Cardiovascular and Renal Calcification and Bone: A comparison of the effect of Dietary Fatty Acids

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Abstract

Cardiovascular (CV) and renal calcification is regularly found with osteoporosis and both are conditions of chronic inflammation and oxidative stress. Intake of dietary fatty acids is known to impact on the incidence of CV disease and bone loss but few studies have specifically looked at their impact on CV or renal calcification. This review found that although a very high total fat intake is likely to prove detrimental to both tissues and bone, particularly with low calcium intake, human studies often show mixed results, possibly because fatty acid intake shows a U-shaped dose/response curve, contrary to the expected linear relationship. Nevertheless, intake of fish and fish oil are generally found to protect against ectopic calcification and bone loss, with a low omega 6 to omega 3 ratio (preferably <5:1) proving critical. Fish intake of 3-4 servings a week was believed to be optimal. In arteries, the relationship between fish oil intake and other markers of sub-clinical atherosclerosis, such as intima-media thickness, may be stronger than their relationship with arterial calcification. Any association with arterial calcification often lost significance after adjustment for CV risk factors, suggesting that fish oil may act principally by lowering risk factors and calling into question whether CV calcification is a condition of dyslipidaemia.

Introduction

Ectopic calcification has long been found in autopsy studies and is now regularly detected with multi-detector computed tomography (MDCT) and, when located in the cardiovascular (CV) system, has become known as a form of subclinical atherosclerosis. The initiation of ectopic calcification resembles the process of osteogenesis, involving the same cells, proteins and cytokines. Studies have shown osteoblast- and osteoclast-like cells and bone matrix proteins in the arterial wall1, hydroxyapatite (calcium phosphate) deposition and, in cases of more severe calcification, fully formed bone is seen in arteries and valves2. Fracture incidence and low BMD have frequently been shown to correlate with CV calcification3-4 and plaque burden5, particularly in postmenopausal women, and the two conditions have several shared risk factors and biomarkers6. Within arteries, calcification may occur in the tunica media, particularly in chronic kidney disease and diabetes mellitus, or as a thick fibrous atheroma cap7-8. Although this covering of the atheroma may help to prevent plaque rupture, its presence has also been correlated with acute coronary syndrome9. Despite the apolipoprotein E4 genotype being correlated with dyslipidaemia and atherosclerosis, it is not associated with aortic calcification or BMD10. It remains unclear as to why ectopic calcification forms in the first place, with one theory suggesting that calcium phosphate is taken out of bone and deposited in soft tissue rather than being incorporated in bone or excreted in urine, as would normally be the case.

Diet is known to play an important role in the development of atherosclerosis risk factors, with fat intake thought to be particularly important via the pathogenesis of dyslipidaemia. A diet high in saturated and trans fats is thought to contribute to the development of atheroma11-13, while certain omega-6 (ω6) polyunsaturated fatty acids (PUFAs), found mainly in plant oils, may also be atherogenic14. By contrast, monounsaturated fatty acids (MUFAs) from olive oil and omega-3 (ω3) PUFAs from fish oil have been found to reduce atherosclerosis15-16. In particular, a lower ratio of dietary ω3 to ω6 is a critical risk factor for
acute coronary syndrome[15]. Within each PUFA family (Figure 1), there is an intricate pathway of metabolites, dependent upon shared enzymes. In order to explore another possible similarity between ectopic calcification and bone mineralisation, this article compares the effect of dietary fats on both ectopic calcification and bone mineralisation and, where also assessed, other measures of sub-clinical atherosclerosis.

**In vitro studies**

While many in vitro studies have been carried out on ectopic calcification in order to elucidate its pathogenesis, few have involved fats and oils. In human arterial endothelial cells, a magnesium deficiency allowed entry of the trans fatty acids linolealaidic and elaidic acids into cells, which increased the incorporation of calcium and may thereby increase arterial calcification[16]. Omega-3 fats, however, inhibited the mineralisation of vascular cells and activity of alkaline phosphatase (ALP), a marker of bone formation, with much of the benefit deriving from docosahexaenoic acid (DHA)[17]. In human osteoblast-like cells, arachidonic acid (AA), but not eicosapentaenoic acid (EPA) or oleic acid, reduced bone cell adhesion, while EPA and oleic acid, but not AA, increased gene expression of type I collagen and fibronectin, encouraging bone growth[18]. Similarly, an optimum ratio of 4:1 linoleic acid to alpha-linolenic acid increased osteoblastogenesis, the effect being enhanced by the addition of conjugated linoleic acid and calcium[19].

**Animal studies**

**Ectopic calcification**

Dietary cholesterol is regularly used, often with other factors, to induce atherosclerotic lesions and/or calcification of the arteries and heart valves in animal models of hypercholesterolaemia[20]; the calcification tends to appear later than atherosclerotic lesions[21], while arteries may calcify before valves[22]. The necessary conditions for arterial or renal calcification induction appear to be a high fat diet plus cholesterol, while high fat alone or a low fat diet with cholesterol failed to induce calcification[23-24], although high fat actually reduced renal calcification[25]. Finally, a study of bone and renal calcification found that a high fat diet increased serum ALP and renal calcium phosphate deposition, with significant bone calcium loss and increased urinary calcium excretion[26].

Among PUFAs, a diet rich in linoleic acid (LA) decreased heart valve and gastric and renal artery calcification[27], with no difference in severity of calcification between a high or low LA diet[28]. Fish oil and gamma-linolenic acid (GLA), alone or combined, significantly reduced renal calcification, with a possible synergistic effect between the two[29]. EPA could also limit induced abdominal aorta medial (but not iliac) calcification by lowering the expression of osteogenic markers and suppressing matrix metalloproteinase-9 activity[30], although monooester EPA plus LA increased renal and aortic calcification, while high dose EPA given with antioxidant lipoic acid prevented calcification[29].

**Bone**

Both a high fat diet, regardless of its constituents, and a deficiency can be detrimental to bone[30-31], suggesting that fatty acid intake demonstrates a U-shaped dose/response curve. Animal saturated fats can be harmful to bone and reduce calcium absorption[32], although coconut oil (a saturated fat) appears to increase BMD and bone strength[33], while olive oil (a

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<th>Omega-6 (ω6)</th>
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<td>Diet Linoleic acid (LA)</td>
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<td>Docosahexaenoic acid (DHA)</td>
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**Figure 1: Metabolism of polyunsaturated fatty acids.**

MUFA prevented bone loss in ovariectomised rats[34]. Among PUFAs, both LA and ALA can separately strengthen bone[35] but may be more effective when supplemented together[36]. Where oils rich in LA (safflower, soyabean, sunflower, sesame and corn oils) and flaxseed oil (containing principally ALA) are compared, there is either no difference in their positive effect on bone or ALA is more beneficial[37-38]. When LA was compared to fish oil (EPA + DHA), fish oil resulted in consistently improved bone parameters[39-40]. These properties of fish oil may be stronger in ovariectomised but not sham-operated animals[41] but can also increase newly formed bone and bone thickness in males[42]. Only in one study did fish oil give significantly degraded bone properties[43] but the fish oil dose was extremely high.

The balance between ω6 and ω3 appears to be critical, with an ω6:ω3 ratio of 5:1 giving significantly higher BMC than a 10:1 ratio, with optimum results when the total fat intake was low[44]. Another study found that an LA /fish oil ratio of 3:7 gave the highest bone formation rate[45], while incorporation of DHA into tissues showed a U-shaped curve, with the maximum incorporation occurring between the LA:ALA ratios of 4:1 and 2:1[46]. Although some studies have shown that EPA, with or without DHA, can be effective[47], others suggest that it is DHA that is the active constituent with respect to bone[48], with high dose EPA lowering BMD[49]. The metabolites of LA may be more effective than LA itself. Studies show that higher ratios of GLA:EPA were more beneficial, with one showing an optimum ratio of 3:1[50], although DHA was more effective than both[51]. AA, also found in meat, could reduce markers of bone resorption and raise BMC[52], with an optimum ratio for AA:DHA of 5:1, although this ratio was only beneficial at low levels of intake[53].

**Human epidemiological studies**

**Ectopic calcification**

In the only study to consider dietary cholesterol, intake was positively associated with the CAC score in Korean men, whereas total fat was not associated[54]. Studies of the percentage of energy represented by fat intake and CAC prevalence or extent show mixed results, while a large study showed that a food pattern high in total, saturated and trans fats was positively associated with CAC presence and extent and cIMT[55], although other dietary factors may have been confounding. Total and saturated fat intake were
also positively correlated with CAC incidence among type 1 diabetics, although this was no longer significant after adjustment for conventional CV risk factors. A small study of Korean haemodialysis patients, particularly prone to ectopic calcification, found no association of intake of saturated fat or ω6 PUFAs and calcification of the extremities or the abdominal aorta. A prospective study of premenopausal women showed that although there was no association between total fat intake and arterial calcification or the carotid plaque index, saturated fat intake was positively correlated with coronary but not aortic calcification or the carotid plaque index, after adjustment for risk factors. There have been no studies of MUFAs and ectopic calcification.

A large US study investigated intake of long chain ω3 PUFAs and non-fried fish and found no association with CAC presence, although there was a significant inverse association with cIMT. Similarly, a comparison study between Japanese and American men showed a significant inverse association between EPA and DHA intake and CAC and cIMT but after adjustment for CVD risk factors, only the relationship with cIMT in Japanese men continued. The median intake of EPA/DHA in the lowest tertile in Japanese men was still greater than that of the highest tertile in American men, which may explain the protective effect for cIMT in Japanese men only. In a further comparison of middle-aged Japanese and Caucasian men, Japanese men had a higher level of serum long chain ω3 PUFAs and a significantly lower incidence of CAC compared to Caucasians, even after adjusting for age and conventional CV risk factors. Another Japanese study of acute myocardial infarction (MI) patients found that in multivariable analysis, only serum log EPA was inversely correlated with the CAC score, although serum log EPA, DHA and the ω-6: EPA ratios were inversely correlated with soft plaque scores. A large Dutch prospective study found that subjects with a fish intake >19g/d compared to zero had a significantly lower incidence of CAC score of 11-400, the association being entirely attributable to women; the association with CAC score >400 was not significant in either gender. This inverse association in women became non-significant after adjustment for triglycerides, although adjustment for hypertension and other blood lipids did not change the association. Curiously, there was no association with intake of EPA+DHA, although the majority of the fish consumed was cod which does not contain high long chain ω3 fats.

Bone
Epidemiological studies of total and saturated fat intake show mixed results with respect to BMD and fracture risk, with saturated fat being detrimental only at higher intakes. Mixed results were also seen in investigations of MUFA intake, although when analysed by source, olive oil intake was positively associated with BMD, but where MUFAs were principally derived from animal sources, there was no association. Macdonald et al showed that intake of mainly animal-derived MUFAs was only adversely associated with BMD in the lowest tertile of calcium intake.

There is also no clear conclusion from the results of studies of PUFA intake, with most showing no association, although some benefit was seen with higher intake of ω3 PUFAs, although AA may be protective in men. There appears to be an interaction between ω6 and ω3 PUFAs. Fish oil intake above the median resulted in lower BMD among those with LA intake above the median and higher BMD among those with the highest AA intake. Nevertheless, high AA with low fish oil intake may reduce BMD. Again, increased PUFA intake was associated with greater BMD loss only in those in the lowest tertile of calcium intake. In the many studies investigating total fish intake, there is either a beneficial association with BMD or fracture risk or no relationship; the correlation may only be with oily fish intake. Two studies have found that eating fish 3-4 times per week was associated with reduced risk but there was no association with greater fish consumption.

Human intervention studies
There are no intervention studies of fatty acids and ectopic calcification but several related to biomarkers of bone formation or resorption, which may equally apply to bone or ectopic calcification. Many of these involve multiple interventions, making it impossible to isolate the effect of any one fatty acid. In general, lower saturated fat intake and a lower ω6:ω3 ratio proved beneficial, with fish, fish oil and oleic acid increasing bone formation markers and reducing resorption markers. Some studies showed no significant difference from the placebo but one reason for this may be that the placebo is usually an oil, which may itself have a beneficial effect.

Comparison of the effect of fats on CV calcification versus other measures of subclinical atherosclerosis
High intake of saturated, trans and total fats appears to be positively correlated with both CAC presence and cIMT, although fish and fish oil intake may show no significant association with CAC after adjustment for risk factors but a significant protective effect against cIMT and soft plaque scores. This is particularly evident in comparison studies of Japanese and American men, which show a significant inverse association between fish oil intake or serum EPA/DHA and CAC presence or extent and cIMT in univariate analysis but mixed results for CAC after adjustment for CVD risk factors. This suggests that fish oil may operate by lowering CV risk factors, which has more impact on cIMT than on CAC, and was borne out by one of the MESA studies which showed that the inverse association of BMD with CAC was stronger in women without dyslipidaemia, arguing against the hypothesis that dyslipidaemia is the key factor responsible for the inverse association of BMD with atherosclerosis. Furthermore, the median intake of fish oil in the lowest tertile in Japanese men was still greater than that of the highest tertile in American men, suggesting that, with respect to cIMT, fish oil intake has to reach a certain minimum before it becomes effective.

Potential mechanisms
Although both ectopic calcification and bone loss are conditions of ageing and are prevalent in smokers, type 2 diabetics, postmenopausal women and renal patients, there have been numerous additional mechanisms proposed for their association, among which are chronic inflammation and oxidative stress, in particular elevated lipid oxidation products. Oxidised LDL is known to inhibit osteoblast differentiation and mineralisation and promote mineralisation of vascular cells by increasing oxidative stress and receptor activator of nuclear factor kappa B ligand (RANKL). Both ω6 and ω3 fats are prone to oxidation, while heating or processing may convert them to pro-inflammatory trans fats, although little research has been carried out in this area with respect to bone or ectopic calcification. Fatty acids also metabolise to prostaglandins and cytokines, with ω6 fats generating pro-inflammatory cytokines via prostaglandin E2 and ω3 fats generating less inflammatory or anti-inflammatory cytokines via prostaglandin E3.
Conclusion

Ectopic calcification is often associated with osteoporosis and bone fracture, probably through the common mechanism of chronic inflammation and oxidative stress. Studies on fat intake and ectopic calcification are fewer than for bone but most animal studies show that a high total or saturated fat diet promotes ectopic calcification and lower BMD, while PUFAs, particularly fish oil and GLA, can decrease ectopic calcification and improve bone parameters. Human studies, however, show mixed results for total and saturated fats and PUFAs, although very high levels of any fat appears detrimental to both. Where there is a clear effect, the particular fat has the same beneficial or harmful effect on tissues and bone. Some of the reason for the lack of clarity of results may be that most studies only look for a linear relationship, whereas fatty acid intake may show a U-shaped dose/response curve. Although the ω6:ω3 ratio was not assessed in ectopic calcification, the highest bone formation rate occurred with a ratio of below 5:1. There may also be an interaction between types of PUFA, with fish oil plus high LA being detrimental in bone but fish oil plus high AA being beneficial. High PUFA intake also lowered BMD if calcium intake was low. While both ω6 and ω3 fats are necessary for bone, ω3 fats are generally more effective, with DHA being the active component of fish oil for bone health, while EPA appears more beneficial in arteries. Similarly, oily fish intake protects against CAC and bone loss and fracture. A high intake of total and saturated fats was also positively correlated with cIMT, another measure of subclinical atherosclerosis, while fish and fish oil were inversely associated; the association may be stronger for cIMT and soft plaque scores than for CAC.

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