INTRODUCTION
Tibolone is a tissue specific steroid that, inside the body, rapidly metabolizes into three compounds, two of which have estrogenic effects and one that has both progesterogenic and androgenic effects. Many studies have shown convincingly that tibolone prevents post-menopausal bone loss, without stimulating breast or endometrium.
Bone mass is a good predictor of strength in the main loading direction, but off-axis the relative importance of the trabecular architecture seems more important. Since most falls exert loading impacts in off-axis directions, this might explain why bone mass is a poor predictor of fraction risk and stresses the importance of knowing how drugs that prevent bone loss affect trabecular architecture.
In this study we investigated the effects of long-term suboptimal tibolone dose on the 3D trabecular architecture in aged ovariectomised rats. We implemented a longitudinal study setup using in-vivo micro-CT, which allows detecting subtle changes in trabecular architecture in individual rats.

METHODS
126 ten-month old female Wistar rats were used. 18 Animals were used as a baseline control, and the remaining animals were randomized over 3 treatment groups (sham operated: SHAM; ovariectomised: OVX; ovariectomised and treated with tibolone: OVX+T) and 4 time-points (4, 14, 34 and 54 weeks). A daily dose of 2 mg/kg tibolone were administered orally to the rats in the OVX+T group. At each time point urinary deoxypyridinoline per urinary creatine (DPD/Cr) and plasma osteocalcin were determined in urine and plasma of the animals sacrificed at that time point as markers of bone metabolism.
Out of the 54 weeks groups, 21 animals (7 from each group) were selected randomly and the proximal tibia of the right leg was scanned once at each time point (0, 4, 14, 34 and 54 weeks) with an in-vivo micro-CT scanner (Skyscan 1076). All data sets were repositioned and reoriented using registration software. After registration, all data sets were segmented using local thresholds. Metaphyseal trabecular bone was separated from the cortex and morphometric parameters (BV/TV, Tb.Th, SMI and Conn. Dens.) were calculated. All animal handling formed part of a larger experiment for which approval was obtained from the Animal Ethics Committee.

RESULTS
Treatment with tibolone significantly reduced bone loss associated with estrogen deficiency, to levels between OVX and sham control (figure 1a). Similar patterns were observed for the measures of trabecular topology. OVX resulted in more rod-like trabeculae (SMI increased 50% at week 34 in the OVX group). Treatment with tibolone reduced this to 18%, while no changes were observed for the SHAM controls. Also, treatment with tibolone strongly reduced the decrease in connectivity of the trabecular network (-55% at week 34) to levels between SHAM (-45%) and OVX (-90%). Tibolone treatment resulted in different patterns of change in trabecular thickness as compared to the other morphometric parameters. While in the SHAM animals mild bone loss was associated with a mild but gradual increase in trabecular thickness, and in the OVX animals rapid bone loss resulted in strong increases in trabecular thickness in the few remaining trabeculae, treatment with tibolone completely prevented any changes in trabecular thickness (figure 1b). The two biochemical turnover markers (DPD/Cr and osteocalcin) were significantly increased with OVX and tibolone completely prevented this increase for all time-points.
Visual inspection of the registered follow up scans showed that bone loss patterns in the tibolone treated animals occurred in very similar patterns as in the other groups (figure 2). Trabecular bone was lost preferentially in the more distal and central regions of the marrow cavity, while close to the growth-plate bone loss consisted of removal of trabecular connections. Close to the growth-plate, trabeculae were removed that did not have a neighbor on the opposite side of the growth-plate, while trabeculae that did have a neighbor were preserved. This resulted in the alignment of trabeculae across the growth-plate.

DISCUSSION
Treatment with tibolone significantly reduced the effects of OVX, both on bone mass as on bone architecture parameters. It is intriguing that tibolone completely inhibited the increase in trabecular thickness with aging as observed in the other two groups. In both SHAM and OVX groups, bone loss was associated with an increase in trabecular thickness, suggesting a mechanical compensation mechanism with remaining trabeculae that carry more load. It might be that treatment with tibolone blocks such a compensation mechanism, though the observation of aligning trabeculae suggests that bone adaptation is still intact. From ongoing studies we have evidence that the mineralized tissue itself is increased in the tibolone treated animals. A study involving mechanical testing on vertebrae of the tibolone treated rats found an increase in tissue properties (1). In addition direct testing of individual trabeculae demonstrated a higher elastic modulus of the trabecular tissue (2). Finally, this was further supported by calibrated high resolution micro-CT data (using EVX micro-CT) that showed higher mineral levels in the trabeculae of the tibolone treated animals (2). We hypothesize that this increased tissue stiffness in the tibolone treated animals makes it unnecessary to increase the structural stiffness by adding more material on the remaining trabeculae such as occurs in the SHAM and the OVX groups.
While tibolone has both estrogenic and progesterogenic effects in the body, only estrogenic activity is responsible for its effects on bone (3). Though the effects of progesterone are still unclear some studies have shown a stimulating effect on osteoblasts. Further, the estrogen metabolites of tibolone have a high affinity for ERα, but only a low affinity for ERβ (4). It is likely that the absence of progesterone and the different activation patterns for the two estrogen receptors could influence the tissue quality. Our data has shown that treatment with tibolone differentially prevented the effects of ovariectomy on various parameters of bone architecture in the tibia of mature rats and completely inhibited the increase in trabecular thickness that was associated with bone loss in both SHAM and OVX animals, while bone loss was prevented partially.

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