Author's response to reviews

Title: The association between lean mass and bone mineral content in the high disease activity group of adult patients with juvenile idiopathic arthritis

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Version: 2 Date: 16 January 2014

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Reviewer #1:

1. Were the control patients supplemented with vitamin D and calcium? How long were the JIA subjects treated with vitamin D and calcium? What was the dose?

Response:
The control subjects were not supplemented with vitamin D and calcium. The JIA patients were supplemented with 1000mg calcium and 800 IU vitamin D daily, for at least six months prior to evaluation. This information was added to Subjects and methods (Study design, participants). Data were added to Results and Table 1.

Page 5
The control subjects were not supplemented with vitamin D and calcium. The JIA patients were supplemented with 1000 mg calcium and 800 IU vitamin D daily, for at least six months prior to evaluation.

Page 8
No statistical differences of demographic characteristics between the patient and control group were found except for higher serum 25-hydroxyvitamin D levels in the JIA patients.

2. Was the selection of the control group a random selection of those who volunteered?

Response:
The volunteers (100 subjects) were selected randomly from classmates, friends and acquaintances of JIA patients. The 84 controls were chosen from the volunteer group so that the age of the volunteers as much coincided with patient age. This was added to Subjects and methods (Study design, participants).

Page 5
The control sample of young men and women with no fracture was recruited by invitation in the same district of Prague. The volunteer group (100 subjects) was selected randomly from classmates, friends and acquaintances of JIA patients. From these invitations, three eligible age- and gender- matched control participants (only 2 control participants in 9 females) were selected for each JIA case.

3. There is some need for editing. For instance in the abstract:
   delete “an” in the background sentence “adult patients with an active juvenile idiopathic arthritis (JIA) untreated with tumor necrosis factor alpha inhibitors”
   “measures” should be “measured” in the sentence In patients with clinically active JIA (disease activity score 28, 6.36 ± 0.64, C reactive protein, 18.36 ± 16.95 mg/l), aBMD was reduced in all measures sites
   Quality of written English: Needs some language corrections before being published.

Response:
We rephrased the relevant paragraphs and the manuscript was extensively edited professionally by the translator.
Reviewer #2:

1. The authors should tell more information about the relationship between muscles and BMC (Schoneau et al. 2002) and about weight and BMC, BMD.

   Response:
   These informations were added to Background.
   Page 4.
   Decrease in bone mass in JIA is also associated with muscle atrophy. A linear relationship was described between muscle cross-sectional area and bone mineral content (BMC) of radial diaphysis in healthy children and adolescents [11]. The bone-muscle unit plays an important role especially in the growing bones of children and adolescents. It is the muscle forces, not body weight, that load the load-bearing bones. Bones adapt their strength to maintain the strain caused by physiological loads close to a set point and the largest physiological loads are caused by muscle contractions [12], and muscle strength thus strongly influences postnatal bone strength [13]. In JIA, inflammation, low physical activity as well as the GC therapy may be responsible for muscular atrophy.

2. Is fat mass or lean mass affected by GC use as only five patients were actually never-users of GCs.
   Are there differences between men and women.
   Are these notions to be considered in interpreting the results?

   Response:
   We thank the reviewer for this comment. Results were calculated according to the reviewer’s suggestions. Tables 3, 4 and 5 and Figure 1 are added.
   Page 8 and 9 (Results)
   In the total body as well as in the trunk and extremities (both legs and arms), no significant differences in tissue mass were observed between JIA patients and control subjects. However, in all measured regions, lean mass and BMC fraction was significantly lower, and fat mass fraction was significantly higher in JIA patients compared to controls.
   Significant differences in body composition between JIA and controls were also evident in both genders (Table 3). BMC was reduced in all the measured sites, lean mass was reduced as well and fat mass was increased in all measured areas except for the trunk.
   In women with JIA, body composition at the total body and legs was significantly different from that in women untreated with glucocorticoids and from that in women in the control group (Fig. 1).
   In JIA patients, significant correlations were observed between indices of composition of legs, and physical performance and disease duration (Table 4). Significant correlations were also observed in these patients between the indices of body composition by gender (Table 5). Thanks to the inclusion of a number of female patients treated with GCs, it was possible to observe a significant negative correlation
between GCs usage and BMC of legs, and between GCs usage and DAS 28. The association between lean mass and BMC in legs of women with JIA treated with GCs, not treated with GCs, and in healthy women as well as in men with JIA and control subjects is given in Figure 2.

Using multiple linear regression analysis, the fraction of BMC was significantly predicted by total body lean mass both in women (total body, \( p = 0.002 \)) and in men (total body, \( p = 0.022 \), and in legs, \( p = 0.008 \)), while current GC therapy, DAS28, and duration of disease did not contribute significantly to the prediction of the BMC in the patients with JIA.

Page 10 (Discussion)
The differences between body composition of total body and legs in the subgroup of women with JIA treated and not treated with GCs indicate a negative effect of GCs on the lean mass and BMC, and the positive effect on fat tissue. This is in good agreement with several cross-sectional and longitudinal studies demonstrating substantial effects of GCs on muscle atrophy and body composition in patients with medical illnesses such as Crohn’s disease, multiple sclerosis, systemic lupus erythematosus, glucocorticoid-sensitive nephrotic syndrome and post-renal transplantation [18-23]. The significant positive correlation between the activity of the disease and GC use could be explained by the necessity of GC therapy in patients with severe course of disease. However, while 9 out of 19 women patients were on GC therapy, the BMC fraction was significantly predicted by GC use rather than by DAS28. The importance of lean mass is further supported by the significant correlation between disease duration and increase of fat mass and reduction of bone and lean mass and deteriorated physical performance of legs evaluated using the chair test. In a study where lean mass and cortical and trabecular bone forearm BMD were measured using peripheral quantitative computed tomography, JIA patients had significantly reduced muscle cross-sectional area and this reduction significantly correlated with muscle strength and bone geometry abnormalities and, particularly, with reduced thickness of the cortical bone [24]. Similar conclusions were derived from the measurement of muscle and bone mass of the tibia [25].

3. In JIA there is no general agreement about remission. Disease activity should be discussed more thoroughly and use of DAS 28 in patients with JIA.

Response:
DAS 28 in JIA patients was used because the patients were adult and before TNFα blockers treatment, therefore we had to use the criteria of the Czech Rheumatology Society for therapy with TNFα inhibitors. As a criterion of disease activity, we had to use DAS 28. The improved description has been added to the text.

Page 5
According to the criteria of the Czech Rheumatology Society, the basic indication for therapy with TNFα inhibitors is an unsatisfactory response to therapy with one disease-modifying anti-rheumatic drug (DMARD) (preferably methotrexate, alternatively sulphasalazine or leflunomide). DMARD therapy before TNFα blockers initiation must be at least 3-6 months with adequate dosage (methotrexate dose 20-
30mg). The other basic condition is a disease activity score 28 (DAS 28) of at least $\geq 3.9$ [14].

4. Is the precision of Lunar Prodigy reliable enough to compare fat mass and lean mass between the groups?

**Response:**
As required by the opponent we expressed body composition (BMC, lean and fat mass) in %. Thus, we added paragraph into the section of Subjects and Methods and subsection Bone densitometry:

**Page 6**
The availability of DXA enables the precise measurement of body composition in terms of lean and fat mass and bone mineral content of the total body, trunk, legs and arms. In our study we calculated % for lean mass, fat mass and BMC evaluation. BMC, lean mass and fat mass were measured using whole-body absorptiometry software of the bone densitometer (Prodigy, GE, U.S.A.) and were expressed in grams. Percentages of BMC, lean mass and fat mass were calculated by dividing each absolute value by total mass. For instance percentage trunk fat was calculated by dividing trunk fat mass by total fat mass and was designated (%) trunk fat. A strong correlation between body weight and total body mass as measured by DXA ($r=0.98$) was obtained in a preliminary study. The coefficients of variation of measurements of BMC, lean and fat mass were 0.9, 1.0 and 2.0%, respectively.

5. Quality of written English: Not suitable for publication unless extensively edited.

**Response:**
The manuscript was extensively edited by the translator.