We are IntechOpen, the world’s leading publisher of Open Access books
Built by scientists, for scientists

6,500
Open access books available

177,000
International authors and editors

195M
Downloads

154
Countries delivered to

TOP 1%
Our authors are among the most cited scientists

12.2%
Contributors from top 500 universities

WEB OF SCIENCE™
Selection of our books indexed in the Book Citation Index in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com
1. Introduction

Dietary proteins represent 10 to 20% of energy consumption. The recommended daily minimum intake of protein and amino acids in adults is 0.8 g per kg of body weight. However, no upper limit has been identified. In industrialized countries, the main sources of protein are milk, eggs and meat. The nutritional value of protein is influenced by several factors, especially the amino acid (AA) composition, protein digestibility, protein digestion kinetics and the ability to transfer AA for protein synthesis. Diets based on either animal or vegetable products supply proteins of differing quality in differing quantities. Plant proteins are often deficient or low in some specific indispensable AAs. Soy protein is reported as a “complete” protein but its overall indispensable AA content is relatively low (~85% lower than milk) (Wilson & Wilson, 2006).

Epidemiological data support a positive association between protein intake and bone health. Protein is the precursor of AAs used for bone matrix protein synthesis. Moreover, studies evaluating the relationship between dietary protein and bone turnover support the hypothesis that high protein intake may decrease bone resorption. IGF-1 is a key mediator of bone growth (Geusens & Boonen, 2002) and dietary protein is an important regulator of circulating IGF-1 levels (Bonjour et al., 2001). However, protein is also known to be calciuric, though the origin of the increased calcium excretion is debated. According to the acid ash hypothesis, the protein-induced acid load would have a deleterious effect on bone (Barzel & Massey, 1998). Finally, some proteins, due to their amino acid content, have specific effects on bone metabolism by acting directly on bone cells or through indirect pathways.

Even if the effect of nutrition on bone is not as dramatic as that of pharmaceuticals, it can be of great help when considering bone loss. Indeed nutrition is a lower-cost, longer-term, higher-compliance and wider-spread approach than pharmaceutical treatment. The nutritional intervention can be done alone preventively or in combination with a therapy in more severe cases. Some nutritional components such as calcium and vitamin D are recognized to have a positive effect on bone. However, the effect of protein on bone health is much more debated.

This chapter reviews the literature on the subject, giving an overview of the data available and comparing the proposed mechanisms of action. Attention is drawn to protein quality and to the specific effect of some soy, collagen and milk peptides on bone metabolism.
2. Action of protein on bone

The mechanism underlying the debate on the effect of protein on bone is the hormonal anabolic effect. It is based on the increase of bone anabolic hormone IGF-1 with the consumption of protein.

2.1 Bone protein metabolism and turnover

Type I collagen is the major structural protein distributed throughout the whole body accounting for 25% of total body protein and for 80% of total conjunctive tissue in humans. The different factors involved in bone strength include not only mineral density, crystal characteristics, micro architecture, geometry and morphology (size and shape), but also protein matrix and collagen fiber quality. Cortical bone strength, whose main role is to protect bone integrity, is influenced not only by porosity, presence of micro damage and mineral composition, but also by the orientation of collagen fibers, extent and nature of collagen cross-linking and number and composition of cement lines (Burr, 2002; Currey, 2001; Seeman & Delmas, 2006; Turner, 2006). Collagen is an important component of bone, being the main extra cellular matrix protein for calcification and playing a role in osteoblast differentiation (Takeuchi et al., 1996, 1997).

Markers of bone resorption, which measure the release of peptide resulting of the degradation of mature modified type 1 collagen into the serum and urine, are commonly used to evaluate the relationship between dietary protein and bone turnover. However, to better understand the effect of feeding on bone collagen turnover, a better knowledge of the physiology of bone collagen synthesis is needed (Babraj Smith 2005).

2.2 Hormonal anabolic effect

Protein has an anabolic effect on bone metabolism by upregulating Insulin-like Growth Factor 1 (IGF-1). IGF-1 is a peptide hormone mainly produced by the liver under the action of the Growth Hormone. A small percentage of IGF-1 is also produced locally by different cell types throughout the body, including osteoblasts (Ohlsson et al., 2009). There is good evidence of the anabolic effect of IGF-1 on bone and muscle, especially when considering age-related disorders (Perrini et al., 2010). Studies measuring IGF-1 levels under different protein intakes consistently report that a high protein intake increases IGF-1. This fact has been shown over a six-month period in young exercising subjects (Ballard et al., 2005) and evidence also exists in older populations of both sexes (Arjmandi et al., 2005; Dawson-Hughes et al., 2004; Hunt et al., 2009).

There is some evidence that this hormonal response is based on the quality of the protein ingested. Dawson-Hughes and colleagues showed that a fivefold increase of aromatic AA intake (phenylalanine and histidine) for 24 days induced an increase of IGF-1 level. On the other hand, a fivefold increase of branched chain AA (leucine and isoleucine) induced no effect on IGF-1, indicating that the protein intake effect on IGF-1 is probably AA-dependent (Dawson-Hughes et al., 2007). Moreover, two studies comparing milk and soy protein reported that soy induces a greater increase of IGF-1 (Arjmandi et al., 2003; Khalil et al., 2002). However, it is important to note that the soy protein contained isoflavone in both studies, thus the effect on IGF-1 could be attributed to this molecule rather than to the AA content.
The effect of protein intake on IGF-1 level could involve Calcium-Sensing Receptor (CaSR) which was also reported to be implicated in the calciuric effect of protein (Conigrave et al., 2008). *In vitro* investigations showed that CaSR is expressed in hepatocytes (Canaff et al., 2001). The activation of CaSR in the liver by AA could explain the increased IGF-1 level due to protein consumption. Moreover, osteoblasts also express CaSR and produce IGF-1 under its regulation. CaSR also upregulates numerous bone forming mechanisms and inhibits bone resorption (Marie, 2010). Aromatic AA supplementation induces an increase in IGF-1 while branched chain AAs have no effect (Dawson-Hughes et al., 2007). This is consistent with the findings of Conigrave *et al*., that aromatic AAs are the most potent activators of CaSR, while branched chain AAs are the less potent ones (Conigrave *et al*., 2000).

There is no doubt that protein induces an increase of IGF-1 level. By doing so and according to what is known of IGF-1, protein is likely to have an anabolic effect on bone. The proposed mechanism would be that a high protein intake (especially one rich in aromatic AAs) would activate CaSR in hepatocytes and osteoblasts and stimulate IGF-1 production, which would in turn exert an anabolic effect on bone.

### 2.3 Bone turnover markers

Bone turnover markers are measured in blood or urine; they give information on bone formation or on bone resorption. Both types of markers are needed in a study in order to assess bone turnover.

The recent meta-analysis of Darling *et al*. concludes that there is no clear evidence of an effect of protein intake on bone markers (Darling *et al*., 2009). Some studies reported an increase in bone resorption while bone formation remained stable (Kerstetter *et al*., 1999; Roughhead *et al*., 2003; Zwart *et al*., 2005). However, in one of them, the authors report an increase of hydroxyproline (a bone resorption marker) that could be related to the protein source: a collagen-rich meat (Roughhead *et al*., 2003). On the other hand, three studies considering both formation and resorption markers concluded that there was a positive effect of protein on bone. One of them observed a decrease of desoxypyridinoline (a bone resorption marker) with stable formation, indicating a positive decoupling (Hunt *et al*., 2009). In the second study, both formation and resorption markers increased in a protein-supplemented group compared to control during 6 months of exercise. According to the authors, bone formation will increase faster than bone resorption over time (Ballard *et al*., 2005). In the final study, a one year soy protein supplementation only increased bone formation markers while resorption markers remained at the same level (Arjmandi *et al*., 2005). Finally, two intervention studies reported a decrease of resorption with protein supplementation but provided no data on bone formation (Dawson-Hughes *et al*., 2004; Ince *et al*., 2004).

### 3. Protein and calcium metabolism

#### 3.1 Urine calcium

Urinary calcium excretion is the major pathway for body calcium loss, along with that of feces and sweat. A high urinary calcium level has been linked to increased bone loss (Giannini *et al*., 2003).
Numerous studies have reported a positive linear relationship between dietary protein and urinary calcium (Kerstetter & Allen, 1990; Kerstetter et al., 2003; Whiting et al., 1997). The rise in urinary calcium with protein intake has also been observed in more recent studies (Ceglia et al., 2009; Hunt et al., 2009; Jajoo et al., 2006). In one study, no effect of protein on calcium was observed (Roughead et al., 2003). The mechanism underlying the effect of protein on calcium excretion is debated. First, a high protein diet induces an increase of glomerular filtration rate which will exacerbate any increased calcium excretion (Kerstetter et al., 1998). According to the acid ash hypothesis, the calciuric effect of a high protein intake would be caused by the protein-induced acid load. A meta-analysis by Fenton et al. of over 25 studies concluded that changes in acid excretion modulate calcium excretion (Fenton et al., 2008). Other studies showed that urinary calcium could be reduced by decreasing the acid load (L. Frassetto et al., 2005; Lemann et al., 1991). The systemic acidosis generated by the protein load inhibits TRPV5 calcium channel expression in the kidney. Because TRPV5 participates in renal calcium reabsorption, its inhibition increases urinary calcium (Nijenhuis et al., 2006).

Another mechanism for the protein-induced calcium excretion was proposed by Conigrave and colleagues, involving the extracellular CaSR (Conigrave et al., 2008). CaSR found on cells of the parathyroid glands and in the renal tubules are sensitive not only to calcium, but also to some AAs (Conigrave et al., 2000). Through this double sensitivity, a link is formed between protein and calcium metabolism. According to these findings, AAs would activate the CaSRs, reducing parathormone (PTH) production and decreasing renal calcium reabsorption, leading eventually to an increased calcium excretion (Conigrave et al., 2008). Indeed, PTH level has been shown to be influenced by protein intake (Kerstetter et al., 1997, 1998, 2006).

Urinary calcium loss due to a high protein diet was attributed to enhanced bone resorption (Kerstetter & Allen, 1990); however, this view has been discussed as the link between protein and bone resorption is unclear (Bonjour, 2005; M. Thorpe & Evans, 2011). Urinary calcium excretion is mainly related to bone as the main calcium storage compartment. However, an increase of the excreted calcium could also be related to an increase in calcium absorption through the diet. Hence the effect of protein on intestinal calcium absorption should also be considered.

### 3.2 Calcium absorption

While many studies claim that high-protein diets cause calciuria, there is no clear relationship between protein intake and calcium absorption. Some studies report a positive effect of protein on calcium absorption (Cao et al., 2011; Hunt et al., 2009; Kerstetter et al., 1998, 2005). One study of 13 women observed an increase in calcium absorption that explained 93% of the calcium excretion (Kerstetter et al., 2005). Another study reported an increased calciuria and a non-significant increase in calcium absorption with protein intake. But the net difference between calcium absorption and excretion with high or low protein intake remained stable (Cao et al., 2011). However, other studies observed a decrease of calcium absorption with protein (Dawson-Hughes & Harris, 2002; Heaney, 2000), or no effect at all (Roughead et al., 2003). It should be pointed out that all studies showing a positive effect were short term studies (2 to 7 weeks) whereas the two studies with a negative effect were long-term studies (years). It is thus possible that protein increases calcium absorption at first and that this effect is reversed in the long run.
The effect of protein on calcium absorption could also be conditioned by the calcium intake itself. Two studies tested the effect of protein intake on intestinal calcium absorption at different levels of calcium intake. One observed a positive effect of protein for 675 mg Ca/d and no effect at 1510 mg Ca/d (Hunt et al., 2009) whereas another study observed a negative effect at 871 mg/d and no effect at 1346 mg/d.

Moreover, Kerstetter et al. repeatedly showed that, in the context of low protein intake, there is both an increase of PTH and a decrease in calcium absorption (Kerstetter et al., 1997, 1998, 2006). Those two findings seem incompatible because PTH is known to increase calcium absorption (Heaney, 2007). However, Conigrave et al., as part of his theory on amino acid-sensing CaSR, proposes another mechanism not involving PTH. CaSR is also present in the gastro-intestinal tract, where it promotes gastric acid secretion directly and indirectly through gastrin release (Conigrave & Brown, 2006). High-protein diets induce the acidification of the intestinal content which helps calcium salts to dissolve into Ca\(^{2+}\), thereby facilitating its absorption even if PTH level is low (Conigrave et al., 2008).

### 3.3 The acid-ash theory

According to the acid-ash theory, some foods such as protein would induce a metabolic acidosis whereas other foods such as vegetables and fruits would counteract this process. The acidosis resulting from a high protein diet would be deleterious to bone and induce bone loss.

Dietary proteins, and more specifically their sulphur AA content, are part of the acid-forming nutrients. Consequently, high protein consumption would lead to metabolic acidosis (Frassetto et al., 1998; Kerstetter et al., 2006). pH is closely regulated in the body and any increase of the acid load is buffered through different pathways. The main one is through lung excretion of CO\(_2\), followed by kidney acid excretion. Bone has also been proposed to take part by releasing Ca\(^{2+}\) (Lemann et al., 2003). This last pathway is debated because the lungs’ and the kidneys’ buffering capacity are considered to be sufficient to compensate for the protein-induced acidosis (Bonjour, 2005). The idea that an acidic diet is deleterious for bone is supported by observational studies linking acid production to a reduced BMD (New et al., 2004; Rahbar et al., 2009; Wynn et al., 2008). Intervention studies on this topic usually correct the acid diet of the subjects by supplementing them with alkalinizing molecules. The results consistently show an improvement of bone health with the supplementation. This improvement occurs at the level of urinary calcium excretion (Frassetto et al., 2005; Lemann et al., 1991), bone turnover markers (Dawson-Hughes et al., 2009; He et al., 2010; Maurer et al., 2003) or Bone Mineral Density (BMD) (Domrongkitchaiporn et al., 2002). Despite all this evidence of the beneficial effect of alkalinizing the diet, it should be noted that according to a recent meta-analysis, no causal link could be established between dietary acid load and bone disease (Fenton et al., 2011). It is also important to consider that the findings supporting the acid-ash hypothesis are based on an alkalinisation of the diet and are not directly related to protein intake.

A decrease of pH is probably one of the main activators of bone-resorbing osteoclasts and an inhibitor of bone matrix deposition by osteoblasts, which explains the acid-induced bone reabsorption (Arnett, 2008). Extrapolation of these results to the clinical level is risky as it compares the in vitro pH variation to a high protein-induced acidosis. Indeed, the pH...
Dietary variation induced by protein intake is likely to be too small to induce any effect in the bone micro-environment (Bonjour, 2005).

Although they lead to different conclusions, the acid-ash theory and the hormonal anabolic theory are not mutually exclusive. A dual-pathway model for the effect of protein on bone has been proposed (M. Thorpe & Evans, 2011). Although it has been recently criticized in a meta-analysis (Fenton et al., 2011), it seems that there is a beneficial effect of diet alkalisation. This can be achieved by lowering acidic or by increasing alkaline food consumption. When considering protein, the second method is more beneficial. Indeed, even if protein is an acid nutrient, it is also an activator of bone anabolic hormones and its consumption should not be lowered. This is especially true in populations already consuming a low protein diet such as the elderly. The acid load resulting from the protein consumption should be compensated by an increased consumption of alkalinizing fruits and vegetables.

Some diets designed to promote weight loss rely on high protein consumption for a quick effect on weight. It is hard to estimate what is the effect of such diets on bone. Indeed, hyperproteic diets imply also potentially confounding effect such as spontaneous caloric restriction and a possible lack of micronutrients. Moreover, the wide variety of hyperproteic diets and the potential lack of compliance of the subjects further increase the difficulty of designing an adequate study on the subject.

4. Protein and bone parameters

4.1 Bone Mineral Density

BMD is not the only determinant of skeletal fragility; the spatial distribution of the bone mass (as cortical and trabecular bone) and the intrinsic material properties of bone are also major components (Bouxsein & Seeman, 2009). Measuring BMD or Bone Mineral Content (BMC) is the easiest way of directly assessing bone strength in humans.

Most observation studies report a positive association between protein and bone density (Hannan et al., 2000; Promislow et al., 2002) or between protein and BMD change (Dawson-Hughes & Harris, 2002; Vatanparast et al., 2007). Specific studies showing a positive correlation between protein and bone density cover a broad population: premenopausal women (Teegarden et al., 1998), postmenopausal women (Devine et al., 2005; Ilich et al., 2003; Rapuri et al., 2003; M. Thorpe et al., 2008b), men (Whiting et al., 2002) and children (Alexy et al., 2005; Chevalley et al., 2008).

On the other hand, two studies on premenopausal women concluded that protein intake had no relation with BMD (Beasley et al., 2010; Mazess & Barden, 1991). One study reported a negative association with BMC (Metz et al., 1993). Finally, a case-control study compared 134 osteoporotic women with 137 controls and identified total protein intake as a risk factor for osteoporosis. A meta-analysis by Darling and colleagues conclude that there is no evidence of a negative effect of protein intake on bone when looking at observation studies; in fact there is probably a small positive effect of protein on bone (Darling et al., 2009).

There are very few interventional studies comparing high vs. low protein intake. One of them focused on the effect of an AA and carbohydrate supplement during bed rest on 13
men. The study lasted 28 days and the authors reported a decrease in BMC in the supplemented subjects whereas BMC in control subjects remained stable (Zwart et al., 2005). It should be noted that energy intake was different between the two groups due to an energy-free placebo. Another study supplemented hospitalized elderly men and women for 38 days with 20.4g of protein. Along with other positive effects (lower complication rate, shorter hospital stay), the authors reported a decrease in BMD loss with the protein supplement (Tkatch et al., 1992). Finally, a year-long study supplementing 62 postmenopausal women with 25g of soy protein observed no effect on total hip BMD or BMC (Arjmandi et al., 2005).

4.2 Fracture risk

Fracture risk is the ultimate clinical outcome when considering bone. A longitudinal study of 32,050 postmenopausal women observed a reduced relative risk of hip fracture when protein intake was increased (Munger et al., 1999). Another one observed that a reduction in wrist fracture is positively associated with the consumption frequency of high protein food in 1,865 perimenopausal women (D. L. Thorpe et al., 2008a). Finally, in a case-control study on 1,167 cases of hip fractures and 1,334 controls of both sexes aged 50-89, the odds ratio for hip fracture decreased with the increase of protein intake (Wengreen et al., 2004).

According to these three studies, protein would have a beneficial effect on bone, but some other studies found conflicting results (Dargent-Molina et al., 2008; Feskanich et al., 1996; Meyer et al., 1997). However, it should be noted that the negative relationship between protein and fracture risk was significant only in the lowest quartiles of calcium intake (<400 mg/d) and that no association was observed for higher calcium intake (Dargent-Molina et al., 2008; Meyer et al., 1997). Pooling four studies in a meta-analysis, Darling found no significant effect of protein on the risk ratio of fracture (Darling et al., 2009). To our knowledge, it seems that as long as calcium intake is sufficient there is a protective effect of protein on bone that lowers the fracture risk.

The question of the effect of protein on fracture healing has also been addressed in some randomised controlled trials. All four trials were conducted by the same group and reported that a protein supplement improved the patients’ condition after a low-trauma femoral neck fracture (Delmi et al., 1990; Tkatch et al., 1992) or hip fracture (Chevalley et al., 2008; Schurch et al., 1998). All the clinical trials had very similar design: patients were about 80 years old and the protein supplement given was 20g. According to the two studies on femoral neck fracture, patients taking the protein supplementation had better clinical outcomes and reduced rate of complication and mortality during the hospital stay and 6 months after (Delmi et al., 1990; Tkatch et al., 1992). After hip fracture, the protein supplement increased IGF-1, attenuating bone loss at 6 months and shortening hospital stay (Schurch et al., 1998). The IGF-1 increase is independent of the type of protein given (casein, whey protein or whey protein and amino acids) (Chevalley et al., 2010). The beneficial effect of protein is probably linked to both the increased IGF-1 level and the correction of the patient nutritional state.

5. Effect of specific protein source on bone

Each protein bares some information in the form of its chain of amino acids. This information can relate to specific effects of some proteins. When protein goes through the
intestinal barrier, 50% are completely degraded as simple amino acids, 40% are partly degraded as peptides and 10% remain intact (Mallegol et al., 2005). This observation means that half of the digested protein reaching blood is still bearing some information in the form of the AA chain. Much research has tried to investigate what might be the best source of protein for bone health.

5.1 Animal vs. vegetable protein

Some observational studies considered the nature of protein source when measuring the effect on bone. One of them studied the relationships between the animal/vegetable protein ratio and bone parameters on 1035 postmenopausal women, showing that the ratio is positively associated with bone loss and hip fracture risk (Sellmeyer et al., 2001). However, the fact that a ratio was used in this study instead of the absolute values has been criticized (Bonjour, 2005; M. Thorpe & Evans, 2011). Other observational studies provide conflicting results. BMD was positively associated with animal protein intake and negatively to vegetable intake in one study (Promislow et al., 2002) but the opposite association was found when considering bone ultrasound attenuation (Weikert et al., 2005). Low levels of both protein types have been associated with deleterious effects on bone: low animal protein is related to bone loss (Hannan et al., 2000) and low vegetable protein is related to low BMD (Beasley et al., 2010). These results suggest that a minimum intake of both proteins is required regardless of the source. Finally, when considering fracture risk, a positive relationship was found with both animal (Dargent-Molina et al., 2008; Feskanich et al., 1996; Meyer et al., 1997) and vegetable protein (Munger et al., 1999; D. L. Thorpe et al., 2008a). It should be noted that the studies finding a positive relationship between animal protein and fracture risk also found a positive one with total protein. On the other hand, those finding a positive relationship between vegetable protein and fracture risk reported a negative one with total protein. Hence the results for animal and vegetable protein are not obtained in the same context.

The intervention studies comparing animal and vegetable protein always focus on specific types of protein, usually soy and casein. Hence it is the specific effect of those proteins that is evaluated and not the one of the animal and vegetable food groups. The only whole-diet intervention study was based on meat or protein-rich vegetables such as nuts and grains. Only urinary parameters were measured and urinary calcium was similar for the two diets (Massey & Kynast-Gales, 2001). More studies of this type are needed to address this issue.

Mechanistically, the reason for differentiating animal from vegetable protein is their differing sulphur AA content and the consequent modulation of potential acid load (Sellmeyer et al., 2001). The influence of sulphate content on protein efficiency has been emphasized in a cross-sectional study on 161 postmenopausal women. The results show a positive association of total protein intake with lumbar spine and total hip BMD; however, in lumbar spine this benefit is suppressed by the sulphur-containing AAs (M. Thorpe et al., 2008b). The authors conclude that an excessive consumption of sulphur AAs is likely to be deleterious for bone. However, as underlined by Massey, the assumption that animal protein contains more sulphur AA than vegetable protein is not always true. As an example, potential acidity from sulphur AA in milk or beef is lower than that of whole wheat or white rice (Massey, 2003). Hence, even if sulphur AAs were proven to have a negative effect on bone, the extrapolation to animal and vegetable food groups is likely to give incorrect
results because of the broad variety of foods in these groups. In future research, it would be more accurate to consider an estimation of the sulphate content of each protein-containing food rather than focusing only on a crude animal-vegetable distinction.

5.2 Milk proteins

Milk contains many bioactive factors, including growth hormones, enzymes, antimicrobials, anti-inflammatory agents, transporters and peptide or nonpeptide hormones. Milk, because it contains bioactive molecules, extends beyond applications in infant nutrition and was considered as a possible source of factors with anabolic effects on bone.

5.2.1 Milk Basic Proteins (MBP)

Clinical trials showed that MBP supplementation increased BMD and decreased bone resorption biomarkers in healthy women (Aoe et al., 2001; Uenishi et al., 2007; Yamamura et al., 2002), menopausal women (Aoe et al., 2005), and healthy older women (Aoyagi et al., 2010). In particular, MBP suppresses osteoclast-mediated bone resorption and leads to reduced osteoclast number in animal studies (Morita et al., 2008). Morita et al. reported that the protein fraction responsible for the observed activities of MBP is the bovine angiogenin which acts as a bone resorption-inhibitory protein (Morita et al., 2008).

However, not all studies have shown a beneficial effect of MBP. In one study, 84 healthy young women were divided into three groups receiving placebo, whole milk, or milk containing 40 mg MBP for 8 months. Compared with the baseline values, total BMD significantly increased in all groups. There was a significant decrease of bone resorption marker N-teleopeptides of type-I collagen (NTx) while bone formation remained stable in both milk groups. (Zou et al., 2009).

HPLC analysis of the major proteins in the MBP fraction identified the presence of the glycoprotein lactoferrin in most of these fractions (Naot et al., 2005).

5.2.2 Lactoferrin

Lactoferrin (LF) is an 80 kDa iron-binding glycoprotein of the transferrin family. This molecule has been demonstrated to inhibit in vitro osteoclast-mediated bone resorption (Lorjet et al., 2002). LF was also demonstrated to have in vitro anabolic, differentiating and anti-apoptotic effects on osteoblasts, and to inhibit osteoclastogenesis. Moreover in vivo local injection of LF above the hemicalvaria increases bone formation and bone area in adult mice (Cornish et al., 2004). LF has a role in host non-specific defense (Gahr et al., 1991; Legrand et al., 2004). In addition to its direct antimicrobial effects, LF is believed to modulate the inflammatory process mainly by preventing the release of inflammatory cytokines which induce recruitment and activation of immune cells at inflammatory sites (Legrand et al., 2005).

Investigations of our group and others established that LF at physiological concentrations can stimulate proliferation of primary osteoblasts and osteoblastic-cell lines and increase osteoblast differentiation (Blais et al., 2009; Cornish, 2004; Takayama & Mizumachi, 2008). Studies using 3-week culture of primary rat osteoblasts show that LF dose-dependently increases the number of nodules and the area of mineralized bone formed (Cornish, 2004). Our in vitro experiments demonstrated that LF could directly act on bone cells. Bovine LF

www.intechopen.com
(bLF) at low physiological concentrations (5 µg/ml) stimulates growth and activity of osteoblastic MC3T3-E1 cells and primary culture of murine osteoblast bone cells (Blais et al., 2009). Low density lipoprotein receptor-related protein 1 and 2, which are present on osteoblastic cells, have been shown to be partially responsible for LF’s mitogenic effect in osteoblasts (Grey et al., 2004). Moreover, Grey et al., showed that LF is able to protect osteoblastic cells from apoptosis induced by serum withdrawal (Grey et al., 2006).

LF action on osteoclasts is strikingly different since it produces an important arrest of osteoclastogenesis (Cornish, 2004; Lorget et al., 2002). However, LF does not modulate mature osteoclast activity. bLF at a concentration ranging from 10 to 1000 µg/ml was found to inhibit pre-osteoclastic established RAW cell growth. These results were confirmed in mixed primary culture of murine bone cells (Blais et al., 2009). In contrast, there was no effect of LF on bone resorption when tested on isolated mature osteoclasts, or in organ cultures that can detect mature osteoclast activity (Grey et al., 2006).

In vivo bone effects of LF were first studied using local injection of LF above the hemicalvaria which increased bone formation and bone area in adult mice (Cornish, 2004). Few recent studies using ovariectomized (OVX) rodents as a model for post menopausal bone loss measured the effect of dietary supplementation on bone (Blais et al., 2009; Guo et al., 2009; Malet et al., 2011). LF administered orally to OVX rats for three months protected them against the OVX-induced reduction of bone volume and BMD and increased the parameters of mechanical strength, increased bone formation and reduced bone resorption (Guo et al., 2009). Our studies using OVX mice demonstrated that the dietary bLF transfer into peripheral blood. LF supplementation increases BMD and bone strength compared to the OVX control group. This study supports a direct effect of LF on bone cells (Blais et al., 2009).

Recent animal studies demonstrated that estrogen deficiency causes bone loss by mechanisms associated with inflammatory and oxidative processes (Grassi et al., 2007; Lean et al., 2003; Muthusami et al., 2005). TNFα is one of the cytokines responsible for the augmented osteoclastogenesis (Suda et al., 1999). Indeed, ovariectomy causes an increase in TNF production from T-cells which in turn increases macrophage colony-stimulating factor and RANKL levels, leading to osteoclastogenesis (Cornish et al., 2004; Suda et al., 1999). Moreover the presence of increased levels of TNFα was reported in the bone marrow of OVX animals and in blood cells of postmenopausal women (Oh et al., 2007; Shanker et al., 1994). Postmenopausal osteoporosis should also be regarded as the result of an inflammatory process (Weitzmann & Pacifici, 2007). It has been shown that bLF plays a role in host non-specific defense and modulates the inflammatory process mainly by preventing the release of inflammatory cytokines that induce recruitment and activation of immune cells at inflammatory sites (Debbabi et al., 1998; Legrand et al., 2006). Indeed, bLF enriched diet ingestion can reduce release of pro-inflammatory cytokines and increase anti-inflammatory cytokine production. Our studies showed that bLF ingestion decreases bone loss and bone resorption markers. A decreased TNFα mRNA expression associated with a TNFα production inhibition on peripheral T-lymphocytes was observed with a bLF supplementation in OVX mice. Furthermore, bLF can prevent lymphocyte activation and cytokine release in the bone micro-environment. Production and release of TNFα were strongly down-regulated by LF. These immune modulations were spatially and temporally correlated with reduced bone loss. We suggested that bLF modulates the inflammatory process via specific TNFα
inhibition in order to improve bone loss (Malet et al., 2011). Recently a clinical trial of 38 healthy postmenopausal women randomized into placebo or ribonuclease-enriched-LF (R-ELF) groups evaluated bone health status over a period of 12 months. The authors demonstrated that R-ELF supplementation reduced bone resorption and increased osteoblastic bone formation; however BMD was not evaluated (Bharadwaj et al., 2009).

In conclusion, LF has a positive effect on bone health and might be useful in pathological states of reduced bone density. The molecular mechanisms are not fully understood but our studies suggest that dietary bLF supplementation can have a beneficial effect on postmenopausal bone loss not only via a direct effect but also by modulating immune function. The development of pharmaceutical or nutriceutical compounds that are based on LF will require a better understanding of LF’s mechanism of action on bone.

5.3 Collagen

Collagen has a unique triple helix configuration with a repeating sequence (Gly-X-Y)\textsubscript{n}, with X and Y being mostly proline and hydroxyproline (Hyp) (Bos et al., 1999; Ramshaw et al., 1998). Some studies suggest that a hydrolyzed collagen-enriched diet improves bone collagen metabolism and BMD. Oral administration of Hydrolyzed Collagen (HC) increased BMC and BMD in rats and mice fed a calcium or protein deficient diet (Koyama et al., 2001; Wu et al., 2004).

5.3.1 Ingestion of collagen

Oral administration of HC was demonstrated to increase the quantity of type I collagen and proteoglycans in the bone matrix of ovariectomized rats (Nomura et al., 2005). Moreover, in patients with osteoporosis, oral intake of HC with calcitonin had a stronger inhibitory effect on bone resorption than calcitonin alone (Adam et al., 1996). Oesser et al. demonstrated the intestinal absorption and cartilage accumulation of collagen-derived peptides (Oesser et al., 1999). It has been generally assumed that collagen–rich diets interact with the bone matrix. Indeed, collagen-derived di- and tripeptides rich in hydroxyproline such as Hyp, Pro-Hyp, Pro-Hyp-Gly or Gly-Pro-Val have been detected in human blood following the ingestion of HC (Iwai et al., 2005). The PEPT1 proton-dependent transporter assures the transport of Pro-Hyp across the intestinal barrier (Aito-Inoue et al., 2007).

A study of Ohara et al. compared quantity and structures of food-derived gelatin hydrolysates in human blood from fish scale, fish skin and pork skin type I collagen in a single blind crossover study (Ohara et al., 2007). Amounts of free Hyp and Hyp-containing peptide were measured over a 24-h period. Hyp-containing peptides comprised approximately 30% of all detected Hyp. However, efficiency of HC ingestion depends not only on collagen origin but also on the molecular size of the HC. Collagen needs to be hydrolysed to be able to interact with bone metabolism.

5.3.2 In vivo studies

Our in vivo studies indicate that ingestion of HC diet induced the growth of the external diameter of the bone cortical zone in OVX mice (Guillerminet et al., 2010, 2011). The increased cortical area was correlated with a significant increase in the femur external
diameter, without modification of the size of the medullar area. Therefore, the increased size of the cortical area was induced by a periosteal apposition of bone on the mouse femur. Due to this increase in bone size, the ultimate strength of femurs of OVX-mice ingesting HC was significantly greater than the control OVX mice. The increase of the external diameter was also related to a higher level of bone ALP during the first month of HC ingestion. However, the effect was transient; after three months no significant ALP increase was reported. Moreover, HC ingestion was able to increase the bone non-mineral content. There was no significant modification of Young's modulus but bone stiffness increased. Assuming that the stiffness of bone is correlated to the amount of type I collagen present (Burr, 2002; Mann et al., 2001), and since some previous studies showed an increase of type I collagen and proteoglycan excretion for mice fed hydrolyzed collagen, we can propose that HC ingestion increases type I collagen formation in mouse bone.

5.3.3 In vitro studies

The in vitro results obtained with primary tissue culture of murine bone cells confirmed that HC was able to stimulate cell growth and ALP activity. In our studies, all the tested collagens were able to increase osteoblast activity but the 2kDA porcine HC was the most efficient in vitro (Guillerminet et al., 2010). Similar observations were also reported with osteoblasts grown on collagen type I films compared to a plastic support with an improvement in various bone markers including increased ALP activity and an accelerated and uniform mineralization of the bone matrix (Lynch et al., 1995). Moreover, our work using the in vitro BD BioCoat™ Osteologic™ bone cell culture system showed that PCH-N hydrolyzed collagen did not modify osteoclast growth but reduced osteoclast differentiation (Guillerminet et al., 2010). This effect, combined with increased osteoblast activity is likely to modulate bone turnover leading to the growth of the external diameter of cortical bone.

5.3.4 Mechanism of action

Several potential mechanisms can be proposed to explain the influence of HC-derived peptides on bone metabolism. Some results have suggested that ingestion of type I hydrolyzed collagen leads to the production and absorption of collagen-derived peptides similar to peptides released from type I collagen in situ during bone resorption. Those peptides also act on bone cell metabolism (Adam et al., 1996). Osteoblast activity involves three steps including proliferation, matrix protein synthesis (type I collagen and proteoglycans) and mineralization of the bone matrix (Owen et al., 1991; Quarles et al., 1992; Stein & Lian, 1993). Several hormones and cytokines can modulate osteoblast and osteoclast differentiation and activity. The cytokine TGF-β which is stored in a latent form in the bone matrix, and secreted during the bone resorption phase, is believed to exert such an effect (Oreffo et al., 1989). TGF-β stimulates type I collagen and proteoglycan production while inhibiting that of hydroxyapatite. Interestingly, the type I collagen-derived peptide DGEA (asparagine, glycine, glutamine and alanine), was shown to interact with α2β1 integrin located on the osteoblast cell membrane. This interaction leads to inhibition of TGF-β and consequently bone matrix protein synthesis (Oesser et al., 1999; Takeuchi et al., 1996, 1997; Xiao et al., 1998). Moreover, Hyp is an aromatic AA, and an increase of its concentration can, as suggested previously, increase IGF-1 levels which consequently attenuates bone loss.
Taken together, the results indicate that hydrolyzed collagen modulates bone formation and mineralization of the bone matrix by stimulating osteoblast growth and differentiation while reducing osteoclast differentiation. These effects lead to growth of the external diameter of the cortical zone.

5.4 Isoflavone-containing soy protein

Soy contains isoflavones able to bind to estrogen receptors (Folman & Pope, 1969). They have received considerable interest as a possible alternative to conventional Hormone Replacement Therapy (HRT). However, the efficiency of phytoestrogens such as soy isoflavones on bone is still to be proven.

Epidemiological studies suggest that populations with high soy intake (such as Asian populations) have a lower incidence of osteoporotic fractures (Adlercreutz & Mazur, 1997; Schwartz et al., 1999). Asian women typically consume about 20g of soy daily which provides 40 mg of isoflavones (Chen et al., 1999; Ho et al., 2003). However, lower rates of fracture in these populations may not be fully attributed to soy consumption as ethnic related variation in fracture rates can also be explained by differences in bone structure (Bouxsein, 2011).

Many animal studies show that soy protein and/or its isoflavones have positive effects on bone mineral density (BMD) (Arjmandi et al., 1998a, 1998b). However, clinical trial results ranged from no significant changes (Alekel et al., 2000; Dalais et al., 1998; Gallagher et al., 2004; Kreijkamp-Kaspers et al., 2004; Potter et al., 1998) to a slight increase (Chiechi et al., 2002; Lydeking-Olsen et al., 2004; Potter et al., 1998) in BMD. The bone protective effects of soy and/or its isoflavones are at best inconclusive.

5.4.1 Types of isoflavones

The major isoflavones in soy foods include genistein and diadzein. Genistein 2 has one-third of the potency of estradiol 1 when it interacts with estrogen receptor-b (ER-b), and one-thousandth of the potency of estradiol 1 when it interacts with ER-a. Hence Genistein 2 can induce a small estradiol-like response in bone tissues (Adlercreutz & Mazur, 1997; Zhou et al., 2003). Another isoflavone, called equol, is not present in soybean but is a metabolic product of the biotransformation of diadzein by gut bacteria (Setchell et al., 2002). 80% of the Asian population are equol producers (Fujimoto et al., 2008; Morton et al., 2002). In contrast, as few as 25% of individuals in North America and Europe are able to make S-equol (Lampe et al., 1998).

5.4.2 Isoflavone and bone fracture

A number of reviews describe the effects of dietary soy and isoflavones on bone (Jackson et al., 2011; Messina, 2010; Reinwald & Weaver, 2010). Among the studies exploring the effect of isoflavone-containing food on BMD in postmenopausal women, few report a relationship between soy consumption and the risk of bone fracture. A clinical trial conducted by Marini et al. found that in postmenopausal osteopenic Italians receiving 54mg/day genistein for two years, spinal BMD increased by 5.8% (n=150), whereas it decreased in the placebo group by 6.3% (n=154). Similar effects were reported for the hip (Marini et al., 2007). However,
recently published long-term trials do not confirm these results; only the trial conducted by Alekel et al. reports a modest effect at the femoral neck with 120mg/d isoflavone but no effect with 80mg/d (Alekel et al., 2009).

In contrast clinical trials investigating associations between soy-food intake and BMD in Japanese or Chinese healthy postmenopausal women report that higher isoflavone consumption is associated with lower risk of bone fracture (Ho et al., 2003; Ikeda et al., 2006; Kaneki et al., 2001). Analysis of fracture incidence in the Shanghai cohort (Zhang et al., 2005) and of hip fracture in the Singapore cohort (Koh et al., 2009) shows in both studies one-third reductions in fracture risk when comparing high- with low-soy consumers.

5.4.3 Factors modulating the effect of soy on bone health

The effectiveness of dietary adaptation of western populations which rarely consumed soy must be considered. East Asian participants in epidemiological studies did not require an adaptation period or an interruption of life-long dietary habits like a western population would. Hence the observation cannot be extrapolated from one population to another.

It may be less difficult to determine bone effects following a life-long intake of traditional foods compared with intermittent intakes of soy. Traditional soy foods are a complex blend of isoflavones, protein, lipids, vitamins, minerals and other bioactive compounds that may act individually and/or synergistically to exert healthy effects. Supplements included in western diets provide quantities of individual soy components. Types of whole soy food consumed (fermented vs. nonfermented) and/or ethnicity (equol producers) may also affect outcome interpretation of soy bone effects.

Long-term observational studies in Asian populations support a benefit of traditional soy food consumption on bone health in this population. The health effects of soy-bean phytoestrogens in non-Asian postmenopausal women are promising. No conclusive evidence supports that the isoflavones from the sources studied do have beneficial effects on bone health. More researches are needed to clarify the role of dietary phytoestrogen in osteoporosis prevention.

6. Conclusion

Protein acts on bone metabolism at different levels and through different mechanisms. There is little evidence that a high-protein diet will increase bone loss. Protein is well-known to be calciuric, yet there are conflicting data on whether the excreted calcium comes from an increase of calcium absorption or from bone resorption. The direct effects of protein on bone turnover markers and BMD seem to be positive when considering observational studies, but interventional studies do not provide significant outcomes to conclude. Finally, when considering fracture rate, there seems to be a small positive effect of protein on bone as long as calcium levels remain adequate.

Two mechanisms are proposed to explain the action of protein on bone: the acid-ash theory and the hormonal anabolic effect through IGF-1 and CaSR. The hormonal anabolic mechanism supports the fact that protein is beneficial to bone by increasing IGF-1. On the other hand, the acid-ash theory considers that the acid load due to protein consumption is harmful to bone. If both mechanisms occur at the same time, it is possible to benefit from the
protein-induced IGF-1 without the negative effect of the acid load by compensating the diet with adequate alkalinizing foods.

Dietary protein quality adds complexity to the protein debate. It has been hypothesized that animal protein would be more deleterious to bone than vegetal protein. However, studies show no real difference between those two protein sources. Similarly, long-term observational studies support a benefit of traditional soy food consumption on bone health, but no conclusive evidence supports the hypothesis that this is due to the isoflavones. On the other hand, some peptides obtained from protein digestion have been shown to be helpful to prevent bone loss. Recent results indicate that HC could be of potential interest for nutritional intervention in the prevention of bone loss. Moreover, LF has been reported to have a positive effect on bone health and might be useful in pathological states of reduced bone density. The molecular mechanisms are not fully understood but our studies suggest that dietary bLF supplementation can have a beneficial effect on postmenopausal bone loss not only by acting on bone cells but also by modulating immune function.

7. References

Adam, M.; Spacek, P.; Hulejova, H.; Galianova, A. & Blahos, J. (1996). [Postmenopausal osteoporosis. Treatment with calcitonin and a diet rich in collagen proteins]. Cas Lek Cesk, Vol. 135, No. 3, (Jan 31 1996), pp. 74-78

Adlercreutz, H. & Mazur, W. (1997). Phyto-oestrogens and Western diseases. Ann Med, Vol. 29, No. 2, (Apr 1997), pp. 95-120

Aito-Inoue, M.; Lackeyram, D.; Fan, M.Z.; Sato, K. & Mine, Y. (2007). Transport of a tripeptide, Gly-Pro-Hyp, across the porcine intestinal brush-border membrane. J Pept Sci, Vol. 13, No. 7, (Jul 2007), pp. 468-474

Alekel, D.L.; Germain, A.S.; Peterson, C.T.; Hanson, K.B.; Stewart, J.W. & Toda, T. (2000). Isoflavone-rich soy protein isolate attenuates bone loss in the lumbar spine of perimenopausal women. Am J Clin Nutr, Vol. 72, No. 3, (Sep 2000), pp. 844-852

Alekel, D.L.; Van Loan, M.D.; Koehler, K.J.; Hanson, L.N.; Stewart, J.W.; Hanson, K.B.; Kurzer, M.S. & Peterson, C.T. (2009). The soy isoflavones for reducing bone loss (SIRBL) study: a 3-y randomized controlled trial in postmenopausal women. Am J Clin Nutr, Vol. 91, No. 1, (Jan 2009), pp. 218-230

Alexy, U.; Remer, T.; Manz, F.; Neu, C.M. & Schoenau, E. (2005). Long-term protein intake and dietary potential renal acid load are associated with bone modeling and remodeling at the proximal radius in healthy children. Am J Clin Nutr, Vol. 82, No. 5, (Nov 2005), pp. 1107-1114

Aoe, S.; Koyama, T.; Toba, Y.; Itabashi, A. & Takada, Y. (2005). A controlled trial of the effect of milk basic protein (MBP) supplementation on bone metabolism in healthy menopausal women. Osteoporos Int, Vol. 16, No. 12, (Dec 2005), pp. 2123-2128

Aoe, S.; Toba, Y.; Yamamura, J.; Kawakami, H.; Yahiro, M.; Kume, M.; Itabashi, A. & Takada, Y. (2001). Controlled trial of the effects of milk basic protein (MBP) supplementation on bone metabolism in healthy adult women. Biosci Biotechnol Biochem, Vol. 65, No. 4, (Apr 2001), pp. 913-918

Aoyagi, Y.; Park, H.; Park, S.; Yoshiuchi, K.; Kikuchi, H.; Kawakami, H.; Morita, Y.; Ono, A. & Shephard RJ (2010). Interactive effects of milk basic protein supplements and habitual physical activity on bone health in older women: A 1-year randomized controlled trial. Int Dairy J, Vol. 20, No. (Mar 2010), pp. 724-730
Arjmandi, B.H.; Birnbaum, R.; Goyal, N.V.; Getlinger, M.J.; Juma, S.; Alekel, L.; Hasler, C.M.; Drum, M.L.; Hollis, B.W. & Kukreja, S.C. (1998a). Bone-sparing effect of soy protein in ovarian hormone-deficient rats is related to its isoflavone content. *Am J Clin Nutr*, Vol. 68, No. 6 Suppl, (Dec 1998a), pp. 1364S-1368S

Arjmandi, B.H.; Getlinger, M.J.; Goyal, N.V.; Alekel, L.; Hasler, C.M.; Juma, S.; Drum, M.L.; Hollis, B.W. & Kukreja, S.C. (1998b). Role of soy protein with normal or reduced isoflavone content in reversing bone loss induced by ovarian hormone deficiency in rats. *Am J Clin Nutr*, Vol. 68, No. 6 Suppl, (Dec 1998b), pp. 1358S-1363S

Arjmandi, B.H.; Khalil, D.A.; Smith, B.J.; Lucas, E.A.; Juma, S.; Payton, M.E. & Wild, R.A. (2003). Soy protein has a greater effect on bone in postmenopausal women not on hormone replacement therapy, as evidenced by reducing bone resorption and urinary calcium excretion. *J Clin Endocrinol Metab*, Vol. 88, No. 3, (Mar 2003), pp. 1048-1054

Arjmandi, B.H.; Lucas, E.A.; Khalil, D.A.; Devareddy, L.; Smith, B.J.; McDonald, J.; Arquitt, A.B.; Payton, M.E. & Mason, C. (2005). One year soy protein supplementation has positive effects on bone formation markers but not bone density in postmenopausal women. *Nutr J*, Vol. 4, No. 2005), pp. 8

Arnett, T.R. (2008). Extracellular pH regulates bone cell function. *J Nutr*, Vol. 138, No. 2, (Feb 2008), pp. 415S-418S

Ballard, T.L.; Clapper, J.A.; Specker, B.L.; Binkley, T.L. & Vukovich, M.D. (2005). Effect of protein supplementation during a 6-mo strength and conditioning program on insulin-like growth factor I and markers of bone turnover in young adults. *Am J Clin Nutr*, Vol. 81, No. 6, (Jun 2005), pp. 1442-1448

Barzel, U.S. & Massey, L.K. (1998). Excess dietary protein can adversely affect bone. *J Nutr*, Vol. 128, No. 6, (Jun 1998), pp. 1051-1053

Beasley, J.M.; Ichikawa, L.E.; Ange, B.A.; Spangler, L.; LaCroix, A.Z.; Ott, S.M. & Scholes, D. (2010). Is protein intake associated with bone mineral density in young women? *Am J Clin Nutr*, Vol. 91, No. 5, (May 2010), pp. 1311-1316

Bharadwaj, S.; Naidu, A.G.; Betageri, G.V.; Prasadaraa, N.V. & Naidu, A.S. (2009). Milk ribonuclease-enriched lactoferrin induces positive effects on bone turnover markers in postmenopausal women. *Osteoporos Int*, Vol. 20, No. 9, (Sep 2009), pp. 1603-1611

Blais, A.; Malet, A.; Mikogami, T.; Martin-Rouas, C. & Tome, D. (2009). Oral bovine lactoferrin improves bone status of ovariectomized mice. *Am J Physiol Endocrinol Metab*, Vol. 296, No. 6, (Jun 2009), pp. E1281-1288

Bonjour, J.P. (2005). Dietary protein: an essential nutrient for bone health. *J Am Coll Nutr*, Vol. 24, No. 6 Suppl, (Dec 2005), pp. 526S-536S

Bonjour, J.P.; Ammann, P.; Chevalley, T. & Rizzoli, R. (2001). Protein intake and bone growth. *Can J Appl Physiol*, Vol. 26 Suppl, No. 2001), pp. S153-166

Bos, K.J.; Rucklidge, G.J.; Dunbar, B. & Robins, S.P. (1999). Primary structure of the helical domain of porcine collagen X. *Matrix Biol*, Vol. 18, No. 2, (Apr 1999), pp. 149-153

Bouxsein, M.L. (2011). Bone structure and fracture risk: do they go arm in arm? *J Bone Miner Res*, Vol. 26, No. 7, (Jul 2011), pp. 1389-1391

Bouxsein, M.L. & Seeman, E. (2009). Quantifying the material and structural determinants of bone strength. *Best Pract Res Clin Rheumatol*, Vol. 23, No. 6, (Dec 2009), pp. 741-753

Burr, D.B. (2002). The contribution of the organic matrix to bone's material properties. *Bone*, Vol. 31, No. 1, (Jul 2002), pp. 8-11

Canaff, L.; Petit, J.L.; Kisiel, M.; Watson, P.H.; Gascon-Barre, M. & Hendy, G.N. (2001). Extracellular calcium-sensing receptor is expressed in rat hepatocytes. Coupling to
intracellular calcium mobilization and stimulation of bile flow. J Biol Chem, Vol. 276, No. 6, (Feb 9 2001), pp. 4070-4079

Cao, J.J.; Johnson, L.K. & Hunt, J.R. (2011). A diet high in meat protein and potential renal acid load increases fractional calcium absorption and urinary calcium excretion without affecting markers of bone resorption or formation in postmenopausal women. J Nutr, Vol. 141, No. 3, (Mar 2011), pp. 391-397

Ceglia, L.; Harris, S.S.; Abrams, S.A.; Rasmussen, H.M.; Dallal, G.E. & Dawson-Hughes, B. (2009). Potassium bicarbonate attenuates the urinary nitrogen excretion that accompanies an increase in dietary protein and may promote calcium absorption. J Clin Endocrinol Metab, Vol. 94, No. 2, (Feb 2009), pp. 645-653

Chen, Z.; Zheng, W.; Custer, L.J.; Dai, Q.; Shu, X.O.; Jin, F. & Franke, A.A. (1999). Usual dietary consumption of soy foods and its correlation with the excretion rate of isoflavonoids in overnight urine samples among Chinese women in Shanghai. Nutr Cancer, Vol. 33, No. 1, 1999), pp. 82-87

Chevalley, T.; Bonjour, J.P.; Ferrari, S. & Rizzoli, R. (2008). High-protein intake enhances the positive impact of physical activity on BMC in prepubertal boys. J Bone Miner Res, Vol. 23, No. 1, (Jan 2008), pp. 131-142

Chevalley, T.; Hoffmeyer, P.; Bonjour, J.P. & Rizzoli, R. (2010). Early serum IGF-I response to oral protein supplements in elderly women with a recent hip fracture. Clin Nutr, Vol. 29, No. 1, (Feb 2010), pp. 78-83

Chiechi, L.M.; Secreto, G.; D’Amore, M.; Fanelli, M.; Venturelli, E.; Cantatore, F.; Valerio, T.; Laselva, G. & Loizzi, P. (2002). Efficacy of a soy rich diet in preventing postmenopausal osteoporosis: the Menfis randomized trial. Maturitas, Vol. 42, No. 4, (Aug 30 2002), pp. 295-300

Conigrave, A.D. & Brown, E.M. (2006). Taste receptors in the gastrointestinal tract. II. L-amino acid sensing by calcium-sensing receptors: implications for GI physiology. Am J Physiol Gastrointest Liver Physiol, Vol. 291, No. 5, (Nov 2006), pp. G753-761

Conigrave, A.D.; Brown, E.M. & Rizzoli, R. (2008). Dietary protein and bone health: roles of amino acid sensing receptors in the control of calcium metabolism and bone homeostasis. Annu Rev Nutr, Vol. 28, No. 2008), pp. 131-155

Conigrave, A.D.; Quinn, S.J. & Brown, E.M. (2000). L-amino acid sensing by the extracellular Ca2+-sensing receptor. Proc Natl Acad Sci U S A, Vol. 97, No. 9, (Apr 25 2000), pp. 4814-4819

Cornish, J. (2004). Lactoferrin promotes bone growth. Biometals, Vol. 17, No. 3, (Jun 2004), pp. 331-335

Cornish, J.; Callon, K.E.; Naot, D.; Palmano, K.P.; Banovic, T.; Bava, U.; Watson, M.; Lin, J.M.; Tong, P.C.; Chen, Q.; Chan, V.A.; Reid, H.E.; Fazzalari, N.; Baker, H.M.; Baker, E.N.; Haggarty, N.W.; Grey, A.B. & Reid, I.R. (2004). Lactoferrin is a potent regulator of bone cell activity and increases bone formation in vivo. Endocrinology, Vol. 145, No. 9, (Sep 2004), pp. 4366-4374

Currey, J. (2001). Sacrificial bonds heal bone. Nature, Vol. 414, No. 6865, (Dec 13 2001), pp. 699

Dalais, F.S.; Rice, G.E.; Wahlqvist, M.L.; Grehan, M.; Murties, A.L.; Medley, G.; Ayton, R. & Straus, B.J. (1998). Effects of dietary phytoestrogens in postmenopausal women. Climacteric, Vol. 1, No. 2, (Jun 1998), pp. 124-129

Dargent-Molina, P.; Sabia, S.; Touvier, M.; Kesse, E.; Breet, G.; Clavel-Chapelon, F. & Bouton-Ruault, M.C. (2008). Proteins, dietary acid load, and calcium and risk of postmenopausal fractures in the E3N French women prospective study. J Bone Miner Res, Vol. 23, No. 12, (Dec 2008), pp. 1915-1922
Darling, A.L.; Millward, D.J.; Torgerson, D.J.; Hewitt, C.E. & Lanham-New, S.A. (2009). Dietary protein and bone health: a systematic review and meta-analysis. *Am J Clin Nutr*, Vol. 90, No. 6, (Dec 2009), pp. 1674-1692

Dawson-Hughes, B. & Harris, S.S. (2002). Calcium intake influences the association of protein intake with rates of bone loss in elderly men and women. *Am J Clin Nutr*, Vol. 75, No. 4, (Apr 2002), pp. 773-779

Dawson-Hughes, B.; Harris, S.S.; Palermo, N.J.; Castaneda-Sceppa, C.; Rasmussen, H.M. & Dallal, G.E. (2009). Treatment with potassium bicarbonate lowers calcium excretion and bone resorption in older men and women. *J Clin Endocrinol Metab*, Vol. 94, No. 1, (Jan 2009), pp. 96-102

Dawson-Hughes, B.; Harris, S.S.; Rasmussen, H.; Song, L. & Dallal, G.E. (2004). Effect of dietary protein supplements on calcium excretion in healthy older men and women. *J Clin Endocrinol Metab*, Vol. 89, No. 3, (Mar 2004), pp. 1169-1173

Dawson-Hughes, B.; Harris, S.S.; Rasmussen, H.M. & Dallal, G.E. (2007). Comparative effects of oral aromatic and branched-chain amino acids on urine calcium excretion in humans. *Osteoporos Int*, Vol. 18, No. 7, (Jul 2007), pp. 955-961

Debbabi, H.; Dubarry, M.; Rautureau, M. & Tome, D. (1998). Bovine lactoferrin induces both mucosal and systemic immune response in mice. *J Dairy Res*, Vol. 65, No. 2, (May 1998), pp. 283-293

Delmi, M.; Rapin, C.H.; Bengoa, J.M.; Delmas, P.D.; Vasey, H. & Bonjour, J.P. (1990). Dietary supplementation in elderly patients with fractured neck of the femur. *Lancet*, Vol. 335, No. 8696, (Apr 28 1990), pp. 1013-1016

Devine, A.; Dick, I.M.; Islam, A.F.; Dhaliwal, S.S. & Prince, R.L. (2005). Protein consumption is an important predictor of lower limb bone mass in elderly women. *Am J Clin Nutr*, Vol. 81, No. 6, (Jun 2005), pp. 1423-1428

Domrongkitchaiporn, S.; Pongskul, C.; Sirikulchayanonta, V.; Stitchantrakul, W.; Leeprasert, V.; Onghiphadhanakul, B.; Radinahamed, P. & Rajatanavin, R. (2002). Bone histology and bone mineral density after correction of acidosis in distal renal tubular acidosis. *Kidney Int*, Vol. 62, No. 6, (Dec 2002), pp. 2160-2166

Fenton, T.R.; Eliasziw, M.; Lyon, A.W.; Tough, S.C. & Hanley, D.A. (2008). Meta-analysis of the quantity of calcium excretion associated with the net acid excretion of the modern diet under the acid-ash diet hypothesis. *Am J Clin Nutr*, Vol. 88, No. 4, (Oct 2008), pp. 1159-1166

Fenton, T.R.; Tough, S.C.; Lyon, A.W.; Eliasziw, M. & Hanley, D.A. (2011). Causal assessment of dietary acid load and bone disease: a systematic review & meta-analysis applying Hill’s epidemiologic criteria for causality. *Nutr J*, Vol. 10, No. 2011), pp. 41

Feskanich, D.; Willett, W.C.; Stampfer, M.J. & Colditz, G.A. (1996). Protein consumption and bone fractures in women. *Am J Epidemiol*, Vol. 143, No. 5, (Mar 1 1996), pp. 472-479

Folman, Y. & Pope, G.S. (1969). Effect of norethisterone acetate, dimethylstilboestrol, genistein and coumestrol on uptake of [3H]oestradiol by uterus, vagina and skeletal muscle of immature mice. *J Endocrinol*, Vol. 44, No. 2, (Jun 1969), pp. 213-218

Frassetto, L.; Morris, R.C., Jr. & Sebastian, A. (2005). Long-term persistence of the urine calcium-lowering effect of potassium bicarbonate in postmenopausal women. *J Clin Endocrinol Metab*, Vol. 90, No. 2, (Feb 2005), pp. 831-834

Frassetto, L.A.; Todd, K.M.; Morris, R.C., Jr. & Sebastian, A. (1998). Estimation of net endogenous noncarbonic acid production in humans from diet potassium and protein contents. *Am J Clin Nutr*, Vol. 68, No. 3, (Sep 1998), pp. 576-583
Dietary Protein and Bone Health

Fujimoto, K.; Tanaka, M.; Hirao, Y.; Nagata, Y.; Mori, M.; Miyanaga, N.; Akaza, H. & Kim, W.J. (2008). Age-stratified serum levels of isoflavones and proportion of equol producers in Japanese and Korean healthy men. *Prostate Cancer Prostatic Dis*, Vol. 11, No. 3, (2008), pp. 252-257

Gahr, M.; Speer, C.P.; Damara, B. & Sawatzki, G. (1991). Influence of lactoferrin on the function of human polymorphonuclear leukocytes and monocytes. *J Leukoc Biol*, Vol. 49, No. 5, (May 1991), pp. 427-433

Gallagher, J.C.; Satpathy, R.; Rafferty, K. & Haynatzka, V. (2004). The effect of soy protein isolate on bone metabolism. *Menopause*, Vol. 11, No. 3, (May-Jun 2004), pp. 290-298

Geusens, P.P. & Boonen, S. (2002). Osteoporosis and the growth hormone-insulin-like growth factor axis. *Horm Res*, Vol. 58 Suppl 3, No. 2002, pp. 49-55

Giannini, S.; Nobile, M.; Dalle Carbonare, L.; Lodetti, M.G.; Sella, S.; Vittadello, G.; Minicuci, N. & Crepaldi, G. (2003). Hypercalciuria is a common and important finding in postmenopausal women with osteoporosis. *Eur J Endocrinol*, Vol. 149, No. 3, (Sep 2003), pp. 209-213

Grassi, F.; Tell, G.; Robbie-Ryan, M.; Gao, Y.; Terauchi, M.; Yang, X.; Romanello, M.; Jones, D.P.; Weitzman, M.N. & Pacifici, R. (2007). Oxidative stress causes bone loss in estrogen-deficient mice through enhanced bone marrow dendritic cell activation. *Proc Natl Acad Sci U S A*, Vol. 104, No. 38, (Sep 18 2007), pp. 15087-15092

Grey, A.; Zhu, Q.; Watson, M.; Callon, K.; Palmano, K.; Ross, J.; Naoi, D.; Reid, I.R. & Cornish, J. (2004). The low-density lipoprotein receptor-related protein 1 is a mitogenic receptor for lactoferrin in osteoblastic cells. *Mol Endocrinol*, Vol. 18, No. 9, (Sep 2004), pp. 2268-2278

Guillerminet, F.; Beaupied, H.; Fabien-Soule, V.; Tome, D.; Benhamou, C.L.; Roux, C. & Blais, A. (2010). Hydrolyzed collagen improves bone metabolism and biomechanical parameters in ovariectomized mice: an in vitro and in vivo study. *Bone*, Vol. 46, No. 3, (Mar 2010), pp. 827-834

Heaney, R.P. (2000). Dietary protein and phosphorus do not affect calcium absorption. *Am J Clin Nutr*, Vol. 72, No. 3, (Sep 2000), pp. 758-761

Heaney, R.P. (2007). Vitamin D endocrine physiology. *J Bone Miner Res*, Vol. 22 Suppl 2, No. (Dec 2007), pp. V25-27
Ho, S.C.; Woo, J.; Lam, S.; Chen, Y.; Sham, A. & Lau, J. (2003). Soy protein consumption and bone mass in early postmenopausal Chinese women. *Osteoporos Int*, Vol. 14, No. 10, (Oct 2003), pp. 835-842

Hunt, J.R.; Johnson, L.K. & Fariba Roughhead, Z.K. (2009). Dietary protein and calcium interact to influence calcium retention: a controlled feeding study. *Am J Clin Nutr*, Vol. 89, No. 5, (May 2009), pp. 1357-1365

Ikeda, Y.; Iki, M.; Morita, A.; Kajita, E.; Kagamimori, S.; Kagawa, Y. & Yoneshima, H. (2006). Intake of fermented soybeans, natto, is associated with reduced bone loss in postmenopausal women: Japanese Population-Based Osteoporosis (JPOS) Study. *J Nutr*, Vol. 136, No. 5, (May 2006), pp. 1323-1328

Illich, J.Z.; Brownbill, R.A. & Tamborini, L. (2003). Bone and nutrition in elderly women: protein, energy, and calcium as main determinants of bone mineral density. *Eur J Clin Nutr*, Vol. 57, No. 4, (Apr 2003), pp. 554-565

Ince, B.A.; Anderson, E.J. & Neer, R.M. (2004). Lowering dietary protein to U.S. Recommended dietary allowance levels reduces urinary calcium excretion and bone resorption in young women. *J Clin Endocrinol Metab*, Vol. 89, No. 8, (Aug 2004), pp. 3801-3807

Iwai, K.; Hasegawa, T.; Taguchi, Y.; Morimatsu, F.; Sato, K.; Nakamura, Y.; Higashi, A.; Kido, Y.; Nakabo, Y. & Ohtsuki, K. (2005). Identification of food-derived collagen peptides in human blood after oral ingestion of gelatin hydrolysates. *J Agric Food Chem*, Vol. 53, No. 16, (Aug 2005), pp. 6531-6536

Jackson, R.L.; Greiwe, J.S. & Schwen, R.J. (2011). Emerging evidence of the health benefits of S-equol, an estrogen receptor beta agonist. *Nutr Rev*, Vol. 69, No. 8, (Aug 2011), pp. 432-448

Jajoo, R.; Song, L.; Rasmusussen, H.; Harris, S.S. & Dawson-Hughes, B. (2006). Dietary acid-base balance, bone resorption, and calcium excretion. *J Am Coll Nutr*, Vol. 25, No. 3, (Jun 2006), pp. 224-230

Kaneki, M.; Hodges, S.J.; Hosoi, T.; Fujiwara, S.; Lyons, A.; Crean, S.J.; Ishida, N.; Nakagawa, M.; Takechi, M.; Sano, Y.; Mizuno, Y.; Hoshino, S.; Miyao, M.; Inoue, S.; Horiki, K.; Shiraki, M.; Ouchi, Y. & Orimo, H. (2001). Japanese fermented soybean food as the major determinant of the large geographic difference in circulating levels of vitamin K2: possible implications for hip-fracture risk. *Nutrition*, Vol. 17, No. 4, (Apr 2001), pp. 315-321

Kerstetter, J.E. & Allen, L.H. (1990). Dietary protein increases urinary calcium. *J Nutr*, Vol. 120, No. 1, (Jan 1990), pp. 134-136

Kerstetter, J.E.; Caseria, D.M.; Mitnick, M.E.; Ellison, A.F.; Gay, L.F.; Liskov, T.A.; Carpenter, T.O. & Insogna, K.L. (1997). Increased circulating concentrations of parathyroid hormone in healthy, young women consuming a protein-restricted diet. *Am J Clin Nutr*, Vol. 66, No. 5, (Nov 1997), pp. 1188-1196

Kerstetter, J.E.; Mitnick, M.E.; Gundberg, C.M.; Caseria, D.M.; Ellison, A.F.; Carpenter, T.O. & Insogna, K.L. (1999). Changes in bone turnover in young women consuming different levels of dietary protein. *J Clin Endocrinol Metab*, Vol. 84, No. 3, (Mar 1999), pp. 1052-1055

Kerstetter, J.E.; O’Brien, K.O.; Caseria, D.M.; Wall, D.E. & Insogna, K.L. (2005). The impact of dietary protein on calcium absorption and kinetic measures of bone turnover in women. *J Clin Endocrinol Metab*, Vol. 90, No. 1, (Jan 2005), pp. 26-31

Kerstetter, J.E.; O’Brien, K.O. & Insogna, K.L. (1998). Dietary protein affects intestinal calcium absorption. *Am J Clin Nutr*, Vol. 68, No. 4, (Oct 1998), pp. 859-865
Kerstetter, J.E.; O’Brien, K.O. & Insogna, K.L. (2003). Dietary protein, calcium metabolism, and skeletal homeostasis revisited. *Am J Clin Nutr*, Vol. 78, No. 3 Suppl, (Sep 2003), pp. 584S-592S

Kerstetter, J.E.; Wall, D.E.; O’Brien, K.O.; Caseria, D.M. & Insogna, K.L. (2006). Meat and soy protein affect calcium homeostasis in healthy women. *J Nutr*, Vol. 136, No. 7, (Jul 2006), pp. 1890-1895

Khalil, D.A.; Lucas, E.A.; Juma, S.; Smith, B.J.; Payton, M.E. & Arjmandi, B.H. (2002). Soy protein supplementation increases serum insulin-like growth factor-I in young and old men but does not affect markers of bone metabolism. *J Nutr*, Vol. 132, No. 9, (Sep 2002), pp. 2605-2608

Koh, W.P.; Wu, A.H.; Wang, R.; Ang, L.W.; Heng, D.; Yuan, J.M. & Yu, M.C. (2009). Gender-specific associations between soy and risk of hip fracture in the Singapore Chinese Health Study. *Am J Epidemiol*, Vol. 170, No. 7, (Oct 1 2009), pp. 901-909

Koyama, Y.; Hirota, A.; Mori, H.; Takahara, H.; Kuwaba, K.; Kusubata, M.; Matsubara, Y.; Kasugai, S.; Itoh, M. & Irie, S. (2001). Ingestion of gelatin has differential effect on bone mineral density and body weight in protein undernutrition. *J Nutr Sci Vitaminol (Tokyo)*, Vol. 47, No. 1, (Feb 2001), pp. 84-86

Kreijkamp-Kaspers, S.; Kok, L.; Grobbee, D.E.; de Haan, E.H.; Aleman, A.; Lampe, J.W. & van der Schouw, Y.T. (2004). Effect of soy protein containing isoflavones on cognitive function, bone mineral density, and plasma lipids in postmenopausal women: a randomized controlled trial. *Jama*, Vol. 292, No. 1, (Jul 7 2004), pp. 65-74

Lampe, J.W.; Karr, S.C.; Hutchins, A.M. & Slavin, J.L. (1998). Urinary equol excretion with a soy challenge: influence of habitual diet. *Proc Soc Exp Biol Med*, Vol. 217, No. 3, (Mar 1998), pp. 335-339

Lean, J.M.; Davies, J.T.; Fuller, K.; Jagger, C.J.; Kirstein, B.; Partington, G.A.; Urry, Z.L. & Chambers, T.J. (2003). A crucial role for thiol antioxidants in estrogen-deficiency bone loss. *J Clin Invest*, Vol. 112, No. 6, (Sep 2003), pp. 915-923

Legrand, D.; Elass, E.; Carpentier, M. & Mazurier, J. (2005). Lactoferrin: a modulator of immune and inflammatory responses. *Cell Mol Life Sci*, Vol. 62, No. 22, (Nov 2005), pp. 2549-2559

Legrand, D.; Elass, E.; Carpentier, M. & Mazurier, J. (2006). Interactions of lactoferrin with cells involved in immune function. *Biochem Cell Biol*, Vol. 84, No. 3, (Jun 2006), pp. 282-290

Legrand, D.; Elass, E.; Pierce, A. & Mazurier, J. (2004). Lactoferrin and host defence: an overview of its immuno-modulating and anti-inflammatory properties. *Biometals*, Vol. 17, No. 3, (Jun 2004), pp. 225-229

Lemann, J., Jr.; Bushinsky, D.A. & Hamm, L.L. (2003). Bone buffering of acid and base in humans. *Am J Physiol Renal Physiol*, Vol. 285, No. 5, (Nov 2003), pp. F811-832

Lemann, J., Jr.; Pleuss, J.A.; Gray, R.W. & Hoffmann, R.G. (1991). Potassium administration reduces and potassium deprivation increases urinary calcium excretion in healthy adults [corrected]. *Kidney Int*, Vol. 39, No. 5, (May 1991), pp. 973-983

Loget, F.; Clough, J.; Oliveira, M.; Daury, M.C.; Sabokbar, A. & Offord, E. (2002). Lactoferrin reduces in vitro osteoclast differentiation and resorbing activity. *Biochem Biophys Res Commun*, Vol. 296, No. 2, (Aug 16 2002), pp. 261-266

Lydeking-Olsen, E.; Beck-Jensen, J.E.; Setchell, K.D. & Holm-Jensen, T. (2004). Soy milk or progesterone for prevention of bone loss—a 2 year randomized, placebo-controlled trial. *Eur J Nutr*, Vol. 43, No. 4, (Aug 2004), pp. 246-257

Lynch, M.P.; Stein, J.L.; Stein, G.S. & Lian, J.B. (1995). The influence of type I collagen on the development and maintenance of the osteoblast phenotype in primary and
passaged rat calvarial osteoblasts: modification of expression of genes supporting cell growth, adhesion, and extracellular matrix mineralization. *Exp Cell Res*, Vol. 216, No. 1, (Jan 1995), pp. 35-45

Malet, A.; Bournaud, E.; Lan, A.; Mikogami, T.; Tome, D. & Blais, A. (2011). Bovine lactoferrin improves bone status of ovariectomized mice via immune function modulation. *Bone*, Vol. 48, No. 5, (May 1 2011), pp. 1028-1035

Mallegol, J.; van Niel, G. & Heyman, M. (2005). Phenotypic and functional characterization of intestinal epithelial exosomes. *Blood Cells Mol Dis*, Vol. 35, No. 1, (Jul-Aug 2005), pp. 11-16

Mann, V.; Hobson, E.E.; Li, B.; Stewart, T.L.; Grant, S.F.; Robins, S.P.; Aspden, R.M. & Ralston, S.H. (2001). A COL1A1 Sp1 binding site polymorphism predisposes to osteoporotic fracture by affecting bone density and quality. *J Clin Invest*, Vol. 107, No. 7, (Apr 2001), pp. 899-907

Marie, P.J. (2010). The calcium-sensing receptor in bone cells: a potential therapeutic target in osteoporosis. *Bone*, Vol. 46, No. 3, (Mar 2010), pp. 571-576

Marini, H.; Minutoli, L.; Polito, F.; Bitto, A.; Altavilla, D.; Atteritano, M.; Gaudio, A.; Mazzaferrro, S.; Frisina, A.; Frisina, N.; Lubrano, C.; Bonaiuto, M.; D’Anna, R.; Cannata, M.L.; Corrado, F.; Adamo, E.B.; Wilson, S. & Squadrito, F. (2007). Effects of the phytoestrogen genistein on bone metabolism in osteopenic postmenopausal women: a randomized trial. *Ann Intern Med*, Vol. 146, No. 12, (Jun 19 2007), pp. 839-847

Massey, L.K. (2003). Dietary animal and plant protein and human bone health: a whole foods approach. *J Nutr*, Vol. 133, No. 3, (Mar 2003), pp. 862S-865S

Massey, L.K. & Kynast-Gales, S.A. (2001). Diets with either beef or plant proteins reduce risk of calcium oxalate precipitation in patients with a history of calcium kidney stones. *J Am Diet Assoc*, Vol. 101, No. 3, (Mar 2001), pp. 326-331

Maurer, M.; Riesen, W.; Muser, J.; Hulter, H.N. & Krapf, R. (2003). Neutralization of Western diet inhibits bone resorption independently of K intake and reduces cortisol secretion in humans. *Am J Physiol Renal Physiol*, Vol. 284, No. 1, (Jan 2003), pp. F32-40

Mazess, R.B. & Barden, H.S. (1991). Bone density in premenopausal women: effects of age, dietary intake, physical activity, smoking, and birth-control pills. *Am J Clin Nutr*, Vol. 53, No. 1, (Jan 1991), pp. 132-142

Messina, M. (2010). Insights gained from 20 years of soy research. *J Nutr*, Vol. 140, No. 12, (Dec 2010), pp. 2289S-2295S

Metz, J.A.; Anderson, J.J. & Gallagher, P.N., Jr. (1993). Intakes of calcium, phosphorus, and protein, and physical-activity level are related to radial bone mass in young adult women. *Am J Clin Nutr*, Vol. 58, No. 4, (Oct 1993), pp. 537-542

Meyer, H.E.; Pedersen, J.I.; Loken, E.B. & Tverdal, A. (1997). Dietary factors and the incidence of hip fracture in middle-aged Norwegians. A prospective study. *Am J Epidemiol*, Vol. 145, No. 2, (Jan 15 1997), pp. 117-123

Morita, Y.; Matsuyama, H.; Serizawa, A.; Takeya, T. & Kawakami, H. (2008). Identification of angiogenin as the osteoclastic bone resorption-inhibitory factor in bovine milk. *Bone*, Vol. 42, No. 2, (Feb 2008), pp. 380-387

Morton, M.S.; Arisaka, O.; Miyake, N.; Morgan, L.D. & Evans, B.A. (2002). Phytoestrogen concentrations in serum from Japanese men and women over forty years of age. *J Nutr*, Vol. 132, No. 10, (Oct 2002), pp. 3168-3171
Munger, R.G.; Cerhan, J.R. & Chiu, B.C. (1999). Prospective study of dietary protein intake and risk of hip fracture in postmenopausal women. *Am J Clin Nutr*, Vol. 69, No. 1, (Jan 1999), pp. 147-152

Muthusami, S.; Ramachandran, I.; Muthusamy, B.; Vasudevan, G.; Prabhu, V.; Subramaniam, V.; Jagadeesan, A. & Narasimhan, S. (2005). Ovarietomy induces oxidative stress and impairs bone antioxidant system in adult rats. *Clin Chim Acta*, Vol. 360, No. 1-2, (Oct 2005), pp. 81-86

Naot, D.; Grey, A.; Reid, I.R. & Cornish, J. (2005). Lactoferrin--a novel bone growth factor. *Clin Med Res*, Vol. 3, No. 2, (May 2005), pp. 93-101

New, S.A.; MacDonald, H.M.; Campbell, M.K.; Martin, J.C.; Garton, M.J.; Robins, S.P. & Reid, D.M. (2004). Lower estimates of net endogenous non-carbonic acid production are positively associated with indexes of bone health in premenopausal and perimenopausal women. *Am J Clin Nutr*, Vol. 79, No. 1, (Jan 2004), pp. 131-138

Nijenhuis, T.; Renkema, K.Y.; Hoenderop, J.G. & Bindels, R.J. (2006). Acid-base status determines the renal expression of Ca2+ and Mg2+ transport proteins. *J Am Soc Nephrol*, Vol. 17, No. 3, (Mar 2006), pp. 617-626

Nomura, Y.; Oohashi, K.; Watanabe, M. & Kasugai, S. (2005). Increase in bone mineral density through oral administration of shark gelatin to ovariectomized rats. *Nutrition*, Vol. 21, No. 11-12, (Nov-Dec 2005), pp. 1120-1126

Oesser, S.; Adam, M.; Babel, W. & Seifert, J. (1999). Oral administration of (14)C labeled gelatin hydrolysate leads to an accumulation of radioactivity in cartilage of mice (C57/BL). *J Nutr*, Vol. 129, No. 10, (Oct 1999), pp. 1891-1895

Oh, S.M.; Lee, S.H.; Lee, B.J.; Pyo, C.W.; Yoo, N.K.; Lee, S.Y.; Kim, J. & Choi, S.Y. (2007). A distinct role of neutrophil lactoferrin in RelA/p65 phosphorylation on Ser536 by recruiting TNF receptor-associated factors to IkappaB kinase signaling complex. *J Immunol*, Vol. 179, No. 9, (Nov 1 2007), pp. 5686-5692

Ohara, H.; Matsumoto, H.; Ito, K.; Iwai, K. & Sato, K. (2007). Comparison of quantity and structures of hydroxyproline-containing peptides in human blood after oral ingestion of gelatin hydrolysates from different sources. *J Agric Food Chem*, Vol. 55, No. 4, (Feb 21 2007), pp. 1532-1535

OHLSSON, C.; Mohan, S.; Sjogren, K.; Tivesten, A.; Isgaard, J.; Isaksson, O.; Jansson, J.O. & Svensson, J. (2009). The role of liver-derived insulin-like growth factor-I. *Endocr Rev*, Vol. 30, No. 5, (Aug 2009), pp. 494-535

OREFØ, R.O.; Mundy, G.R.; Seyedin, S.M. & Bonevaid, L.F. (1989). Activation of the bone-derived latent TGF beta complex by isolated osteoclasts. *Biochem Biophys Res Commun*, Vol. 158, No. 3, (Feb 15 1989), pp. 817-823

Owen, T.A.; Aronow, M.S.; Barone, L.M.; Bettencourt, B.; Stein, G.S. & Lian, J.B. (1991). Pleiotropic effects of vitamin D on osteoblast gene expression are related to the proliferative and differentiated state of the bone cell phenotype: dependency upon basal levels of gene expression, duration of exposure, and bone matrix competency in normal rat osteoblast cultures. *Endocrinology*, Vol. 128, No. 3, (Mar 1991), pp. 1496-1504

Perrini, S.; Laviola, L.; Carreira, M.C.; Cignarelli, A.; Natalicchio, A. & Giorgino, F. (2010). The GH/IGF1 axis and signaling pathways in the muscle and bone: mechanisms underlying age-related skeletal muscle wasting and osteoporosis. *J Endocrinol*, Vol. 205, No. 3, (Jun 2010), pp. 201-210
Potter, S.M.; Baum, J.A.; Teng, H.; Stillman, R.J.; Shay, N.F. & Erdman, J.W., Jr. (1998). Soy protein and isoflavones: their effects on blood lipids and bone density in postmenopausal women. *Am J Clin Nutr*, Vol. 68, No. 6 Suppl, (Dec 1998), pp. 1375S-1379S

Promislow, J.H.; Goodman-Gruen, D.; Slymen, D.J. & Barrett-Connor, E. (2002). Protein consumption and bone mineral density in the elderly: the Rancho Bernardo Study. *Am J Epidemiol*, Vol. 155, No. 7, (Apr 1 2002), pp. 636-644

Quarles, L.D.; Yohay, D.A.; Lever, L.W.; Caton, R. & Wenstrup, R.J. (1992). Distinct proliferative and differentiated stages of murine MC3T3-E1 cells in culture: an in vitro model of osteoblast development. *J Bone Miner Res*, Vol. 7, No. 6, (Jun 1992), pp. 683-692

Rahbar, A.; Larijani, B.; Nabipour, I.; Mohamadi, M.M.; Mirzaee, K. & Amiri, Z. (2009). Relationship among dietary estimates of net endogenous acid production, bone mineral density and biochemical markers of bone turnover in an Iranian general population. *Bone*, Vol. 45, No. 5, (Nov 2009), pp. 876-881

Rapuri, P.B.; Gallagher, J.C. & Haynatzka, V. (2003). Protein intake: effects on bone mineral density and the rate of bone loss in elderly women. *Am J Clin Nutr*, Vol. 77, No. 6, (Jun 2003), pp. 1517-1525

Reinwald, S. & Weaver, C.M. (2010). Soy components vs. whole soy: are we betting our bones on a long shot? *J Nutr*, Vol. 140, No. 12, (Dec 2010), pp. 2312S-2317S

Roughead, Z.K.; Johnson, L.K.; Lykken, G.I. & Hunt, J.R. (2003). Controlled high meat diets do not affect calcium retention or indices of bone status in healthy postmenopausal women. *J Nutr*, Vol. 133, No. 4, (Apr 2003), pp. 1020-1026

Schurch, M.A.; Rizzoli, R.; Slosman, D.; Vadas, L.; Vergnaud, P. & Bonjour, J.P. (1998). Protein supplements increase serum insulin-like growth factor-I levels and attenuate proximal femur bone loss in patients with recent hip fracture. A randomized, double-blind, placebo-controlled trial. *Ann Intern Med*, Vol. 128, No. 10, (May 15 1998), pp. 801-809

Schwartz, A.V.; Kelsey, J.L.; Maggi, S.; Tuttleman, M.; Ho, S.C.; Jonsson, P.V.; Poor, G.; Sisson de Castro, J.A.; Xu, L.; Matkin, C.C.; Nelson, L.M. & Heyse, S.P. (1999). International variation in the incidence of hip fractures: cross-national project on osteoporosis for the World Health Organization Program for Research on Aging, *Osteoporos Int*, Vol. 9, No. 3, (1999), pp. 242-253

Seeman, E. & Delmas, P.D. (2006). Bone quality—the material and structural basis of bone strength and fragility. *N Engl J Med*, Vol. 354, No. 21, (May 25 2006), pp. 2250-2261

Sellmeyer, D.E.; Stone, K.L.; Sebastian, A. & Cummings, S.R. (2001). A high ratio of dietary animal to vegetable protein increases the rate of bone loss and the risk of fracture in postmenopausal women. Study of Osteoporotic Fractures Research Group. *Am J Clin Nutr*, Vol. 73, No. 1, (Jan 2001), pp. 118-122

Setchell, K.D.; Brown, N.M. & Lydebek-Olsen, E. (2002). The clinical importance of the metabolite equol—a clue to the effectiveness of soy and its isoflavones. *J Nutr*, Vol. 132, No. 12, (Dec 2002), pp. 3577-3584
Shanker, G.; Sorci-Thomas, M. & Adams, M.R. (1994). Estrogen modulates the expression of tumor necrosis factor alpha mRNA in phorbol ester-stimulated human monocyctic THP-1 cells. *Lymphokine Cytokine Res*, Vol. 13, No. 6, (Dec 1994), pp. 377-382

Stein, G.S. & Lian, J.B. (1993). Molecular mechanisms mediating proliferation/differentiation interrelationships during progressive development of the osteoblast phenotype. *Endocr Rev*, Vol. 14, No. 4, (Aug 1993), pp. 424-442

Suda, T.; Takahashi, N.; Udagawa, N.; Jimi, E.; Gillespie, M.T. & Martin, T.J. (1999). Modulation of osteoclast differentiation and function by the new members of the tumor necrosis factor receptor and ligand families. *Endocr Rev*, Vol. 20, No. 3, (Jun 1999), pp. 345-357

Takayama, Y. & Mizumachi, K. (2008). Effect of bovine lactoferrin on extracellular matrix calcification by human osteoblast-like cells. *Biosci Biotechnol Biochem*, Vol. 72, No. 1, (Jan 2008), pp. 226-230

Takeuchi, Y.; Nakayama, K. & Matsumoto, T. (1996). Differentiation and cell surface expression of transforming growth factor-beta receptors are regulated by interaction with matrix collagen in murine osteoblastic cells. *J Biol Chem*, Vol. 271, No. 7, (Feb 16 1996), pp. 3938-3944

Takeuchi, Y.; Suzawa, M.; Kikuchi, T.; Nishida, E.; Fujita, T. & Matsumoto, T. (1997). Differentiation and transforming growth factor-beta receptor down-regulation by collagen-alpha2beta1 integrin interaction is mediated by focal adhesion kinase and its downstream signals in murine osteoblastic cells. *J Biol Chem*, Vol. 272, No. 46, (Nov 14 1997), pp. 29309-29316

Teegarden, D.; Lyle, R.M.; McCabe, G.P.; McCabe, L.D.; Proulx, W.R.; Michon, K.; Knight, A.P.; Johnston, C.C. & Weaver, C.M. (1998). Dietary calcium, protein, and phosphorus are related to bone mineral density and content in young women. *Am J Clin Nutr*, Vol. 68, No. 3, (Sep 1998), pp. 749-754

Thorpe, D.L.; Knutsen, S.F.; Beeson, W.L.; Rajaram, S. & Fraser, G.E. (2008a). Effects of meat consumption and vegetarian diet on risk of wrist fracture over 25 years in a cohort of peri- and postmenopausal women. *Public Health Nutr*, Vol. 11, No. 6, (Jun 2008a), pp. 564-572

Thorpe, M. & Evans, E.M. (2011). Dietary protein and bone health: harmonizing conflicting theories. *Nutr Rev*, Vol. 69, No. 4, (Apr 2011), pp. 215-230

Thorpe, M.; Mojtahedi, M.C.; Chapman-Novakofski, K.; McAuley, E. & Evans, E.M. (2008b). A positive association of lumbar spine bone mineral density with dietary protein is suppressed by a negative association with protein sulfur. *J Nutr*, Vol. 138, No. 1, (Jan 2008b), pp. 80-85

Tkatch, L.; Rapin, C.H.; Rizzoli, R.; Slosman, D.; Nydegger, V.; Vasey, H. & Bonjour, J.P. (1992). Benefits of oral protein supplementation in elderly patients with fracture of the proximal femur. *J Am Coll Nutr*, Vol. 11, No. 5, (Oct 1992), pp. 519-525

Turner, C.H. (2006). Bone strength: current concepts. *Ann N Y Acad Sci*, Vol. 1068, No. (Apr 2006), pp. 429-446

Uenishi, K.; Ishida, H.; Toba, Y.; Aoe, S.; Itabashi, A. & Takada, Y. (2007). Milk basic protein increases bone mineral density and improves bone metabolism in healthy young women. *Osteoporos Int*, Vol. 18, No. 3, (Mar 2007), pp. 385-390

Vatanparast, H.; Bailey, D.A.; Baxter-Jones, A.D. & Whiting, S.J. (2007). The effects of dietary protein on bone mineral mass in young adults may be modulated by adolescent calcium intake. *J Nutr*, Vol. 137, No. 12, (Dec 2007), pp. 2674-2679
Weikert, C.; Walter, D.; Hoffmann, K.; Kroke, A.; Bergmann, M.M. & Boeing, H. (2005). The relation between dietary protein, calcium and bone health in women: results from the EPIC-Potsdam cohort. *Ann Nutr Metab*, Vol. 49, No. 5, (Sep-Oct 2005), pp. 312-318.

Weitzmann, M.N. & Pacifici, R. (2007). T cells: unexpected players in the bone loss induced by estrogen deficiency and in basal bone homeostasis. *Ann N Y Acad Sci*, Vol. 1116, No. (Nov 2007), pp. 360-375.

Wengreen, H.J.; Munger, R.G.; West, N.A.; Cutler, D.R.; Corcoran, C.D.; Zhang, J. & Sassano, N.E. (2004). Dietary protein intake and risk of osteoporotic hip fracture in elderly residents of Utah. *J Bone Miner Res*, Vol. 19, No. 4, (Apr 2004), pp. 537-545.

Whiting, S.J.; Anderson, D.J. & Weeks, S.J. (1997). Calciuric effects of protein and potassium bicarbonate but not of sodium chloride or phosphate can be detected acutely in adult women and men. *Am J Clin Nutr*, Vol. 65, No. 5, (May 1997), pp. 1465-1472.

Whiting, S.J.; Boyle, J.L.; Thompson, A.; Mirwald, R.L. & Faulkner, R.A. (2002). Dietary protein, phosphorus and potassium are beneficial to bone mineral density in adult men consuming adequate dietary calcium. *J Am Coll Nutr*, Vol. 21, No. 5, (Oct 2002), pp. 402-409.

Wilson, J. & Wilson, G.J. (2006). Contemporary issues in protein requirements and consumption for resistance trained athletes. *J Int Soc Sports Nutr*, Vol. 3, No. 2006), pp. 7-27.

Wu, J.; Fujioka, M.; Sugimoto, K.; Mu, G. & Ishimi, Y. (2004). Assessment of effectiveness of oral administration of collagen peptide on bone metabolism in growing and mature rats. *J Bone Miner Metab*, Vol. 22, No. 6, (2004), pp. 547-553.

Wynn, E.; Lanham-New, S.A.; Krieg, M.A.; Whittamore, D.R. & Burckhardt, P. (2008). Low estimates of dietary acid load are positively associated with bone ultrasound in women older than 75 years of age with a lifetime fracture. *J Nutr*, Vol. 138, No. 7, (Jul 2008), pp. 1349-1354.

Xiao, G.; Wang, D.; Benson, M.D.; Karsenty, G. & Franceschi, R.T. (1998). Role of the alpha2-integrin in osteoblast-specific gene expression and activation of the Osf2 transcription factor. *J Biol Chem*, Vol. 273, No. 49, (Dec 4 1998), pp. 32988-32994.

Yamamura, J.; Aoe, S.; Toba, Y.; Motouri, M.; Kawakami, H.; Kumegawa, M.; Itabashi, A. & Takada, Y. (2002). Milk basic protein (MBP) increases radial bone mineral density in healthy adult women. *Biosci Biotechnol Biochem*, Vol. 66, No. 3, (Mar 2002), pp. 702-704.

Zhang, X.; Shu, X.O.; Li, H.; Yang, G.; Li, Q.; Gao, Y.T. & Zheng, W. (2005). Prospective cohort study of soy food consumption and risk of bone fracture among postmenopausal women. *Arch Intern Med*, Vol. 165, No. 16, (Sep 12 2005), pp. 1890-1895.

Zhou, S.; Turgeman, G.; Harris, S.E.; Leitman, D.C.; Komn, B.S.; Bodine, P.V. & Gazit, D. (2003). Estrogens activate bone morphogenetic protein-2 gene transcription in mouse mesenchymal stem cells. *Mol Endocrinol*, Vol. 17, No. 1, (Jan 2003), pp. 56-66.

Zou, Z.Y.; Lin, X.M.; Xu, X.R.; Xu, R.; Ma, L.; Li, Y. & Wang, M.F. (2009). Evaluation of milk basic protein supplementation on bone density and bone metabolism in Chinese young women. *Eur J Nutr*, Vol. 48, No. 5, (Aug 2009), pp. 301-306.

Zwart, S.R.; Davis-Street, J.E.; Paddon-Jones, D.; Ferrando, A.A.; Wolfe, R.R. & Smith, S.M. (2005). Amino acid supplementation alters bone metabolism during simulated weightlessness. *J Appl Physiol*, Vol. 99, No. 1, (Jul 2005), pp. 134-140.
The World Health Organization (WHO) has established dual-energy x-ray absorptiometry (DXA) as the best densitometric technique for assessing bone mineral density (BMD) in postmenopausal women and has based the definitions of osteopenia and osteoporosis on its results. DXA enables accurate diagnosis of osteoporosis, estimation of fracture risk and monitoring of patients undergoing treatment. Additional features of DXA include measurement of BMD at multiple skeletal sites, vertebral fracture assessment and body composition assessment, including fat mass and lean soft tissue mass of the whole body and the segments. This book contains reviews and original studies about DXA and its different uses in clinical practice (diagnosis of osteoporosis, monitoring of BMD measurement) and in medical research in several situations (e.g. assessment of morphological asymmetry in athletes, estimation of resting energy expenditure, assessment of vertebral strength and vertebral fracture risk, or study of dry bones such as the ulna).

How to reference

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Anne Blais, Emilien Rouy and Daniel Tomé (2012). Dietary Protein and Bone Health, Dual Energy X-Ray Absorptiometry, Prof. Abdelah El Maghraoui (Ed.), ISBN: 978-953-307-877-9, InTech, Available from: http://www.intechopen.com/books/dual-energy-x-ray-absorptiometry/dietary-protein-and-bone-health
