Reviewer's report

**Title:** Menstrual irregularity and bone mass in premenopausal women: Cross-sectional associations with testosterone and SHBG

**Version:** 1  **Date:** 8 August 2010

**Reviewer:** Angelica Hirschberg

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This study investigates associations between menstrual irregularity, serum androgen levels and bone mass in a population-based sample of premenopausal women. Menstrual cycle characteristics were obtained by a self-administered questionnaire and bone mass was measured by quantitative ultrasound. Irregular cycles were found to be associated with higher bone mass, which could partially be explained by increased levels of free androgen index (FAI). The study is important but there are several concerns that need to be clarified.

**Major Comments**

In the background section the literature references could be updated. There are papers demonstrating that oligomenorrhea in female top athletes is related to increased bone mass and PCOS (Rickenlund et al. Fertil Steril 2003, 79: 947-955; Hagmar et al. Med Sci Sports Exerc 2009, 41: 1241-1248).

Assessment of menstrual cycle characteristics were classified into regular and irregular based on self-reported data from questionnaires. This classification is a rather simplified way to study menstrual disturbances. Thus, it has been demonstrated that amenorrhea and oligomenorrhea may represent different hormonal mechanisms of menstrual disturbances. Loss of menses i.e. amenorrhea is often associated with a hormonal profile in agreement with hypothalamic inhibition, whereas oligomenorrhea (menses with long intervals) is more often associated with increased androgen levels and a hormonal profile in agreement with PCOS (Rickenlund et al. J Clin Endocrinol Metab 2004, 89: 702-707; Wiksten-Almströmer et al. Acta Obstet Gynecol Scand 2009, 88: 543-549). What do irregular or very irregular menstrual cycles correspond to? What would a woman who has amenorrhea answer? Although there may be insufficient number of cases, it would be interesting to analyse whether the results differ between women with irregular and those with very irregular menses.

Was blood sampling standardized and collected in relation to the menstrual cycle or not? If not, this is an important limitation of the study which should be acknowledged. Furthermore, how was ovarian function determined? Was FSH measured? If not, how do the authors know that not any woman was peri- or postmenopausal?

Another limitation of the study is the lack of evaluation of clinical symptoms of hyperandrogenism, such as hirsutism and acne, which could confirm PCOS.
the Discussion section, page 10 it is mentioned the possibility of undiagnosed PCOS. In order to explore this possibility, the authors excluded patients with increased total testosterone above the upper normal limit. However, the most typical with PCOS is total testosterone within the normal upper range but SHBG below the normal limit resulting in increased level of FAI.

Figure 1 is unclear. BUA, SOS and QUI are measured in different units but the Y-axis is the same. Please, explain what is presented.

Figure 2 is also unclear. What is on the y-axis?

How does bone mass in women with and without irregular cycles relate to normal values? Did any woman have low values corresponding to osteopenia or osteoporosis? This would be valuable information.

Statistically it is not clear which of the investigated factors, hormones or irregular menses, is the strongest predictor of bone mass. This could be found out by stepwise multiple regression analysis. As it stands now it is not possible to conclude whether irregular menses or FAI is the most important factor to explain high bone mass in this population of women of fertile age.

What are the clinical consequences of the study? It is important to state that menstrual disturbances always should be evaluated although it may not be harmful for bone mass.

Minor comments

Age range would be valuable information.

Result section, second paragraph, line 1: BUA was not significantly higher in women with irregular cycles than those with regular cycles.

BUA, SOS and QUI should be presented in either table 1 or figure 1.

**Level of interest:** An article of importance in its field

**Quality of written English:** Acceptable

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**

I declare that I have no competing interests.