January 29, 2016

Manuscript title: Oncogenic role of leptin and Notch Inerleukin-1 leptin crosstalk outcome (NILCO) in Cancer
Manuscript type: review
ESPS manuscript number: 22462

Dear *World Journal of Methodology (WJM)* Editor and Reviewers:

Thank you for providing detailed and thought-provoking comments for our manuscript. We have worked diligently to address all questions/concerns expressed by each reviewer. Thus, our manuscript has been strengthened accordingly. We believe that the current changes have improved our manuscript.

In the revised manuscript we have made the following changes:

We have included the full name of NILCO (Notch Interleukin-1 leptin crosstalk outcome) in the manuscript title as suggested by the *WJM* editors.

Reviewer's code 02445708:
Comment 1:
As I known, the primary task of WJM is to publish papers in the field of methodology. Thus, the major problem of this manuscript is that it does not contain information in that field.
Revision for comment 1:
We have added a paragraph showing methodologies used for determining the role of leptin signaling in cancer progression. The text is included here (also found on page 4 of the manuscript)

This review shows updated information on leptin's oncogenic role in breast, endometrial, and pancreatic cancers. We present experimental data obtained by using different research methodologies (including: cell culture, animal trials, flow cytometry, immunological methods, polymerase chain reaction (PCR), etc.) suggesting that adiposity affects cancer progression. The effect of adiposity on cancer progression, leptin signaling mediated activation of cancer stem cells, and the link between leptin and drug resistance is discussed.

Comment 2:
Minor: Leptin is protein, not peptide (page 6)
Revision for comment 2:

We have now stated that: This small protein binds to the leptin receptor (OB-R) leading to control of leptin ligand/receptor mediated pathways.

Comment 3:
leptin protein receptor? (Fig. 1)
Revision for comment 3:
We have corrected Figure 1 to show OB-R receptor

Reviewer's code 02441737:
Comment 1: It would be of interest to researchers discuss the role of androgens like testosterone, andorstendione, dehydro-epiandrosterone and sex hormone-binding globulin, in the activation of leptin signaling pathway; addition to discussing the variations of both androgens and estrogens and leptin levels during the phases of the menstrual cycle among obese and non-obese women.
Revision for comment 1: We have added an additional sub-chapter to the paper to this comment. The text is included here (and can be found on page of the manuscript).
Androgens, estrogens and leptin in the Menstrual Cycle

Estrogens are the main regulator of the menstrual cycle. Estrogens are mainly produced by the ovaries and regulated by neuroendocrine hormone signaling[[76]]. However, estrogens are also synthesized by adipose tissue[77]. In fact, estradiol levels varied throughout the menstrual cycle between women with different body fat content[78]. Women with both very low and very high body fat had significantly lower estradiol levels during the follicular phase and midcycle during their menstrual cycle[78].

Androgens are produced and accumulated in adipose tissue. They can be converted into estrogen via the actions of aromatase. Excessive size of adipose tissue can convert androgens into estradiol and estrone via aromatase providing an important estrogenic surge in obese patients[71]. Therefore, these molecules could alter female reproductive function and hormonal equilibrium especially after menopause in obese women[79]. Androgens and estrogens influence the menstrual cycle. In normal weight women, testosterone fluctuates throughout the menstrual cycle and peaks during the ovulation phase[79]. Conversely, androstenedione and dehydroepiandrosterone showed no significant variations throughout the menstrual cycle[79]. Androstenedione levels were found to peak at ovulation[80]. Yet, epidemiological studies have shown increased EmCa risk among pre- and postmenopausal women who have elevated plasma

androstenedione and testosterone, and among postmenopausal women who have increased levels of estrone and estradiol. Interestingly, free testosterone levels were significantly higher in obese women when compared to non-obese women and slight variations of testosterone were observed during each phase of the menstrual cycle[81].

Also, the menstrual cycle may be influenced by the levels of serum leptin[82]. In obese women, the highest serum leptin levels are observed during the luteal phase. Similarly, an increase in estradiol levels coincided with the increase in serum leptin levels[82]. However, serum leptin levels were unchanged throughout the menstrual cycle of women with normal weight[82].

Comment 2: Would be of interest that the authors present more information to understand the effects of TAM in obese and non-obese patients with risk for breast cancer.
Revision for comment 2: Additional information describing clinical trial data discussing the effects of TAM in obese and non-obese patients is here (and on pages 13-14 of the manuscript).

A recent finding has reported the role the synergistic relationship between leptin and STAT3 phosphorylation as a mediator of TAM resistance in breast cancer cells[63]. When treated in vitro with 2μM of TAM for 72 hours, ER positive MCF-7 and MCF-7/HER2 cell lines showed a statistically significant decrease in cell viability as measured by MTT assay[63]. However, 72 hour combination treatment of MCF-7 and MCF-7/HER2 cell lines with 2μM TAM + 200 ng/mL leptin had a restorative effect on cell viability[63]. The study used western blot analysis of p-STAT3, OB-R, HER2, and ER to investigate leptin's role in STAT3 phosphorylation in the presence of TAM. STAT3 phosphorylation (activation) increased in MCF-7 cells treated with TAM alone and TAM plus leptin[63]. In contrast, MCF-7/HER2 cells treated with TAM alone had decreased expression of phosphorylated STAT3[63]. More interestingly, leptin restored phosphorylated STAT3 to levels that were comparable to untreated cells in the presence of TAM at 24 hour and 7 day timepoints[63]. This research provided insight on two key mechanistic pathways that could be critical for decreasing TAM resistance in obese breast cancer patients.

However, recent studies investigating the effects of TAM treatment in obese and non-obese ER+ breast cancer patients are showing that TAM may continue to be an effective treatment for obese patients suffering from the disease. Analysis of the data acquired after the National Surgical Adjuvant Breast and Bowel Project (NSABP) protocol B-14, shows that overall mortality rates were reduced in both obese and non-obese women who were treated with TAM when compared to women with similar BMI who received a placebo[64]. Obese patients (n=687) had higher TAM/placebo hazard ratios for breast cancer recurrence, contralateral breast cancer, total mortality, and mortality after breast cancer events compared to underweight (n=83), normal weight (n=1593), and overweight (n=1022) patients[64]. However, TAM effectively reduced breast cancer recurrence and mortality rates across all BMIs, and there was no statistically significant increase in mortality for obese women[64].

A published secondary analysis of the double blind Arimidex, Tamoxifen Alone or in Combination (ATAC) clinical trial shows that overall recurrence rates were equal in ER+ breast cancer patients who were treated with TAM[65]. Similarly, the Austrian Breast and Colorectal Cancer Study Group trail 6 (ABCSG-06) reports that there was no difference in outcomes (disease-free survival, distant recurrence-free survival, and overall survival) between obese and non-obese women who received TAM treatment[66]. However, obese women who received TAM in combination with an aromatase inhibitor had worse outcomes than non-obese counterparts receiving the same treatment[66]. These studies show that there may be strong associations between body weight and TAM efficacy. Azrad et al. have presented additional background on this subject including studies that compare the efficacy of TAM and aromatase inhibitors[67]. A major section of Azrad's review provides data from four clinical studies and each suggests that aromatase inhibitors are less effective than TAM when treating women with hormone-receptor positive breast cancers[67].

Comment 3: It is advisable to present at the bottom of Figure 1, a detailed explanation of the mechanisms of action of leptin.

Revision for comment 3: A detailed explanation of the mechanisms of leptin action has been included in the manuscript and is found on 10 page below Figure 1.

Comment 4: In Table 1, it is recommended that the authors present data about the frequencies of women with EmCa type I, type II and type III; also explain at the foot of the table the meaning of the acronyms used.
Revision for comment 4: We have added an acronym legend to the bottom of Table 1 on page 17 of the manuscript.

We have added data about the frequencies of women with Type I and Type II EmCa on page 16 of the manuscript, the text is also included here: Clinical data has been published showing links between obesity, EmCa type, and race. The study showed that 55.3% (n=871) of the women diagnosed with Type I EmCa were obese, while 36% (n=64) were obese and more likely to belong to a non-white race[100]. Although this study did not clearly define the relationship between obesity and Type II, the data suggest that obesity and health disparities play a role in EmCa.

In the manuscript on page 18, we have stated that all samples from African American women with EmCa were obese BMI and all of the Chinese women with EmCa were lean BMI, the text is also included here: Table 1 shows the expression levels of NILCO components in Type I and Type II EmCa from obese African American women and lean Chinese women.

Comment 5: In the paragraph of pancreatic cancer, correct the term "waste" by the waist.
Revision for comment 5: We have corrected the word "waste" to "waist".

Comment 6: It would be interesting that the authors present a paragraph describing and explaining the results of clinical trials for the alleged effects of weight reduction in leptin signaling pathways in the genesis of breast, endometrial and pancreatic cancers.
Revision for comment 6: In order to address the effects of "weight reduction in leptin signaling pathways in the genesis of breast, endometrial, and pancreatic cancers" in the revised version, we have added an additional chapter titled "Effects of weight loss on leptin level in Cancer". It is included here (the new chapter is found on page 22-23 of the manuscript).

6. Effects of weight loss on leptin levels in Cancer

Despite the recognized role of overweight and obesity on cancer incidence, the majority of the clinical trials addressing BMI reduction are relegated to breast cancer. Few reports are available for endometrial and pancreatic cancers. A retrospective study suggests bariatric surgery may improve quality of life for morbidly obese women suffering from low risk type I endometrial cancer[91]. Clinical insights on the effects of weight loss in endometrial cancer patients include a retrospective study with findings that suggest weight loss after diagnosis and treatment may lead to poor prognosis[92]. Similar findings on the deleterious effects of weight reduction in pancreatic cancer patients show that significant weight loss during or following treatment correlates to poor post-treatment outcomes[93;94].

It is believed that obesity may promote the progression of ER^+ breast cancer in post-menopausal obese women via increased production of the estrogens by adipocytes[95]. In contrast to endometrial and pancreatic cancer data, long-term survival prognosis after treatment is poorer in overweight or obese who suffer from pre or post-menopausal breast cancer[96]. Several clinical studies show trends between weight loss and lower leptin levels in patients with breast cancer. One recent study, randomized patients into low fat (n=73) or low carbohydrate (n=66) diet intervention groups to determine how weight loss affects plasma leptin and adiponectin levels in overweight or obese postmenopausal breast cancer survivors[97]. Following the six month diet intervention the women in both groups, exhibited significant reductions in body weight and fat mass. Results from this trial show that the mean leptin level of the patients prior to intervention was 36ng/mL, more than a 3 fold increase to concentration associated with normal weight (5-10ng/mL)[97]. Interestingly, 50% of the patients had circulating adiponectin levels that are the same as normal weight women[97]. Moreover, the diet interventions decreased leptin levels by 92% of the patients. Thirty two percent of the individuals in the intervention arms experienced a six-month decrease in adiponectin; whereas, the remainder increased somewhat. However, this study did not address the impact of weight loss and adipokine reduction on breast cancer recurrence. Knowing how reduction of body weight and leptin levels affects breast cancer

progression would be instrumental for the design of new chemotherapeutics. Obtaining clinical information on the mechanisms linking body weight loss, cancer progression, and recurrence could be also be key for developing preventative strategies that target leptin mediated breast cancer progression in obese women.

Reviewer's code 02860540:

Comments 1: I only suggest to add in the introduction section or creating a little paragraph an overview about the tumours in which the leptin has been shown to play a role.

Revision for comment 1: We have added additional information describing the types of cancer/tumors that have been shown to be affected by leptin and its receptor. We have included the text here (also found on page 4 of the manuscript):

Leptin has been shown to play a role in several types of cancers. The expression of leptin and its receptor (OB-R) has been reported in many cancer types including: Gliomas, Carcinomas, Adenocarcinomas, and Melanomas[18]. Obesity signals (leptin) have been linked to the progression of several cancers. The connection between obesity signals (leptin and/or OB-R) and cancer progression has been detected in bladder, brain, breast, colon, endometriod, esophageal, kidney, liver, lung, ovarian, prostate, skin, and thyroid cancers[18].

We hope that the revised version of our manuscript is now acceptable to be published in the *World Journal of Methodology.*

Cordially,

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