels include individual plots for the change in knee extensor strength.

Change #8

Original, Line 213-215

We examined whether the observed between-group difference in the 1-year change in knee extensor strength was attributable to the levocarnitine treatment by using the multivariable-adjusted linear regression model which included age, sex, duration of hemodialysis, diabetic kidney disease or not, prior CVD, and knee extensor strength at the beginning of the period were included as potential confounders (Table 2).

Revised, Line 223-228

We examined whether the observed between-group difference in the 1-year change (from 2013 to 2014, or from 2014 to 2015) in knee extensor strength was attributable to the levocarnitine treatment by using the multivariable-adjusted linear regression model which included age, sex, duration of hemodialysis, diabetic kidney disease or not, prior CVD, and knee extensor strength at the beginning of the period as potential confounders (Table 2).

Change #9

Figure 3 has been modified so that the right panels include individual plots for the change in handgrip strength.

Change #10

Original, Line 294-295 (3rd paragraph of Discussion)

levocarnitine treatment was reported to increase the circumference and muscle volume of the thy of hemodialysis patients [22].

Revised, Line 305-306 (3rd paragraph of Discussion)

levocarnitine treatment was reported to increase the circumference and muscle volume of the **thigh** of hemodialysis patients [22].

Change #11

Original, Line 298-314 (5th paragraph of Discussion)

How can we explain the different associations of levocarnitine treatment with the changes in knee extensor strength and handgrip strength in this study? The different responsiveness to the treatment may be attributable to difference in muscle fiber composition. As mentioned above, Giovenali et al [20] performed repeated quadriceps muscle biopsies before and after levocarnitine treatment in hemodialysis patients and showed that atrophy of type 1 and type 2A muscle fibers was improved, whereas no improvement was found in type 2B muscle fibers, suggesting that levocarnitine treatment improved muscle fiber types that generate energy via fatty acid oxidation than glycogen. These fiber types are contained more in quadriceps muscles than brachial biceps muscles, although quadriceps muscles are rich in both types of fibers [33-35]. As compared to type 2B fiber, type 1 and 2A fibers contain more mitochondria in which energy is generated via β -oxidation of long-chain fatty acids which need carnitine for their transport from cytosol into mitochondria [9,11]. Thus, the compositional difference in muscle fibers with different metabolic characteristics may explain the preferential association of levocarnitine treatment with increase in knee extensor strength than handgrip strength observed in this study.

Revised, Line 309-328

How can we explain the different associations of levocarnitine treatment with the changes in knee extensor strength and handgrip strength in this study? The different responsiveness to the treatment may be attributable to difference in muscle fiber composition. As mentioned above, Giovenali et al [20] performed repeated quadriceps muscle biopsies before and after levocarnitine treatment in hemodialysis patients and showed that atrophy of type 1 and type 2A muscle fibers was improved, whereas no improvement was found in type 2B muscle fibers, suggesting that levocarnitine treatment improved muscle fiber types that generate energy via fatty acid oxidation than glycogen. As compared to type 2B fiber, type 1 and 2A fibers contain more mitochondria in which energy is generated via β -oxidation of long-chain fatty acids which need carnitine for their transport from cytosol into mitochondria [9,11]. Thus, **we speculate that** the compositional difference in muscle fibers with different metabolic characteristics may explain the preferential association of levocarnitine treatment with increase in knee extensor strength than handgrip

strength observed in this study. However, we could not find previous reports that compared muscle fiber composition between muscles responsible for knee extensor strength (such as quadriceps muscle) and handgrip strength (such as flexor digitorum superficialis, flexor digitorum profundus, and flexor pollicis longus). Therefore, the above-mentioned explanation is a speculation which needs further studies.

Change #12

Original, Line 315-325, Study limitations

There are several limitations in this study. First, because this study includes only hemodialysis patients in Japan, we are not sure that the results of this study are applicable to other populations. Second, because we excluded patients who discontinued the levocarnitine treatment in three months following the instruction on the package insert, such exclusion may affect the results. Third, the non-carnitine group served as a control group, but the characteristics of the two groups were different unlike randomized controlled trials. To address this issue, statistical adjustment was done using multivariable-adjusted linear regression models. Forth, although the change in knee extensor strength may increase physical activity [36,37], we did not examine such association with levocarnitine treatment.

Revised

There are several limitations in this study. First, because this study includes only hemodialysis patients in Japan, we are not sure that the results of this study are applicable to other populations. Second, because we excluded patients who discontinued the levocarnitine treatment in three months following the instruction on the package insert, such exclusion may affect the results. Third, the non-carnitine group served as a control group, but the characteristics of the two groups were different unlike randomized controlled trials. To address this issue, statistical adjustment was done using multivariable-adjusted linear regression models. **Fourth**, although the change in knee extensor strength may increase physical activity [33,34], we did not examine such association with levocarnitine treatment. **Fifth, because levocarnitine treatment was done only in patients with carnitine deficiency as evidenced by low serum free carnitine levels, it is unknown whether the same treatment is associated with muscle strength in those with normal serum carnitine levels.** Sixth, we did not have repeated measurements of serum carnitine levels. Therefore, we are unable to show how much the supplementation increased serum carnitine levels in the carnitine group. Also, it is unknown whether serum carnitine levels stayed unchanged or decreased over time without supplementation in the non-carnitine group. Seventh, although we measured muscle strength, we did not perform measurement of muscle mass or diagnosis of the presence of sarcopenia. Eighth, there might be the effect of familiarization or learning effect in the increase of knee extension strength measurement. However, it is unlikely, because such effect, if any, should be cancelled out in the between-group comparison. Also, the measurements were done at an interval of one year.

Change #13

Original, Line 333-334 (Last part of Discussion)

In conclusion, this observational study in the carnitine and non-carnitine groups clearly demonstrated that treatment with levocarnitine was associated with increase in knee extensor strength among hemodialysis patients. In addition, levocarnitine treatment was more preferentially associated with the change in knee extensor strength than the change in handgrip strength. These observations, along with previous results by clinical trials, support the potential benefit on knee extensor strength of levocarnitine treatment in hemodialysis patients with carnitine deficiency. Further well-designed trials are needed to draw more solid conclusions.

Revised, Line 353-362

In conclusion, this observational study in the carnitine and non-carnitine groups clearly demonstrated that treatment with levocarnitine was associated with increase in knee extensor strength among hemodialysis patients **in the carnitine group**. In addition, levocarnitine treatment was more preferentially associated with the change in knee extensor strength than the change in handgrip strength. These observations, along with previous results by clinical trials, support the potential benefit on knee extensor strength of levocarnitine treatment in hemodialysis patients with carnitine deficiency. Further well-designed trials are needed to draw more solid conclusions. **This study could be a good starting point for such clinical trials.**

Change #14

Reference numbers have been updated. References #33-35 have been removed, and #36-37 have been changed to #33-34.

Change #15

Table 1. Body weight has been added to the table because this information may be important to interpret the dose of levocarnitine per kg body weight.

Change #16

A typographical error has been corrected.

Original, Line 353

The data will be availabe upon reasonable request ...

Revised, Line 381

The data will be **available** upon reasonable request ...