INTRODUCTION:
Expansion of adipose tissue in bone marrow at the expense of osteogenesis is an age-related phenomenon. Similarly, it occurs in consequence of bone marrow oedema and osteonecrosis. It may thus contribute to the pathogenesis of disease entities like systemic osteoporosis and as well focal manifestations like “transient osteoporosis” and osteonecrosis. The reason most likely is an altered balance of the respective differentiation pathways. However, the molecular basis for this phenomenon is largely unknown. Individual transcription factors are key regulators for adipogenesis and osteogenesis such as peroxisome proliferator activated receptor gamma 2 (PPARgamma2) or cbfa1. However, still both differentiation pathways are incompletely understood. Plasticity between osteogenesis and adipogenesis could contribute to the increase of adipocytes in aging and disease states. The underlying molecular mechanisms of this transdifferentiation process are unknown. Knowledge on the nature of key factors for transdifferentiation could help to define new therapeutic targets for stimulation of osteogenesis. We established a cell culture system which allows for reprogramming (i.e. transdifferentiation) of osteoblasts into adipocytes and vice versa beyond the stage of committed precursors during the differentiation pathways originating from mesenchymal stem cells.

METHODS:
Human mesenchymal stem cells were isolated from the femoral head of patients undergoing total hip arthroplasty. Cells were plated at high density in DMEM/F12 medium with 10% fetal calf serum (FCS) and 50µg/ml ascorbate. Medium was changed twice weekly during propagation of the adherent cells. Upon confluency the cells were split into appropriate wells or chamber slides and were subsequently differentiated into osteoblasts or adipocytes as follows: For osteogenesis primary human osteoblasts have been used and were cultured in DMEM medium since residual lipid markers were still detectable. For control analysis. Some adipocytes however, did not respond to the osteogenic expression of osteoblast markers such as osteocalcin in RT-PCR analysis. Some adipocytes however, did not respond to the osteogenic medium since residual lipid markers were still detectable. For control purpose in addition to the transdifferentiation of adipocytes into osteoblasts the direct differentiation of mesenchymal stem cells into osteoblasts was performed.

RESULTS:
For transdifferentiation of osteoblasts into adipocytes mesenchymal stem cells were cultured in osteogenic medium for at least 2 weeks. At this time point alkaline phosphatase staining revealed a homogenous increase in staining within the cells. Also osteocalcin and alkaline phosphatase were highly expressed by RT-PCR analysis. These committed osteoblasts were then cultured in the adipogenic medium for 2 weeks in order to induce the transdifferentiation (reprogramming). After a few days lipid droplets developed and after 2 weeks within the adipogenic medium cells displayed a homogenous oil-red O staining. Direct adipogenic differentiation of mesenchymal stem cells was performed in parallel in order to compare the effectiveness of the adipogenic differentiation with the efficacy of the transdifferentiation process. The same homogenous stainings were observed. After adipogenic transdifferentiation of the committed osteoblasts no RNA markers for osteoblasts remained detectable whereas the lipid markers (LPL and PPARgamma2) were highly expressed.

For transdifferentiation of adipocytes into osteoblasts the mesenchymal stem cells were cultured into the adipogenic medium for up to 2 weeks. Then cells received the osteogenic medium for 4 weeks. Again, RNA marker expression and stainings of the initially performed adipogenesis revealed the homogenous differentiation into adipocytes. After the transdifferentiation of adipocytes into osteoblasts marked deposition of mineral (alizarin red staining) was observed as well as expression of osteoblast markers such as osteocalcin in RT-PCR analysis. Some adipocytes however, did not respond to the osteogenic medium since residual lipid markers were still detectable. For control purpose in addition to the transdifferentiation of adipocytes into osteoblasts the direct differentiation of mesenchymal stem cells into osteoblasts was performed.

DISCUSSION:
Our results indicate that the plasticity between osteogenesis and adipogenesis extends during the individual differentiation pathways up to the terminal differentiation stage of osteoblasts and adipocytes. In an established differentiation system from human bone marrow mesenchymal stem cells terminal differentiated adipocytes could be transdifferentiated to express markers of differentiated osteoblasts including mineralization (alizarin red staining). Also osteoblasts beyond the onset of osteocalcin expression were able to be efficiently reprogrammed into adipocytes. Controls revealed the adipogenic and osteogenic potential of the individual primary cultures used for the transdifferentiation pathways. The transdifferentiation process of committed osteoblasts into adipocytes was as efficient as the direct differentiation of adipocytes from mesenchymal stem cells. In contrast the transdifferentiation of terminally differentiated adipocytes into osteoblasts was incomplete. Rare publications in the literature refer to transdifferentiation between osteoblasts and adipocytes (in terms of reprogramming committed precursors and/or terminally differentiated cells). In some reports primary human osteoblasts have been used and were cultured in adipogenic medium. The observed adipocyte formation, however, in some reports represents a differentiation pathway, since trabecular bone derived cells also represent multipotent cells able to differentiate into adipocytes, osteoblasts and others. The homogenous reprogramming observed in our cell system as well as the similar kinetics of e.g. adipocyte formation from committed osteoblasts compared to the control of direct adipogenic differentiation from the mesenchymal stem cells performed in parallel provides strong evidence for the existence of the transdifferentiation (i.e. reprogramming) between adipocytes and osteoblasts. Transdifferentiation could contribute to the increase in adipogenesis in aging and disease states. Since the molecular mechanisms of this phenomenon are unclear at present, appropriate cell culture systems could provide the basis for the elucidation of the underlying molecular pathways. Thereby, novel targets could be detected for therapeutic interventions in order to stimulate osteogenesis.