Author’s response to reviews

Title: Normal/high-fat milk consumption is associated with higher lean body and muscle mass in Japanese women aged between 40 and 60 years: a cross-sectional study

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Response to the Editor and the Reviewers

We truly appreciate valuable suggestions and comments made by the editor and the reviewers. Below are our point by point responses.

First of all, in the course of the revision, we found an error in the categorization of a participant concerning the low-fat milk consumption status. According to the change in the number of women in each group, we reanalyzed the data, which was shown as Table 3 (formerly Table 2).

Editor Comments:

Overall the background needs more work; it starts with literature about menopause, while it is not the focus of this study. The background should present available literature regarding aim of the study and if there is any gap in the literature; and rationale for conducting the study.

> The change in body composition induced by menopause, and the modification thereof by women’s dietary habits was the initial focus of our study, which had been incorporated into the Introduction section. It was revised according to the reviewers’ suggestion.

Methods: please add more information about the original study and study design; e.g. how were participants recruited? What was inclusion criteria? How data were collected etc
Some information concerning the recruitment etc. was added to the Methods section.

Definition of low and normal/high fat milk consumers and non-consumers is not clear. It seems that non-consumers of low fat milk include those who are high fat milk consumers. If this is the case, there is a problem with analysis and interpretation of the findings.

The definition of consumers and non-consumers was detailed in the Methods section.

Please add information about sample size calculation and if there was enough power in data analysis and study findings.

As this is a cross-sectional analysis of the baseline data obtained in a previous study, sample size calculation had not been performed.

Please add information about the study ethics approval.

The information about the study ethics approval was added to the Methods section.

Amy Hector (Reviewer 1): The study conducted by Sukenobe et al. investigated the association between low and normal/high fat milk consumption and body composition in Japanese women aged 40 to 60 years. The authors reported that compared with non-consumers, women who consumed normal/high fat milk had significantly higher lean body and muscle mass, which was associated with vitamin D intake from milk.

Comments:

Introduction Line 17: The effect between milk intake and body composition? Or the association?

The phrase was corrected as “the association between milk intake and body composition”.

Definitions Line 11: Please define consumers vs non-consumers further. How much milk per serving was consumed before the participants were considered to drink milk two or more times per week? This could have significant effects on the dose of protein and vit D consumed. On average, how many times per week did the “consumers” in the low and normal/high fat milk group actually consume milk?

Brief-type self-administered diet history questionnaire (BDH-Q) inquired about the intake of a food item on a 7-point scale. Concerning normal/high- and low-fat milk, the scale is not about the volume, but about the frequency of intake: twice a day or more (7); once a day (6); 4 to 6 times a week (5); 2 to 3 times a week (4); once a week (3); less than once a week (2); none (1). According to the participants’ responses, they were classified as “consumers” if they scored 4 or more, and as “non-consumers” if they scored 3 or less. The Methods section was reorganized to add this information.

Were multivitamin or calcium/vitamin D supplements taken into account?
The aim of the previous study of which the baseline data the present study utilized was to investigate the effect of a dietary supplement. Therefore, women who used other types of supplements had been excluded from the study. That piece of information was added to the Methods section.

Body Composition: what is the measurement error of this machine? Are the differences in body composition in this study outside of the range of measurement error?

The measurement error of the bioimpedance analyzer we used was reported as less than 0.5kg, and the difference between groups in the current study was 1.1 to 1.2kg, which was regarded as significant. The piece of information was added to the Methods section.

It would be helpful to explain the difference between LBM and muscle mass

A sentence was added to the Methods section: “The lean body mass is calculated as body weight minus fat mass, and it includes the weight of organs, skin, bones, water, and muscles.”

Dietary Habits:

A major concern in this study is the lack of information regarding dietary intakes of the participants. E.g. protein/fat/carbohydrate intake per day. Without this information, it is difficult to attribute the difference in body composition only to milk intake when differences in total protein intake may also affect these results.

The estimated average daily intake of protein was not significantly different between consumers and non-consumers of normal/high- and low-fat milk consumers. The data were inserted into the first paragraph of the Results section.

Statistical Analyses: Could you please further explain the ad hoc computer algorithm. What were these "98 different nutritional factors" and where is the data? It seems there are three comparisons you are trying to make: non-consumers vs low fat consumers vs normal/high fat consumers. Is a t-test appropriate in this case?

The list of 98 nutrients was added as a new Table 1. When we compared the two groups of consumers and non-consumers of normal/high-fat and low-fat milk separately, we used unpaired t-test, Mann-Whitney U test, and chi-square test where we assumed it appropriate.

Results: Please clarify how these numbers were calculated: "28 versus 57 women were classified as low fat milk consumers versus non-consumers, respectively, and 40 versus 45 women were classified as normal/high fat milk consumers versus non-consumers, respectively." Why does the number of non-consumers change? It might be worth reporting the number of low fat consumers, normal/high fat, and non-consumers separately and provide the data in this manner. This is particularly confusing given your definition "participants were considered consumers if they drank milk at least twice a week and non-consumers if they drank milk at most once a week". Should the milk consumers not then be split further into low and normal/high consumers?
As is mentioned above, BDH-Q inquired the frequency of milk intake in two separate items: normal/high-fat milk and low-fat milk. Therefore, the participants were divided into two groups and were compared in two different ways. Also in the latter part of the paper, “exclusive” normal/high-fat milk consumers and “exclusive” low-fat milk consumers were compared.

What was the average MHR-QOL of each subset of consumers?

The MHR-QOL values of each group were inserted into the Tables.

"absorbed more vitamin D from milk": No blood tests were conducted to monitor vitamin D absorption or status in these women.

The word “absorbed” was replaced by “ingested”.

Tables 1 and 2: Ages 40-60 is a wide age range. Is it possible to report the numbers of participants in their 40's, 50's and 60's or the length of time spent in menopause? These factors may also affect the results of the study. For example, how do you know the normal/high fat consumers on average have less time spent in menopause than the non-consumers, therefore the LBM is higher due to this instead of milk intake?

As are shown in Tables 2&3 (formerly Tables 1&2), the age and the distribution of menopausal status are not significantly different between consumers and non-consumers of either normal/high- or low-fat milk Therefore, we did not assume that age or years since menopause contributed to the observed difference in muscle and lean body masses.

Discussion: Is milk not fortified with vitamin D? Without directly measuring, how can you be sure participants in the low fat consumer category are consuming lower vitamin D overall or their vitamin D status is lower? How do you know the fat intake of the participants from other dietary sources was not sufficient for vit D absorption?

Vitamin D-fortified milk is not available in Japan. The discrepancy between the effects of normal/high- and low-fat milk consumption on muscle and lean body masses implies that there could be factor(s) in a milk fat that are associated with body composition, of which vitamin D is one of the plausible candidates.

Tables 1 and 2: Considering resistance exercise is an important stimulus for muscle growth, it would be important to stratify the physical activity by resistive and aerobic-type work.

Unfortunately, we did not inquire the type of exercise, resistive or aerobic, in the current project.

Table 2: There is a big difference in the proportion of participants who are premenopausal in consumers and non-consumers. Also, why does the proportion of menopausal status of the non-consumers not add up to 100%? There is a big difference in the proportion of individuals who exercise regularly in table 2 which may affect body composition results.
> As is mentioned above, the values in Table 3 (formerly Table 2) were recalculated and were corrected.

Fig 1: How exactly was the vitamin D intake from milk (ug) calculated?

> Because the calculation formulae used within the BDH-Q algorithm are patent-protected, we could not answer this question properly.

Zuzanna Goluch-Koniuszy, PhD., D.Sc., Eng. (Reviewer 2): The research topic undertaken by the authors is valid and current. In my opinion, the reviewed article is educational. However, it requires minor corrections and additions in the sections MATERIALS AND METHODS and RESULTS, as suggested below.

Comments:

TITLE: Correct

ABSTRACTS: Correct.

INTRODUCTION: Correct.

MATERIALS AND METHODS

The authors did not explain in what conditions the anthropometric measurements, necessary for body composition estimates with the use of bioelectrical impedance analysis (BIA), were made. What was the equipment used to measure body height? Were anthropometric measurements and body composition estimates were done in the morning? Were the patients prepared for measurements (on an empty stomach, after urination and defecation)? Did they do any stretching exercises? Were they administered any diuretic medicine or/and liquids including caffeine 24 hours before the body composition estimates?

The measurement of body height and weight need to be detailed, as an error of 2,5 cm may affect measurement of the amount of water in the body by 1 litre, and an error of 1 kg in body weight affects the measurement of the total water content by 0,2 l, which also affects the measurement of body fat content by 0,7 kg. This information has to be included so that the reader does not doubt the reliability of the performed measurements and the results of the body composition estimates.

Moreover, please add information that the body composition estimate with bioelectrical impedance analysis (BIA) was made on Tanita MC190-EM apparatus in a tetrapolar setting.

> The reviewer’s comment is appreciated. The necessary information was added to the Methods section.

RESULTS
The authors did not provide information whether, in spite of the observable differences between groups of women (depending on the consumption milk with various fat content), the content of lean body mass, body fat and body water were appropriate for the subjects’ age and sex. It has been proven that BMI is not univocal to the appropriate body composition.

> The reviewer’s comment is appreciated. The necessary information was added to the Methods section.

DISCUSSION: Correct

REFERENCES:
Number 16: incomplete
> The information of the reference was completely provided.
Number 29: please provide DOI
> The information of the reference was completely provided.
Number 31: please correct the spelling of D3
> The spelling was corrected.

Almost 40% of literature is more than a decade old. Please refer to the manuscript for the publications of the last ten years.
> On some topics, it was difficult to find newer and more relevant references.

TABLES: Tables 1 and 2 are missing the unit for Visceral fat level
> The visceral fat level was a parameter calculated by the bioimpedance analyzer to be shown in arbitrary units.

FIGURE: Correct.