Author’s response to reviews

Title: A Pilot Study of FDG PET/CT Detects a Link Between Brown Adipose Tissue and Breast Cancer

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Author’s response to reviews: see over
Object: MS: 4501248021055759 - Research article
Analysis 18F-FDG PET/CT Scans of Breast Cancer patients: Initial Translational Study to Evaluate Link Between Brown Adipose Tissue and Breast Cancer
Dr. Cao et al.,

Thank you for consideration of our manuscript for publication in your journal.

We have revised our manuscript according to the reviewer’s comments. Please find below a point-by-point response. Changes are highlighted in the text, unless otherwise indicated.

Reviewer's report

Reviewer number: 1

1. The authors underline that study is of the pilot type, and it is understandable. Nevertheless, Introduction and Discussion sections are not written in ‘in-depth’ style. For example, literature related to the study of BAT in cancer patients is practically not mentioned (excluding such specific tumors like hibernomas). Meanwhile, this literature is not abundant but it exists.

   • We have made significant revisions to the Background (pg. 4-6) and combined Results and Discussion section (pg. 8-12) to reflect a more in depth and comprehensive review of the current literature (albeit few) regarding the study of BAT in cancer patients.

2. This reviewer could not find in the text of manuscript any information on the status of patients in regard of the treatment: were these patients primary (that is, not treated) or not? It is very important since any treatment (surgery, chemo- or hormonal therapy, etc) may influence body composition and adipose tissues status.

   • We have addressed this comment on pg. 12 of the manuscript: due to the retrospective nature of this study, complete clinical data were not available for each patient. Therefore, we acknowledge several limitations to this study including the unavailability of the actual menopause status, and the timing of prior medication and treatment history of every patient.
3. The authors inform that they included patients with fasting blood glucose level lesser than 220 mg/dl or 12.2 mmol/l. Why was it so? Does it mean that part of the patients had diabetes? What part? Did diabetes influence the incidence of BAT discovery?

- We have addressed this comment on pg. 7 of our manuscript. It is a routine practice that a patient with a glucose level of more than 220 mg% should not be injected with 18F-FDG for the scan. This is a guideline for PET/CT imaging. High glucose may interfere with 18F-FDG tracer uptake. We have revised the text to reflect this point. Pg. 7 now reads “As routine practice, patients with fasting blood glucose level greater than 220 mg/dl were excluded from study as high glucose may interfere with 18F-FDG tracer uptake based on the imaging guidelines”

4. The authors inform that mean weight of BC patients was 66 kg and non-breast cancer patients – 70 kg. They say nothing about body mass index value (which may be important here), do not discuss that excessive BMI is connected in opposite way with breast cancer risk in pre- and postmenopausal period and do not mention that in non-breast cancer patients the factor of prediagnostic body weight loss may be of importance.

- We have addressed this comment on pg. 7 of our manuscript indicating the importance of the relationship between BMI and BAT: “Notably, BAT is inversely correlated with obesity and body mass index (BMI). Unfortunately, we do not have the complete BMI status for all patients in the study, and thus were not able to discuss this parameter in depth in our results.”

5. In discussion, the authors mention that more often finding of BAT in breast cancer patients younger that 55 yrs, possibly, may be related to estrogens, but do not present mean age of the patients in groups younger and older 55 yrs and do not give data on menstrual status of females in the first of these groups.

- We do not have the complete menstrual status for all patients in the study. We discussed this and other limitations on page 12: “Therefore, we acknowledge several limitations to this study including the unavailability of the actual menopause status and prior medication and treatment history of every patient.”

6. The authors mention their previous important studies on presence of BAT-like tissue in mammary glands of adult mice but do not explain how this may be related to the finding of BAT in such places like neck, supraclavicular area, etc. The mentioned possibility of paraendocrine influences helps here not much.

- This comment is now detailed and some limitations were added in the manuscript on page 11: In contrast to rodents, there is only limited understanding of the distribution of
BAT within the human breast. Multilocular adipocytes resembling brown adipocytes have been detected in postmortem human infant breasts. [21,22]. Albeit rare, the observation of brown fat tumors (hibernomas) in the breast have been described [23-27]. We also acknowledge that activation of BAT tissue within the breasts in female patients undergoing FDG PET/CT is typically not seen. This could be due to the heterogeneity of cell types within the breast compared to the homogenous cell types in BAT. Interestingly, studies have shown that exposure of humans and rodents to cold activates thermogenic activity in brown adipose tissue (BAT) [28,29]. Biopsies from mice also show that this BAT activation causes an obvious transition from subcutaneous white adipose tissue (WAT) into brown-like adipose tissue (BRITE or BEIGE). If this phenomenon also occurs in humans, than the breast cancer patients positive for active BAT in common regions are more likely to show brown-like adipose tissue within their subcutaneous breast adipose tissue biopsies.

7. Finally, the explanation of mechanistic pathway between BAT and breast cancer (including an assumption that BAT may influence breast cancer progression) is practically not given.

- This comment is also addressed beginning at the bottom of pg. 10: Mechanisms for a potential association between active BAT and breast cancer are not entirely clear and currently under investigation. We suggest two alternate hypotheses. First, BAT could participate as an active participant in the progression of breast cancer. Given its high vascularity and ability to secrete bioactive molecule [10,11]. BAT could potentially cause nearby pre-cancerous epithelial cells to proliferate more rapidly, accelerating the progression of breast cancer. Alternatively, BAT could participate as a passive participant in the progression of breast cancer. Considering cancer cells are also known to secrete bioactive molecules to support its own progression [30], activation of BAT could be secondary to the breast cancer.
METHODS

The authors mention that if a patient had multiple PET/CT scans only the first was considered for analysis, which is appropriate. There is no mention at what time point in the course of the patient's disease that the FDG-PET/CT scans included in the analysis were being performed. It is possible that results could be different in patients at initial diagnosis compared to those who have received prior therapy (including hormonal based therapy). The breakdown of indications should be included and commented upon. In addition, for subsequent treatment scans, types of therapy might also be relevant, particularly given the authors' conclusion that hormones might have a role in brown fat and breast cancer development.

- This is an important comment that is addressed by several of the reviewers. We have addressed this and other limitations of the study on pg. 12: “While care was taken to control for some of the known factors that could potentially affect FDG uptake in BAT including matching study participants for age, sex, weight, and same day of scan between groups, due to the retrospective nature of this study, complete clinical data were not available for each patient.

RESULTS

1) Could the authors provide the mean or median ages in the subgroups based on age (ages < and >55 for breast cancer and control groups), just to confirm that these were not significantly different.

- We have indicated in the manuscript on pg. 9: There were no significant differences between the mean ages in the subgroups: [age ≤ 55 yrs: Breast cancer 46.3 +/- 6.7 vs. Non breast cancer 47.9 ± 6.5, p=0.288] and [age > 55 yrs: Breast cancer 65.9 ± 8.3 vs. Non breast cancer 66.1 ± 7.4, p=0.878].

2) The reviewer realizes that this is a retrospective study and the amount of clinical data may be limited, however, instead of using the arbitrary age cut off of 55, it might be more interesting to determine the actual menopausal status of the patient and separate the groups this way. It is possible that younger patients with prior history of chemotherapy could be postmenopausal and some older women are pre or perimenopausal. This might lend more strength to the argument of hormonal effects on brown fat. Menopausal status is probably more easily determined in a breast cancer patients as this is usual part of the breast cancer history for treatment options than for the control group.

- This is an important point that is raised by several of the reviewers. We have addressed this and other limitations of the study on pg. 12: “While care was taken to control for some of the known factors that could potentially affect FDG uptake in BAT including matching study participants for age, sex, weight, and same day of scan between groups, due to the retrospective nature of this study, complete clinical data were not available for each patient. Therefore, we acknowledge several limitations to this study
including the unavailability of the actual menopause status, and the timing of prior medication and treatment history of every patient.

3) Medications, for example beta-blockers, have also been shown to affect brown fat uptake. This may not be possible, but if available it would be helpful to show that at least beta blocker use was the same in each group.

- This and other limitations have been addressed on pg. 12 as indicated above in the above response.
Reviewer number: 3

Major comments
1. This study is based on the assumption that FDG PET is a known valid imaging study with well-established performance characteristics. However, FDG PET/CT may markedly underestimate true prevalence of BAT. FDG uptake may be only the activity during the examination period. FDG PET/CT done in cancer patients may not conclusively analyze the prevalence of brown fat tissue (N Engl J Med 2009;306:1500, Diabetes 2009;58:1526, FASEB J 2009;23:3113, Obesity 2011;19:13).

   Currently FDG PET/CT is the only imaging modality that can detect BAT. We agree that it only presents BAT activity during the time of examination, as BAT can be affected by factors like temperature, sympathetic nerve tone, and others. In the current study, all known factors that can potentially affect BAT activity were controlled in the breast cancer and control groups. To the best of our knowledge, there are no data regarding the distribution of FDG uptake on PET scan and true BAT prevalence. Thus we have indicated in the manuscript on pg. 10: We recognize that in our study, the uptake tissue was not biopsied to validate the presence of BAT. Therefore, FDG PET/CT could potentially underestimate or overestimate the true prevalence of BAT. Nevertheless, the correlation of uptake in hypermetabolic BAT is well-supported and a recognized feature of FDG PET. Further, we anticipate that any “underestimation or overestimation” would apply to both the cancer and control groups [15-17,19,20].

2. Some information relevant to the evaluation of the results is omitted in the article. What were the indications (i.e. diagnosis, staging, chemotherapy response evaluation, or surveillance) of the FDG PET/CT scans? It is important because it seems that docetaxel chemotherapy, which is frequently used in breast cancer, could affect BAT activity (Eur J Nucl Med Mol Imaging 2006,33:785). Moreover, because the effects of adrenergic antagonists (for example, propranolol) and agonists on BAT activity are well known, the authors should provide the information about this medications, or, if unavailable due to the retrospective study design, should at least mention this issue as a study limitation

   • This and other limitations have been addressed on pg. 12 as indicated above in the above response for reviewer 2- question #2.

3. Majority of the control patients had colorectal cancer. Obesity is a well known risk factor for colorectal cancer, whereas it is linked to breast cancer only after menopause. Lower prevalence of brown fat tissue in patients with colon cancer may be related to obesity because subjects with BAT tend to be those who are leaner (Ann N Y Acad Sci 2011;1212:E20). It is possible that obesity is a confounding variable in this study. Although both breast cancer and control group were matched in reference to body weight, it does not measure obesity.
• We have addressed this comment on pg. 12 of the manuscript: Additionally, considering the majority of the control patients had colorectal cancer, it is important to note that obesity is a well known risk factor for colorectal cancer [30]. Lower prevalence of BAT tissue in patients with colon cancer may be related to obesity because subjects with BAT tend to be those who are leaner [31]. Thus, while both breast cancer and control group were matched in reference to body weight, it does not measure obesity making it a possible confounding variable in this study.

4. The primary analysis of this study is image interpretations based on objective image features assessed by visual analysis. The data to support proposed the cutoff of FDG PET/CT interpretation need to be provided.

• In the literature, interpretation of BAT is mainly based on visual evaluation as we did in this study. No cut off value of SUV has been reported to diagnose BAT or not.

5. The information provided to the readers of FDG PET/CT should be described in detail. If the readers were informed of the purpose of this study (comparison between breast and other cancers), the interpretation would have been biased.

• The readers are blind to the history. We agree that in some cases, the readers may have an overall impression whether the case is breast cancer or control based on the pattern of whole body PET/CT images (for example, breast lesion or axillary nodal metastasis). We have addressed this comment on pg. 8 of the manuscript: The interpretation of a positive active BAT site on PET/CT was based on the imaging findings of focal FDG uptake in adipose tissue that is visually more intense than the surrounding muscle activity, which is simple “yes” or “no” with no case showing equivocal findings. No SUVmax threshold value was set to define a positive BAT.

All patients with breast cancer in this study underwent FDG PET/CT as part of routine standard of care staging. It is likely that the readers were able to identify breast cancer patients because of the presence of hot FDG uptake in breast. In fact, the readers might not be blind to the diagnosis of patients.

• It is confirmed in the manuscript on pg. 7 of the manuscript: “.. 2 nuclear medicine physicians blinded to the clinical history…”

6. It is not clear whether image interpretation was performed independently by two nuclear medicine physicians. If it was done independently, assessment of interreader variability should be done.

• We have clarified in the manuscript at the bottom of pg. 7: Training and expertise of the two nuclear medicine physicians reading FDG PET/CT was 7 years and 3 years, respectively, with an interpersonal variation of 0.
7. Information on the training and expertise of the two nuclear medicine physicians reading FDG PET/CT should be provided

- We have added to the manuscript on pg. 7: Training and expertise of the two nuclear medicine physicians reading FDG PET/CT was 7 years and 3 years, respectively.

8. The numbers of positive FDG PET/CT scans according to seasons (i.e. summer, winter, or spring/autumn) should also be given because the true prevalence of BAT is strongly affected by the seasonal factor.

- We have written on the top of pg. 7: For comparison, each breast cancer patient was assigned a paired-control of a non breast cancer patient (mainly colon cancer) who had a PET/CT scan on the same day. Thus, any variability in season would be taken into account and not a confounder to the study.
Breast cancer patients seem to have a 3-fold higher prevalence of increased FDG uptake in brown fat tissue compared to non-breast cancer patients. However, there is no sufficient explanation offered for that finding.

- We have addressed this comment in the manuscript on pg. 10: Mechanisms for a potential association between active BAT and breast cancer are not entirely clear and currently under investigation. We suggest two alternate hypotheses. First, BAT could participate as an active participant in the progression of breast cancer. Given its high vascularity and ability to secrete bioactive molecule [9,10]. BAT could potentially cause nearby pre-cancerous epithelial cells to proliferate more rapidly, accelerating the progression of breast cancer. Alternatively, BAT could participate as a passive participant in the progression of breast cancer. Considering cancer cells are also known to secrete bioactive molecules to support its own progression [18], activation of BAT could be secondary to the breast cancer.

One needs to separate the presence of brown fat tissue (which cannot be verified) and the activation of brown fat tissue seen on FDG-PET. Subsequently, the observation of this paper is that brown fat tissue is more frequently activated in breast cancer patients as compared to other cancer patients. Please discuss your findings from this perspective.

- We have clarified throughout the manuscript that our findings reflect “active” BAT and not just simply the presence of BAT (which may or may not be activated). For example top of pg. 3 in Background section: “….determine the prevalence of active BAT ….”. Additionally, the discussion of our findings from this perspective is addressed in the above response.

The authors claim a potential link between the prevalence of brown fat tissue and breast cancer. This is purely hypothetical and should be rethought taking the comments above into account.

- The authors are aware of this and have provided detail on how future studies could aid to further clarify this issue in the manuscript at the bottom of pg. 11 and into 12: the future, it will be worthwhile to study a larger patient population prospectively that would afford the opportunity to obtain accurate records to control for these and other potential confounding variables in the evaluation of the results. Ideally, this work will be conducted along with histological and immunohistochemical studies that may whether markers of BAT exist locally in breast adipose.

The authors cite a previous paper from their group describing significant differences in the deposition of brown fat tissue in the adult mammary fat pad of a mouse model in BRCA positive versus control mice. I have not seen activation of brown fat tissue within the breasts neither in breast cancer patients nor in any other
female patients undergoing FDG-PET. The link between activation of brown fat tissue and breast cancer as described in the introduction is very weak and should be reworded.

- We have revisited and revised the title of the manuscript to better interpret this study and have clarified the text to address this issue on pg. 11 of the manuscript: In contrast to rodents, there is only limited understanding of the distribution of BAT within the human breast. Multilocular adipocytes resembling brown adipocytes have been detected in postmortem human infant breasts. [21,22]. Albeit rare, the observation of brown fat tumors (hibernomas) in the breast have been described [23-27]. We also acknowledge that activation of BAT tissue within the breasts in female patients undergoing FDG PET/CT is typically not seen. This could be due to the heterogeneity of cell types within the breast compared to the homogenous cell types in BAT. Interestingly, studies have shown that exposure of humans and rodents to cold activates thermogenic activity in brown adipose tissue (BAT) [28,29]. Biopsies from mice also show that this BAT activation causes an obvious transition from subcutaneous white adipose tissue (WAT) into brown-like adipose tissue (BRITE or BEIGE). If this phenomenon also occurs in humans, than the breast cancer patients positive for active BAT in common regions are more likely to show brown-like adipose tissue within their subcutaneous breast adipose tissue biopsies. However, in the current study, we did not explore this hypothesis. Therefore, it would be most ideal to examine breast tissue slides from patient biopsies by H&E or for immunohistochemical markers for BAT to determine if there are cells that resemble the morphology and molecular characteristics of brown adipocytes, respectively.

The hypothesis in the introduction is flawed as FDG-PET is not visualizing the presence of brown fat tissue but only its activation if present.

- We have clarified throughout the manuscript that it is indeed “activation” of BAT and not simply the presence.

Breast cancer patients receive different kind of treatments including anti-hormonal therapies. There might be an influence of estrogen levels in breast cancer patients, particularly in the pre-menopausal group. More information is necessary regarding the type of treatment and the duration of treatment prior to FDG-PET particularly in the breast cancer group.

- This and other limitations have been raised by the reviewers above and has addressed on pg. 12 as indicated (for example see response for reviewer 2-question #2.

**Minor comments:**
The study matches controls with non-controls on the same day for sex, age, and body weight. As ambient temperature is a contributor to brown fat uptake, was this accounted for between the two groups?

- The temperature is controlled in the 2 groups, although we do not know the true temperature each day. We have written on pg. 7: For comparison, each breast
cancer patient was assigned a paired-control of a non breast cancer patient (mainly colon cancer) who had a PET/CT scan on the same day.

No SUV threshold was set to define a positive BAT, introducing subjectivity in positive BAT identification. Was there any oversight or double-reading between the two PET readers to make sure that the level of sensitivity was the same for calling a positive scan?

- There is no SUV cut off value to define BAT in the literature. Diagnosis of BAT on PET/CT is based on visual subjective evaluation. However, the reading is almost always very clear without equivocal cases. There is no interobserver variation with a 100% consent. We have indicated on pg 7 of the manuscript: Image interpretation was performed independently by the two nuclear medicine physicians with an interpersonal variation of 0.

The results state that 39 breast cancer patients were < or = to 55, while 56 patients were greater than 55. This results in a total of 95 patients when 96 patients should be accounted for.

- After careful review of the data, we agree it should be 57 not 56. This has been corrected on pg. 9 of the manuscript.

The paper makes multiple distinctions between pre/peri-menopausal and post-menopausal breast patients and the difference in BAT between the two groups. A further discussion of this finding and possible explanations would be of value.

- We acknowledge that due to the lack of data for menopause status it is not possible to provide an in depth discussion on possible explanations. Therefore, we have provided actions for a future study that would provide more insight (pg 12): “Therefore, we acknowledge several limitations to this study including the unavailability of the actual menopause status, and the timing of prior medication and treatment history of every patient…..”

The paper states that the BAT positive group had a 2-fold higher HER2 positive rate than HER2 negative patients; however this was not statistically significant.

- We have clarified in the manuscript on pg. 12: “…this did not reach statistical significance.”

The title is somewhat misleading as it is not clear to what the phrase “translational study” is referring in this retrospective chart review.

- To provide clarity, we have revised our title: “A Pilot Study of FDG PET/CT Detects a Link Between Brown Adipose Tissue and Breast Cancer”
The limitations of the study should be addressed.

- This has been addressed on pg. 12 as indicated above in similar remarks from other reviewers.

Numerous spelling and grammatical errors are noted throughout the paper, which significantly detracts from the content of the paper
- Done