GENERAL SUMMARY
Bones adapt their mass and structure to the demands of mechanical usage in order to achieve an optimal resistance to mechanical failure with a minimum use of material. This process is known as functional adaptation of bone and occurs during bone remodeling. In the process of bone remodeling, osteoclasts resorb the bone, while osteoblasts deposit new bone. This interplay between osteoclasts and osteoblasts is tightly coordinated by the mechanosensing osteocytes. When osteocytes are mechanically loaded, they start to produce signaling molecules, such as nitric oxide (NO), that affects bone formation. In addition to mechanical loading, several nutrients are also able to affect NO production, but the combined effect of mechanical usage and nutrients on the production of NO by bone cells has not been elucidated.

This thesis focuses on the effect of nutrients on the response of mechano-sensitive bone cells to mechanical stimuli. The aim was to identify nutritional factors that alter the bone cell response to mechanical loading. We also assessed whether the combination of certain nutrients and mechanical stimuli can counteract the effects of inflammatory cytokines on mechanosensitive bone cells, since bone loss associated with systemic inflammatory diseases may involve elevated cytokines levels.

In this thesis the following scientific questions were addressed:

1. What are the pathways leading to the release of signaling molecules, specifically bone morphogenetic proteins, by bone cells in response to mechanical loading?

2. How do dietary components that are used as agents in osteoporosis treatment, such as 1,25-dihydroxyvitamin D₃ and fluoride, affect the response of osteocytes to mechanical stimulation?

3. Could nutrients such as oleuropein potentially counteract inflammation-induced bone loss by restoring the altered response of bone cells to mechanical stimuli in an environment of inflammation?

To seek answers to these questions we first assessed whether osteocytes produce BMPs in response to mechanical loading (Chapter 3). We demonstrated that mechanical loading by pulsating fluid flow (PFF) upregulates BMP7 gene and protein expression in osteocytes in vitro, likely via the vitamin D receptor (VDR). BMP2 gene and protein expression was not affected by PFF.

1,25-dihydroxyvitamin D₃ (1,25(OH)₂D₃) mediates bone mass. The genomic responses to 1,25(OH)₂D₃ are mediated by the vitamin D receptor (VDR), a steroid hormone nuclear receptor. In addition, 1,25(OH)₂D₃ seems to mediate cellular responses via a variety of receptor types located within or near the plasma membrane. In chapter 4 we aimed to determine whether 1,25(OH)₂D₃ affects the production of NO by osteoblasts that are either or not mechanically stimulated by PFF. We expected 1,25(OH)₂D₃ to stimulate NO production by osteoblasts via the VDR, rather than via rapid membrane receptor-mediated
mechanisms. We showed that $1,25(OH)_2D_3$ stimulates inducible NOS expression and NO production by osteoblasts in the absence of mechanical stimulation, likely via genomic VDR action. However the mechanical loading-induced NO production might be affected by $1,25(OH)_2D_3$ independent of genomic VDR action, since $1,25(OH)_2D_3$ diminished PFF-induced NO production in bone cells lacking the VDR.

The bone anabolic agent fluoride has been used for decades to enhance bone mass in osteoporosis, but it is known to disrupt the cytoskeleton. Since the response of bone cells to mechanical loading is mediated by the cytoskeleton, we investigated whether fluoride affects the response of bone cells to mechanical loading by PFF, and whether this is cytoskeleton-mediated. Fluoride inhibited the mechanical loading-stimulated NO production, and decreased the amount of the cytoskeletal component F-actin. Treatment of young hamsters with fluoride resulted in more elongated, smaller osteocytes in interdental bone (Chapter 5). This suggests that fluoride inhibits the mechano-response of bone cells, which might occur via cytoskeletal changes.

The inflammatory cytokine interleukin-1b (IL-1b) reduces the response of osteocytes to mechanical stimuli. Phenolic dietary compounds counteract the effects of inflammatory cytokines. We showed that the phenolic compound oleuropein affects the response of IL-1b–treated osteocytes to mechanical stimulation by PFF. We also found that the NO response to mechanical loading was lower in human osteoblasts from osteoporotic patients than in control bone cells. In MLO-Y4 osteocytes, IL-1β reduced mechanical loading-stimulated NO production, which could not be reversed by oleuropein treatment. IL-1b also decreased F-actin content and COX-2 gene expression in response to mechanical loading Oleuropein prevented the effect on COX-2 expression, but not on F-actin content. Our results suggest that oleuropein may reduce the inflammation-induced bone loss in vivo by reducing the inhibitory effect of IL-1b on PFF-stimulated COX-2 gene expression (Chapter 6).

Glucose-dependent insulintropic peptide (GIP) is a gut-hormone that is released by entero-endocrine K-cells in the duodenum and jejunum in response to carbohydrate and fat ingestion. GIP is known to have an effect on bone mass and bone micro-architecture, but its mechanism of action is unclear. In view of GIP's role in nutrient absorption, it might play a role in linking nutrient ingestion to bone formation. We aimed to determine whether GIP modulates the response of mechanosensitive osteoblasts to mechanical stimulation. Treatment with GIP decreased the mechanical loading-stimulated NO production by osteoblasts suggesting that GIP affects the mechano-response of osteoblasts (Chapter 7).

In this thesis we have identified $1,25(OH)_2D_3$, sodium fluoride, oleuropein and GIP to have an effect on the NO response of bone cells to mechanical loading. These results could contribute to a better understanding of osteoporosis. The increasing number of osteoporosis patients asks for new therapeutic approaches in the near future. These
approaches preferably need to be found in the prevention of osteoporosis, and should be non-invasive. Daily exercise and a balanced diet, possibly fortified with bone formation stimulating nutrients, might be such a new approach. Therefore a better understanding of the pathways involved in mechano-transduction and regulating factors in daily nutrition are necessary to develop such strategies. The results described in this thesis contribute to a better understanding of interactions between bone, daily loading and diet, which might be of importance for the development of new strategies to prevent osteoporosis.