Acute response of biochemical bone turnover markers and the associated ground reaction forces to high-impact exercise in postmenopausal women

AUTHORS: Rizky S. Prawiradilaga1,2, Anders O. Madsen1, Niklas R. Jørgensen3,4, Eva W. Helge1

1 Department of Nutrition, Exercise and Sports, University of Copenhagen, Denmark
2 Faculty of Medicine, Universitas Islam Bandung, Indonesia
3 Department of Clinical Biochemistry, Rigshospitalet Glostrup, Denmark
4 OPEN, Odense Patient data Explorative Network, Odense University Hospital/Institute of Clinical Research, University of Southern Denmark, Odense, Denmark

ABSTRACT: The aim of the study was to examine the acute response of biochemical bone turnover markers (BTM) to high-impact jumping exercise, and to quantify the ground reaction forces (GRF) achieved during each jumping exercise, in postmenopausal women. In a randomized controlled cross-over study over three days, 29 postmenopausal women (age (mean±SD): 60.0±5.6 years) were randomly assigned to 6 x 10 repetitions of three different jumps: countermovement jump (CMJ), drop jump (DJ), diagonal drop jump (DDJ). A fourth day without jumping served as a control (CON). Blood samples were collected before (PRE), after (POST), and 2 hours after (2Hr) exercise. Bone turnover was evaluated by bone formation markers (procollagen type-1 amino-terminal propeptide (P1NP) and osteocalcin (OC)) and the bone resorption marker C-terminal telopeptide of type-1 collagen (CTX). Peak anteroposterior (Fx), mediolateral (Fy), and vertical (Fz) GRF were measured using a force platform. From PRE to POST, P1NP increased (p<0.01) by 7.7±1.8%, 9.4±1.3%, and 10.6±1.6% for CMJ, DJ, and DDJ, which were higher (p<0.01) than CON. OC increased (p<0.05) by 5.5±1.8% for DJ, which was higher (p<0.05) than CON. CTX was not significantly changed at POST. There were no significant differences in BTM Δ-values between the jumps at any time point. For the CMJ, the combined three-axis peak GRF was positively associated with the PRE to POST Δ-change in P1NP (r=0.71, p<0.05). The acute, jump-induced increase in P1NP and OC without any rise in CTX may indicate increased bone formation. Moreover, the study shows a dose-response relationship between GRF and the acute P1NP response after countermovement jumps.


INTRODUCTION

Osteoporosis is a chronic bone disease of increasing public health concern. The disease is characterized by low bone strength due to reduced bone mass and impairment of bone micro-architecture, putting the patient at increased risk of bone fractures [1]. It is estimated to affect 200 million women worldwide, triggering more than 8.9 million fractures annually [2].

Thus, depending on the women’s life stage, osteogenic exercise has different effects and aims [3]. For adolescents, the exercise is aimed at increasing peak bone mass, for premenopausal women it is aimed at increasing bone mineral density (BMD), and for postmenopausal women it is aimed at reducing the age-related bone loss [3,4]. In postmenopausal women, a combination of resistance and high-impact or odd-impact training is found to be effective in improving bone health [3,5].

The osteogenic effect of training is mostly estimated by dual-energy x-ray absorptiometry (DXA) [6]. Additionally, the International Osteoporosis Foundation (IOF) has recommended the use of biochemical bone turnover markers (BTM) as markers of fracture risk assessment and evaluation of treatment effectiveness in clinical settings [7], and the assessment of BTM [8] is a promising method to evaluate an osteogenic response in bone turnover acutely or after only a few weeks of training.

A number of studies have been conducted examining the acute effects of exercise on BTM in adults [8–21], but the results have
been equivocal, and data in postmenopausal women are lacking. Therefore, the present randomized, controlled cross-over study compared the acute biological response of BTM to countermovement jump (CMJ), drop jump (DJ), and diagonal drop jump (DDJ) and the associated ground reaction force (GRF) in postmenopausal women. The overall aim was to investigate whether the jumps differed in the acute osteogenic response, which should be taken into consideration when planning osteogenic training to improve bone mass.

**MATERIALS AND METHODS**

**Experimental approach**

We used a randomized, controlled cross-over design comparing the BTM response after three different high-impact jumping trials and a resting control trial (CON) performed in random order in the early morning over four test days at a similar time. Every trial was separated by at least 48 hours without any moderate-vigorous activities. GRF associated with jumping was measured on a separate test day.

**Ethics**

All participants were fully informed of the procedures and possible discomfort associated with the study before providing their written informed consent to participate. The study was conducted following the Declaration of Helsinki and approved by the local ethics committee of the Capital Region of Denmark, H-4-2012-181.

**Subjects**

Healthy, sedentary postmenopausal women aged 50–70 years, who were more than two years after menopause, non-smokers, and had a body mass index (BMI) <30 kg/m², were invited to participate in the present study. To determine eligibility, prospective subjects underwent bone mineral density (BMD) screening at the lumbar spine and proximal femur (total hip) using DXA. If osteoporosis (T-score < -2.5 SD) or high BMD in relation to age (Z-score > 1.0 SD) at either the lumbar spine or the total hip was found, the participant was excluded. In addition, the following exclusion criteria were applied: the use of medication, hormone therapy or supplements that affect bone metabolism; previous or current medical condition affecting bone health; conditions that make powerful jumping impossible; and/or engagement in regular high-impact and/or resistance training.

Thirty-five women were recruited via a local newspaper and online advertisement (Fig. 1). After the preliminary BMD screening, three

<table>
<thead>
<tr>
<th>Table 1. Participant characteristics at baseline (n=29)</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>60.0 ± 5.6</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>165.2 ± 5.4</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>65.8 ± 7.7</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>24.1 ± 2.6</td>
</tr>
<tr>
<td>Whole-body BMD (g/cm²)</td>
<td>1.099 ± 0.069</td>
</tr>
<tr>
<td>Lumbar spine BMD (g/cm²)</td>
<td>1.075 ± 0.099</td>
</tr>
<tr>
<td>Total hip BMD (g/cm²)</td>
<td>0.923 ± 0.086</td>
</tr>
<tr>
<td>Percent body fat (%)</td>
<td>34.1 ± 7.4</td>
</tr>
<tr>
<td>Total fat mass (kg)</td>
<td>22.3 ± 6.6</td>
</tr>
<tr>
<td>Total lean body mass (kg)</td>
<td>41.5 ± 3.7</td>
</tr>
<tr>
<td>VO₂ max (ml/min/kg)</td>
<td>31.8 ± 5.3</td>
</tr>
</tbody>
</table>

BMI = body mass index, BMD = bone mineral density

---

Fig. 1. Study flow chart
participants were excluded due to osteoporosis, and two participants were unable to jump properly. One participant refrained from further participation due to personal reasons. Twenty-nine of the 35 women who initially responded were, therefore, included. The baseline characteristics of the participants, including VO₂-max as an indication of training status, are reported in Table 1.

**Participant characteristics at baseline**

Proximal femur (total hip), lumbar spine (LS), and whole body (WB) DXA scans (iDXA, Lunar Corporation, Madison, Wisconsin, USA) were performed according to standard procedures to determine BMD (g/cm²). The regions of interest were determined automatically by the software (Encore Version 14.10.022, GE Medical Systems, Madison, Wisconsin, USA). Body composition parameters inclusive body fat percentage (%), lean mass (kg) and fat mass (kg) were derived from WB DXA. Subjects were requested to remove metal objects and void their bladder prior to DXA scanning.

**Training status**

To confirm the participants’ training status, maximal oxygen uptake (VO₂-max) (ml/kg/min) was tested on an electronic ergometer cycle (Monark 839E, Monark Exercise AB, Vansbro, Sweden). Participants were connected to a breath-by-breath gas online analyzing system (Jaeger Oxycon Pro, VIASYS Healthcare, Höchberg, Germany), and direct measurement of VO₂-max was performed according to standard procedures for a progressive test.

**High-impact exercise trials**

A standardized 7-minute low-impact warm-up on a 3.5 cm gymnastics mat preceded each exercise trial. Following the warm-up, the participants were instructed in the actual jump of the day: CMJ, a vertical jump with two-leg launch and landing; DJ, drop jump from a 32 cm box: the landing continued directly into a vertical two-leg jump; DDJ, as DJ, but performed diagonally forward (45°).

All jumps (6 sets of 10 repetitions) were performed on a gymnastics mat, with each set of jumps interspersed with a 90 s rest. The participants were encouraged to “do all their best” in every jump by jumping as high and powerfully as possible with an arm swing in the set-off phase and a sudden stop in the landing. Instructors gave ongoing motivation and corrections if needed.

**Blood sampling and biochemical analyses**

The plasma concentrations of BTM at baseline (PRE), immediately after exercise (POST) and after two hours of rest (2Hr) were measured. As dietary intake can affect BTM [22], the participants showed up in the early morning after an overnight fast and without any vigorous exercise in the preceding 48 hours. Dietary supplements were not allowed. While waiting from POST to 2Hr, participants drank a glass of water. The blood samples were collected from the antecubital vein with a butterfly needle. Each participant had extracted 3 x 6 ml = 18 ml of blood per test day. After each test, the plasma fractions were stored at -80°C until analysis. The bone formation markers procollagen type-1 amino-terminal propeptide (P1NP) and osteocalcin (OC), and the bone resorption marker C-terminal telopeptide of type-1 collagen (CTX), were evaluated by a fully automated immunoassay system (iSYS, Immunodiagnostic Systems Ltd., Bolton, England) by the method of chemiluminescence. The performance of the assay expressed as inter-run coefficients of variation was 9% for OC, 10% for CTX, and 8% for P1NP.

**RESULTS**

**Plasma BTM**

Bone formation and resorption markers are presented in Table II. For all BTM there were no statistically significant differences in Δ-values between the three types of jumps at any time point.

Compared to PRE, P1NP POST was significantly increased for all jumps: 5.4 ± 1.6 µg/L (p<0.005) for CMJ; 6.7 ± 0.9 µg/L (p<0.001)
for DJ; and 7.8±1.1 µg/L (p<0.001) for DDJ. Compared to CON, the increases were all significantly higher for all jumps: 7.2±1.3 µg/L (p<0.01) for CMJ; 8.5±1.2 µg/L (p=0.001) for DJ; and 9.6±1.1 µg/L (p<0.001) for DDJ. P1NP 2Hr for CMJ, DJ, and DDJ did not differ significantly from PRE.

Compared to PRE, OC POST was significantly increased by 1.7±0.7 µg/L (p<0.05) for DJ, but not for CMJ or DDJ. The increase was 2.8±0.8 µg/L (p<0.05) higher than CON. Compared to PRE, OC 2Hr was decreased by 2.3±0.5 µg/L (p<0.01) for CMJ, by 2.5±1.1 µg/L (p<0.005) for DJ, and by 3.0±0.5 µg/L (p<0.001) for CON.

Compared to PRE, CTX POST did not change significantly in any trial. CTX 2Hr was significantly decreased in all trials: by 108.1±22.9 ng/L (p<0.001) for CMJ; by 119.7±25.7 ng/L (p<0.001) for DJ; by 93.8±25.2 ng/L (p<0.001) for DDJ; and by 88.3±15.7 ng/L (p<0.001) for CON.

**Ground reaction forces**

Peak GRF in anteroposterior (Fx), mediolateral (Fy) and vertical (Fz) components of the GRF normalized to body weight, for the three jumps, are presented in Figure 2.

There was a difference (p<0.01) between jumps in Fx and Fy. Subsequent paired comparisons showed that Fx for DJ was

---

**Table 2.** Bone turnover marker (BTM) concentrations at baseline (PRE), immediately after (POST), and 2 hours after exercise (2Hr)

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Jump</th>
<th>PRE</th>
<th>POST</th>
<th>2Hr</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Markers of bone formation</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P1NP (µg/L)</td>
<td>CMJ</td>
<td>70.2 ± 5.6</td>
<td>75.6 ± 6.3***</td>
<td>68.7 ± 6.0</td>
</tr>
<tr>
<td></td>
<td>DJ</td>
<td>71.0 ± 5.5</td>
<td>77.6 ± 5.8****</td>
<td>67.5 ± 6.0</td>
</tr>
<tr>
<td></td>
<td>DDJ</td>
<td>73.0 ± 6.3</td>
<td>80.8 ± 6.8****</td>
<td>70.2 ± 6.0</td>
</tr>
<tr>
<td></td>
<td>CON</td>
<td>71.9 ± 5.3</td>
<td>70.1 ± 5.6</td>
<td>70.6 ± 5.4</td>
</tr>
<tr>
<td>OC (µg/L)</td>
<td>CMJ</td>
<td>31.2 ± 2.3</td>
<td>32.2 ± 2.4</td>
<td>28.9 ± 2.2**</td>
</tr>
<tr>
<td></td>
<td>DJ</td>
<td>30.7 ± 2.2</td>
<td>32.4 ± 2.5***</td>
<td>28.3 ± 2.5**</td>
</tr>
<tr>
<td></td>
<td>DDJ</td>
<td>30.6 ± 2.2</td>
<td>31.8 ± 2.3</td>
<td>29.2 ± 2.2</td>
</tr>
<tr>
<td></td>
<td>CON</td>
<td>31.1 ± 2.1</td>
<td>30.0 ± 2.0</td>
<td>28.1 ± 2.0***</td>
</tr>
</tbody>
</table>

**Marker of bone resorption**

| CTX (ng/L)    | CMJ      | 636.0 ± 83.4 | 635.5 ± 80.3 | 527.9 ± 65.7*** |
|               | DJ       | 645.2 ± 88.3 | 666.2 ± 91.0 | 525.5 ± 69.0*** |
|               | DDJ      | 612.8 ± 85.9 | 632.8 ± 85.4 | 519.0 ± 69.1*** |
|               | CON      | 590 ± 73.6   | 582.4 ± 74.4 | 501.7 ± 65.8*** |

All values are expressed as mean ± SE (n=29). P1NP = procollagen type-1 amino-terminal propeptide. OC = osteocalcin. CTX = C-terminal telopeptide of type-1 collagen. CMJ = countermovement jump. DJ = drop jump. DDJ = diagonal drop jump. CON = control.

* p<0.05 compared to PRE. ** p<0.01 compared to PRE. *** p<0.001 compared to PRE. # p<0.05 compared to C. ## p<0.01 compared to C. ### p<0.001 compared to C.
Acute response of biochemical bone turnover markers

Multiple linear regression analyses of the correlations between BTM Δ-values and the three directions of peak GRF (Fx, Fy, Fz) showed that only for CMJ, ΔP1NP POST correlated significantly with Fz (r=0.61, p<0.01) (Fig. 3a.) and with the combined Fx, Fy, and Fz (r=0.71, p<0.05) (Fig. 3b.). The analyses resulted in the following equation: ΔP1NP = -17.71 + (-16.97*Fx) + 25.25*Fy + 7.33*Fz (R²=0.504). Thus, the combination of Fx, Fy, and Fz explained 50.4% (R²) of the ΔP1NP variation in the CMJ, while Fz explained 37.2%.

DISCUSSION

To the authors’ knowledge, the present study is the first to examine the acute biological response of BTM, and the resultant GRF, of three high-impact jumping exercises in postmenopausal women.

The finding that P1NP was significantly increased at POST for both countermovement jump (7.7±1.8%), drop jump (9.4±1.3%), and diagonal drop jump (10.6±1.6%), which differed significantly from the no-exercise control trial (p<0.01), seems to indicate that the P1NP response was due to exercise and not to diurnal variation. The acute increase in P1NP is consistent with the studies of Brahm et al. [10], Bowtell et al. [9], and Scott et al. [17], who respectively investigated the osteogenic response to resistance, odd-impact, and aerobic training. They found an increase from 9.5 to 31% [9,10,17], which is in a higher range compared to the present study. However, the larger response may be due to the younger age of the participants (21–36 year old men and women [10], 26–46 year old premenopausal women [9], and 28 year old men on average [17]) predisposing to larger maximal muscle strength and larger osteogenic responsiveness. In contrast to these findings, Mouzopoulos et al. [23] showed that the bone formation marker procollagen type-1 carboxy-terminal propeptide (P1CP) decreased by 8.92% after an ultra-marathon run. However, it was hypothesized that this reduction was probably due to intensive mechanical overloading that temporarily may inhibit collagen synthesis [24]. In addition, a study by Cabrera et al. showed that P1CP had less sensitivity and accuracy in response to interventions [25].

Two major strengths of the present study are the supervised and highly controlled jumping exercises and the precise timing of blood sampling on each trial day, minimizing the effect of diurnal variation when comparing BTM concentrations from the four days. It is known that diurnal variation in bone resorption markers represents up to 50% of the variation depending on the time of day [26]. However, no major day-to-day variation in OC [27], P1NP [28], or CTX [29] concentration has been observed when the samples have been collected at a similar time of day. This is supported by our findings since we did not find any significant differences between baseline samples on the individual trial days.

Our finding that P1NP concentrations returned to baseline two hours after exercise is consistent with prior studies [14,17] that showed insignificant Δ-values 2 hours after cessation of high-impact exercise in young males.
Other studies [29,30] examining the change in BTM three hours after a 60-minute bout of aerobic exercise reported that P1NP and P1CP decreased significantly in young subjects. It was speculated that the reason for the decrease could be exercise-induced acidosis that might impair osteoblast activity [31], which might also be the reason for the return of P1NP to baseline two hours after cessation of exercise in the present study. Since we found significant osteogenic improvements after the jumping trials compared to the control trial, the increase in ΔP1NP POST does not seem to be due to circadian rhythms.

OC was only significantly increased at POST for DJ (5.48±1.76%, p<0.05). Nevertheless, there was a non-significant increase in OC POST for DDJ (3.84±1.42%, p=0.057) and CMJ (3.26±1.11%, p=0.076), which together with the increase in P1NP might reflect an immediate anabolic effect of exercise on bone, which is supported by Maimoun et al. [12], who reported a significant increase (11%) in OC after 50 minutes of high-intensity resistance exercise in male cyclists. By contrast, OC decreased significantly (6.8%, p<0.05) following 30 minutes of resistance exercise in young sedentary males and females [10], and decreased after a 245 km ultra-marathon run in young athletes (17.4%, p<0.05) [23]. These inconsistent results confirm that, besides the musculoskeletal intensity of exercise, exercise duration and repetitiveness have an important influence on the biochemical bone marker response to acute exercise. As long exhausting exercise is known to elicit an increase in cortisol, this may induce inhibition of osteoblast function [32], leading to decreased osteocalcin concentrations after a ultra-marathon run, but also desensitization of mechanotransduction mechanisms may be responsible [33].

Our findings of significantly decreased OC concentrations at 2Hr for CMJ and DJ are in agreement with Herrmann et al. [30] and Scott et al. [17], who reported similar OC reductions after three hours of aerobic training. However, the time of the day for blood sampling was not specified in those studies, and no resting control trial was included, which makes it impossible to control for normal diurnal variation, characterized by a decline in the morning, reaching a nadir around noon, and a peak after midnight [34]. Since the 2Hr OC also decreased during the no-exercise (CON) trial in the present study, we hypothesize that the reported 2Hr OC Δ-values are due to diurnal variation, which might also be the case for the reported reductions after three hours [17,30].

CTX, generated by cathepsin K activity, is recommended as a bone resorption marker [35]. In the present study, there was no change in CTX concentrations at POST in any of the jumps, and the significant decrease in 2Hr CTX after all jumps did not differ from the control trial. Other studies [15,17] have also reported a reduced CTX concentration 2 hours following high-impact exercise in men, although with no comparison to a controlled trial to test the diurnal variation. The finding of no significant difference in 2Hr CTX between the four trials in the present study leads us to the conclusion that the CTX reductions were merely due to diurnal variation and not to exercise, which is in agreement with Wichers et al. [36], who reported a decrease in CTX between 08:00 and 11:00 reaching the lowest concentration between 11:00 and 15:00.

The findings of the present study indicate that a single bout (6 sets of 10 repetitions) of high-impact jumping exercise may elicit an osteogenic effect via an increase in the rate of bone formation compared with CON; however, no differences were detected between the CMJ, DJ and DDJ at any time point.

Normally, only the vertical peak GRF (Fz) is evaluated in exercise studies on GRF, but in the present study the anteroposterior (Fx) and the mediolateral peak GRF (Fy) were also assessed. As bone strain [31] and the impact from unusual and odd directions are especially osteogenic [37,38] we considered it logical to evaluate the combined osteogenic impact from GRF in three directions. The significantly highest anteroposterior peak GRF (Fx) was reached in the DDJ (0.874±0.04BW), which includes a forward movement when dropping from the box, unlike the CMJ, which is a vertical jump on the same spot, and the DDJ, which includes a forward diagonal movement. The significantly highest mediolateral peak GRF (FY) was, as expected, reached in the DDJ (0.366±0.038BW), which also induced the highest Δ-value of P1NP POST (from 73.01±6.27 to 80.78±6.77 µg/L, p<0.001) indicating that the DDJ had a superior osteogenic impact. However, there was no correlation (R²=0.09, p=0.21) between the Δ-value of P1NP POST and Fy in DDJ, which may be explained by the relatively low Fy in all jumps compared to especially Fz, but also to Fx. Thus, the isolated impact of Fy may only be of low osteogenic value.

Given that the vertical GRF (Fz) during the three jumping protocols averaged 4 times BW, the impact met the moderate- to high-intensity domain criteria defined by Witzke and Snow for adolescent girls [39]. However, in the present study a lower high-intensity range of GRF may be expected due to lower BMD and weaker bones in post-menopausal women. Thus, a lower GRF would be needed to induce an osteogenic strain.

The finding that Fz did not significantly differ between the jumps, even though Fz in DJ was non-significantly higher than in CMJ (p=0.06), is in agreement with Smale et al. [40], who reported that for postmenopausal women, Fz in CMJ did not differ from Fz in DJ (second landing). However, both results are in disagreement with the study of Weeks and Beck [41] showing that DJ had a significantly higher peak vertical GRF compared to CMJ (DJ 5.5BW vs. CMJ 4.7BW). However, the participants [41] differed markedly from the present study as well as from the study of Smale et al. [40] by being moderately physically active, young adult males and females. Thus, the younger age of the participants could be the reason why Fz was higher than in the present study (DJ 4.0±0.16BW vs. CMJ 3.7±0.16BW) due to better coordination and muscle power in the take-off and landing phase and less variation in jumping performance in the younger group.

To evaluate the overall osteogenic effect of peak GRF in three directions we tested the correlation between the combined three-
Acute response of biochemical bone turnover markers

axis peak GRF (Fx, Fy, and Fz) and ΔP1NP POST, and our finding of a significant correlation for CMJ indicates a clear dose-response relationship between GRF and the acute BTM response with GRF explaining 50.4% (r=0.71) of the variation in the P1NP response. A dose-response relationship is also demonstrated by Rantalainen et al. [14], but to a lesser extent. Thus, they report a significant correlation (r=0.49) between vertical peak GRF and acute BTM in postmenopausal women showed stimulation of bone modelling, reflected by the significant increase in the biochemical formation markers P1NP and OC concomitant with an unchanged resorption marker (CTX). There was no difference in BTM response between the jumps, but a significantly larger increase in P1NP and OC when compared to the resting control condition. In the countermovement jump, the higher correlation between the acute P1NP response and the combined vertical, anteroposterior and mediolateral GRF than with the vertical peak GRF alone indicates that not only vertical but also mediolateral and anteroposterior peak GRF exert an osteogenic stimulus on bone turnover. This dose-response relationship may be important when planning training aimed at improving bone mass and reducing the risk of osteoporosis.

Acknowledgments
We thank all the participants for their time and effort. The authors thank Jens J. Nielsen for assistance in blood draws, Thomas Nyegaard Beck for assistance in VO<sub>2</sub>-max testing, Therese Hornstrup Bondebjerg and Marie Hagman for help in the laboratory, Nadia Guardon for blood sample analysis, Erik B. Simonsen and Pia Melcher for guidance in using the force platform, Christian Ritz for statistical support. We thank the Department of Biomedical Sciences and Xlab at the Faculty of Health and Medical Sciences for their excellent hospitality. RSP gratefully acknowledges a Ph.D. fellowship from Lembaga Pengelola Dana Pendidikan (LPDP)/Indonesia Endowment Fund for Education. The authors have no professional relationships with companies or manufacturers that might benefit from this study.

Disclosures
No conflicts of interest, financial or otherwise, are declared by the author(s).

REFERENCES


