Moderate alcohol consumption and increased bone mineral density: potential ethanol and non-ethanol mechanisms

R. Jugdaohsingh1, M. A. O’Connell2, S. Sripanyakorn1 and J. J. Powell*2
1Gastrointestinal Laboratory, The Rayne Institute, St Thomas’ Hospital, London SE1 7EH, UK
2MRC Human Nutrition Research, Elsie Widdowson Laboratory, Fulbourn Road, Cambridge CB1 9NL, UK

Mounting epidemiological evidence indicates an association between the moderate ingestion of alcoholic beverages and higher bone mineral density (v. abstainers). More limited findings provide some evidence for translation of this association into reduced fracture risk, but further studies are required. Here, these data are reviewed and caveats in their assimilation, comparison and interpretation as well as in the use and application of bone health indices are discussed. Whilst it is concluded that evidence is now strong for the moderate alcohol–bone health association, at least in relation to bone mineral density, mechanisms are less clear. Both ethanol and non-ethanol components have been implicated as factors that positively affect bone health in the light of moderate consumption of alcoholic beverages, and four particular areas are discussed. First, recent findings suggest that moderate ethanol consumption acutely inhibits bone resorption, in a non-parathyroid hormone- and non-calcitonin-dependent fashion, which can only partly be attributed to an energy effect. Second, critical review of the literature does not support a role for moderate ethanol consumption affecting oestrogen status and leading to a knock-on effect on bone. Third, Si is present at high levels in certain alcoholic beverages, especially beer, and may have a measurable role in promoting bone formation. Fourth, a large body of work indicates that phytochemicals (e.g. polyphenols) from alcoholic beverages could influence bone health, but human data are lacking. With further work it is hoped to be able to model epidemiological observations and provide a clear pathway between the magnitude of association and the relative contribution of these mechanisms for the major classes of alcoholic beverage.

Bone health: Moderate alcohol intake: Mechanisms: Silicon: Phytochemicals

It has long been known that excessive alcohol consumption has a negative impact on bone health and that chronic abusive consumption, as with many organs, has catastrophic effects. However, relatively recent epidemiological evidence has suggested that the moderate consumption of alcoholic beverages may have beneficial effects on bone, especially in post-menopausal women. The present review attempts to summarise the current evidence and discusses potential mechanisms (both ethanol-related and non-ethanol-related), but begins by considering the caveats in assimilation of such data.

First, for multiple reasons, the definition of moderate alcohol consumption is not at all clear. Indeed, there are differing guidelines on acceptable intakes of alcoholic beverages between nations (Table 1; International Center for Alcohol Policies, 2003). For example, in the USA guidelines for daily intake indicate ‘up to one standard drink for women and up to two for men’ while in the UK it is ‘up to two to three units for women and up to four for men’. In certain countries it is ≥ 40 g alcohol/d (i.e. up to a half bottle of wine or five UK units). These variable guidelines illustrate the second problem, i.e. the consumer’s ability to identify parity between g alcohol, standard drinks, units etc. The term ‘unit’, as used in the UK, is especially old-fashioned. It is equivalent to 8 g alcohol (i.e. ethanol), which, for example, equates to 125 ml wine with 9% (v/v) ethanol. Few such wines are now drunk in most of the world (with Germany a notable exception), and one

Abbreviations: BMD, bone mineral density; C-terminal, carboxy-terminal; CTx, C-terminal cross-linked telopeptide of type I collagen; DXA, dual-energy X-ray absorptiometry; EGCG, epigallocatechin gallate; ER, oestrogen receptors; HRT, hormone-replacement therapy; N-terminal, amino-terminal; OR, odds ratio; Pn, prenylnaringenin; PTH, parathyroid hormone; RANK, receptor activator of NF-κB; XH, xanthohumol.

*Corresponding author: Professor Jonathan J. Powell, fax +44 1223 437515, email Jonathan.Powell@mrc-hnr.cam.ac.uk
Table 1. International guidelines on acceptable intakes of alcoholic beverages (from International Center for Alcohol Policies, 2003)

<table>
<thead>
<tr>
<th>Country</th>
<th>Standard drink (g ethanol)</th>
<th>Daily consumption guidelines (g)</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Australia</td>
<td>10</td>
<td>≤20</td>
<td>National Health Medical Research Council</td>
</tr>
<tr>
<td>Austria</td>
<td>10</td>
<td>24</td>
<td>Federal Ministry for Labour, Health and Social Affairs</td>
</tr>
<tr>
<td>Canada</td>
<td>13-6</td>
<td>≤27.2</td>
<td>Centre for Addiction and Mental Health, Addiction Research Foundation</td>
</tr>
<tr>
<td>Czech Republic</td>
<td></td>
<td>24</td>
<td>National Institute of Public Health</td>
</tr>
<tr>
<td>Denmark</td>
<td>12</td>
<td>36</td>
<td>National Board of Health</td>
</tr>
<tr>
<td>Finland</td>
<td>11</td>
<td>≤23.6</td>
<td>Oy Alko AB</td>
</tr>
<tr>
<td>France</td>
<td>8–12*</td>
<td>≤20</td>
<td>Ministry of Health, Family and Persons with Disability</td>
</tr>
<tr>
<td>Republic of Ireland</td>
<td>8</td>
<td>24</td>
<td>National Academy of Medicine</td>
</tr>
<tr>
<td>Italy</td>
<td>12</td>
<td>≤24–36</td>
<td>Ministry for Agriculture and Forestry, National Institute for Food and Nutrition</td>
</tr>
<tr>
<td>The Netherlands</td>
<td>9-9</td>
<td>≤39</td>
<td>Stichting Verantwoord Alcoholgebruik</td>
</tr>
<tr>
<td>New Zealand</td>
<td>10</td>
<td>≤30</td>
<td>Alcohol Liquor Advisory Council</td>
</tr>
<tr>
<td>Romania</td>
<td></td>
<td>≤32.5 g for beer and ≤20-7g for wine</td>
<td>Ministry of Health</td>
</tr>
<tr>
<td>South Africa</td>
<td>12</td>
<td>≤36</td>
<td>South African National Council on Alcoholism and Drug Dependence</td>
</tr>
<tr>
<td>Spain</td>
<td>10</td>
<td>≤30</td>
<td>Ministry of Health, Spanish Institute for the Investigation of Beverage Alcohol</td>
</tr>
<tr>
<td>Sweden</td>
<td></td>
<td>≤20</td>
<td>Basque Country: Department of Health and Social Security</td>
</tr>
<tr>
<td>Switzerland</td>
<td>10–12</td>
<td>≤24</td>
<td>Swedish Research Council</td>
</tr>
<tr>
<td>UK</td>
<td>8</td>
<td>≤24–32</td>
<td>Catalonia: Central Authority</td>
</tr>
<tr>
<td>USA</td>
<td>14</td>
<td>≤28</td>
<td>Swiss Institute for the Prevention of Alcohol and Drugs Problems</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Department of Agriculture, Department of Health and Human Services</td>
</tr>
</tbody>
</table>

*For wine 8 g, for beer 12 g.

glass of wine in the UK is often 150–250 ml. Thus, today, a single glass of wine at 250 ml and 13% (v/v) alcohol contains more than three units alcohol. This disparity, in turn, leads on to the third problem, i.e. that the methodology for assessing alcohol intake is still evolving (Dawson, 2003). The metabolism and action of ethanol depends on how rapidly it is consumed and whether it is in the presence or absence of food, amongst other likely variables, and there are limited tools for the measurements of these interactions in free-living subjects. Moreover, as noted earlier, serving size and alcohol levels of wine, some beers and especially mixed drinks with spirits (liquor) can vary greatly, potentially leading to poor recognition by social drinkers of how much ethanol may be contained in a drink. Additionally, for obvious reasons, recall and diary-based methodologies may be limited in assessing alcohol consumption. Although this problem should manifest mostly with binge drinking and chronic abusive consumption rather than with moderate consumption, data collection still requires carefully-considered methodology and should be fit-for-purpose in relation to any associated outcome measure, such as health effects (Dawson, 2003). Unfortunately, many studies have simply relied on assessment of alcohol intake as a poorly-validated "bolt-on" to more established dietary assessment methods. A fourth problem in defining moderate consumption is that ethanol-related health effects (both positive and negative) vary between populations (men v. women, premenopausal v. post-menopausal women etc.); indeed, inter-population variation is commonly observed for the health effects of nutrients per se but is especially pronounced for ethanol. Genetic background greatly influences the metabolism of alcohol, while different tissues have differing sensitivities to ethanol; thus, female cancers have been associated with low levels of alcohol intake whereas cardiovascular protection in older men has been observed even with relatively-high levels of alcohol intake (da Luz & Coimbra, 2004). In the following review, almost all the work refers to alcohol intakes of ≤40 g per capita per d (or, in one study, for the purpose of mechanistic discussion, <0.7 g/kg).

Second, the evidence for an association between moderate alcohol intake and positive effects on bone health has mainly been observed in post-menopausal women. This situation probably reflects the fact that population-based studies of bone health predominate in this group rather than an indication of some special alcohol–bone mineral density (BMD) interaction. Nonetheless, caution should
be exercised when extrapolating findings to men or premenopausal women for many of the reasons outlined earlier and in some of the following text.

Third, much of the methodology that has been used in published studies can be criticised. Numbers are often low, methodology for data collection (see earlier discussion) and/or analysis (see following discussion) is often poor and, in some studies, alcohol has been investigated as part of a great number of nutrients, meaning that a priori hypotheses have not been tested. Methodology is generally incomparable between studies and a common problem has been the use of simple linear regression models to test observations between alcohol intake and BMD. This latter point is important because most studies investigating alcohol intake and health have recognised that a ‘J’-shaped curve tends to be seen such that moderate ingestion offers maximum protection, but increased intake beyond this level shows increased risks (de Lorimier, 2000; da Luz & Coimbra, 2004). For this reason categorical data can be more useful when looking at the detailed association between alcohol intake and health (including bone health), while linear models are really only useful for an initial global overview, especially when studying potential co-variate interactions (Jugdaohsingh et al. 2004). Finally, patterns of alcohol intake are likely to associate strongly with other lifestyle factors, and although many of these factors can be incorporated into epidemiological models, it is unlikely that they can all be incorporated, which adds to the ‘statistical noise’.

In spite of these caveats some relatively-consistent findings have emerged in recent years, which will be summarised together with some speculation on mechanisms, but first an overview of the indicators of bone health will be provided.

**Bone mineral content and density**

Bone mineral content, the amount of mineral in a defined section of bone, can be measured to high precision (≈94%) by a number of techniques; chiefly, X-ray absorptiometry, single-energy X-ray absorptiometry, dual-energy X-ray absorptiometry (DXA), quantitative computed tomography, peripheral quantitative computed tomography and quantitative ultrasound (Kleerekoper & Nelson, 1997; Diez, 2002; Elliott & Binkley, 2004). For this reason categorical data can be provided more accurately in individuals (Cefalu, 2004) but, at the population level, and relevant to this discussion, it remains an extremely important proxy for long-term bone health.

Bone fracture occurs when the stress put on the tissue exceeds the strength of the material. However, bone is a complex composite substance and its strength is described by a number of structural and material properties that determine its quality (Felsenberg & Boonen, 2005). Its structural properties are described by its geometry (i.e. size and shape) and micro-architecture (trabecular interconnection, spacing and thickness, and cortical thickness and porosity; Paschalis et al. 2004; Felsenberg & Boonen, 2005). The material properties are described by the mineral and collagen compositions (extent of mineralisation, crystal size, collagen type and cross-links), the number, size and location of micro-damage and the rate of bone turnover (Felsenberg & Boonen, 2005). DXA measures the extent of mineralisation, which is only one of the components that describe bone quality and strength (Marx, 2004; Felsenberg & Boonen, 2005; Srivastava et al. 2005).

More recent techniques such as quantitative computed tomography, peripheral quantitative computed tomography and three-dimensional magnetic resonance micro-imaging provide more detailed information on the structural
properties and thus bone quality (Malavolta et al. 2004; Felsenberg & Boonen, 2005). They can also distinguish
between cortical (less metabolically active) and trabecular (more metabolically active) bone, provide information
on micro-architecture and give a true volumetric BMD
measurement (i.e. g/cm\(^3\)). However, such methods are not
yet as widely available as DXA in most clinical settings and,
apart from quantitative ultrasound, there is an increase
in the radiation exposure dose (50–60 \(\mu\)Sv with quantita-
tive computed tomography compared with 0.1–1 \(\mu\)Sv with
DXA; Elliott & Binkley, 2004). Thus, currently, DXA
remains the ‘gold standard’ for diagnosis and treatment
monitoring of diseases of bone fragility, but its limitations
must be borne in mind (Bessho, 2003; Fukunaga, 2003;
Khosla, 2003; E Seeman, unpublished results). Factors that
can increase the risk of fracture independently of BMD
also need to be considered (age, body weight, height, BMI,
free oestradiol index (or sex hormone-binding protein
levels), grip strength, lifestyle factors (history of falling,
smoking, alcohol consumption, physical activity, dietary
intake), medical history of fracture, family history of
osteoporosis and use of medication (e.g. steroids, anti-
convulsive drugs, sedatives)), since these factors will
enhance the predictive power of fracture risk (Albrand
et al. 2003; Melton et al. 2003; Felsenberg & Boonen,
2005; Johnell et al. 2005; Miller et al. 2005; Robbins et al.
2005). In addition, such factors should also be considered
in epidemiological models investigating associations with
fracture risk.

Biochemical markers of bone turnover
Bone is a living tissue that constantly undergoes renewal
and repair (or remodelling). The cells responsible for this
process are the bone-forming osteoblasts and the bone-
resorbing osteoclasts. In the adult skeleton the activities
of these cells are normally coupled such that no marked net
increase or loss of bone mass occurs (an average small loss
of 0.4%/year occurs; Srivastava et al. 2005). This coupling
of activities also preserves the bone architecture (Teti,
2005). However, with bone diseases such as osteoporosis
(e.g. as a result of oestrogen deficiency), bone turnover–
remodelling is markedly increased and the activities of
these cells are no longer coupled. Osteoclast activity
dominates, as a result of an increase in osteoclast numbers
over osteoblasts (Manolagas et al. 2002), and a marked net
increase in bone loss occurs (>1–2%/year; Srivastava et al.
2005). Osteoblast activity is also raised as it tries, but fails,
to counteract or compete with the increased bone loss.
Bone formed by these cells may thus be less mineralised
and prone to fracture. This high rate of bone turnover is
 correlated with bone loss in osteoporosis and can explain
50% of the variance in bone mass (Ebeling & Akesson,
2001). Osteoporosis medications, such as bisphosphonates,
work by reducing osteoclast activity and bone loss, thereby
decreasing the rate of bone turnover. Measurement of
the rate of bone turnover is therefore important and the
most practical method for monitoring the efficacy of
osteoporosis therapy and the management of individuals
with bone fragility (Srivastava et al. 2005).

Importantly, the activities of the bone remodelling cells
can be measured, since they release biochemical markers in
blood and urine. Higher levels of these bone markers corre-
late with a higher rate of bone remodelling (Srivastava et al.
2005) and their assessment can be useful in supporting bone
mineral status measurements of bone health.

Bone formation markers
Osteoblasts produce type I collagen as a pro-molecule
(procollagen), which, on the formation of collagen matrix,
releases the carboxy-terminal (C-terminal) and amino-
terminal (N-terminal) extension peptides (Ebeling &
Akesson, 2001). These extension peptides (procollagen
type I C-terminal peptide or procollagen type I N-terminal
peptide) are released into the blood and can be measured
by commercially-available immunoassays, and their levels
are directly proportional to type I collagen synthesis and,
theoretically therefore, bone formation. Type I collagen is
also produced by fibroblasts in other connective tissues
(e.g. skin), but the majority of the extension peptide de-
tected in blood is derived from bone because of the higher
rates and mass of bone turnover compared with other
connective tissues. Measurement of procollagen type I
N-terminal peptide is generally preferred over procollagen
type I C-terminal peptide because of the lack of sensitivity
of the latter in detecting subtle changes in bone turnover
(Ebeling & Akesson, 2001). However, some of the pro-
collagen type I N-terminal peptide released may become
incorporated into bone and then later released during bone
breakdown, and so a proportion of the amount measured
may represent bone resorption (Ebeling & Akesson, 2001).

Following collagen synthesis, osteoblasts undergo cel-

ular differentiation into mature osteoblast cells that ex-
press high alkaline phosphatase activity and secrete
osteocalcin during osteoid formation and mineralisation,
both of which, again, can be measured by commercially-
available immunoassays. Bone-specific alkaline phospha-
tase is reported to show the highest specificity to bone,
compared with the other formation markers, even though
marked cross-reactivity (≤10%) with liver isoenzymes
can occur (Ebeling & Akesson, 2001). Osteocalcin readily
undergoes degradation and so the intact molecule and its
fragments are present in blood, of which the N-MID frag-

ment (so termed because it contains both the N-terminal
and the middle portion of the protein) is the largest (forty-
three or forty-four amino acids). Assays that measure both
the intact and N-MID fragment have been suggested to be
more robust and sensitive than those that measure the
intact molecule alone (Ebeling & Akesson, 2001). Addition-
ally, osteocalcin exists as mature (carboxylated) and
immature (undercarboxylated) forms. The ratio of these
two forms, or the extent of carboxylation, may influence
mineralisation and has been used in the assessment of
fracture risk (see later (p. 297); Ebeling & Akesson, 2001).

These three markers, i.e. the procollagen type I
C-terminal and N-terminal peptides, bone-specific alkaline
phosphatase and osteocalcin, are known as early, mid and
late biochemical markers of bone formation respectively,
and are used as surrogate markers to determine the rate of
bone formation.
Bone resorption markers

Osteoclasts secrete enzymes that break down bone, leading to release into blood and urine of Ca, phosphate and degradation fragments of the type I collagen matrix such as pyridinoline, deoxypyridinoline, C-terminal telopeptides (cross-linked telopeptide of type I collagen (CTx) and cross-linked C-terminal telopeptide of type I collagen), N-terminal telopeptide and helical peptides (fragments of the helical region of the type I collagen α chain; Ebeling & Akesson, 2001). These fragments vary in size (helical peptide>cross-linked C-terminal telopeptide of type I collagen>CTx approximately the same as N-terminal telopeptide>pyridinoline approximately the same as deoxy-pyridinoline), type of cross-link (pyridinoline v. deoxypyridinoline) and the presence (cross-linked C-terminal telopeptide of type I collagen and helical peptides) and absence of the helical peptide chain. Cross-linked C-terminal telopeptide of type I collagen and CTx are suggested to respond to different clinical situations and treatments (Garnero et al. 2003a). The former may be more important in pathological situations such as metastatic bone diseases and multiple myeloma (Garnero et al. 2003a). Osteoclasts release acid phosphatases, and tartrate-resistant acid phosphatase 5b is a specific and direct marker of osteoclast activity (being released by active or activated osteoclasts) and its levels correlate with those of other bone-turnover markers and BMD (Gerdhem et al. 2004). Intact parathyroid hormone (PTH) levels have also been used as an indirect marker of bone resorption. However, the preferred markers of resorption are CTx and N-terminal telopeptide in serum or plasma and pyridinoline and deoxypyridinoline in urine. N-terminal telopeptide is preferred over CTx, since it is more specific to bone (Ebeling & Akesson, 2001). CTx may be derived from other connective tissues, although it has proved to be a useful marker of changes to bone resorption in well-controlled acute-dosing studies (e.g. following ingestion of Ca; Kamel et al. 1998; Guillemant et al. 2000, 2003). Tritiated tetracycline has been used in animal studies to measure the rate of bone resorption (Muhlbauer & Fleisch, 1990; Muhlbauer et al. 2003) but the authors are not aware of its similar use in man.

When monitoring therapy, serum and urine levels of bone-turnover markers are measured in the fasting state, because of the influence of food intake, and at the same time of day, because of diurnal variation (usually in the morning when diurnal variation is lowest; Gerdhem et al. 2004; Roux et al. 2005). Bone-resorption markers in serum show greater diurnal variation than bone-formation markers in serum or either types of marker in urine (Ebeling & Akesson, 2001; Gerdhem et al. 2004; Roux et al. 2005). Changes in levels (20–70%) following the commencement of anti-resorptive treatment are usually seen within a few months (3 months for bone-resorption markers, but not before 6 months for bone-formation markers) compared with the minimum of 1–2 years to see a small change (1–2%) in BMD (Sarkar et al. 2004; Cummings et al. 2005; Roux et al. 2005; Srivastava et al. 2005). Thus, bone markers allow the monitoring of treatment and are used as early indicators of treatment efficacy (e.g. to identify responders and non-responders). Bone markers are also used in the management of patients, since ongoing monitoring has recently been suggested to improve compliance with treatment (Ebeling & Akesson, 2001; Srivastava et al. 2005).

Bone markers, however, show high inter- and intra-individual variation (5–30% for serum and 10–50% for urinary markers) and are sensitive to factors that affect bone turnover such as sex hormone levels, diet, season, time of day and renal and hepatic functions (Woitge et al. 1998; Rapuri et al. 2002; Garnero et al. 2003b; Elliott & Binkley, 2004; Felsenberg & Boonen, 2005; Miller et al. 2005; Roux et al. 2005; Srivastava et al. 2005). Thus, their measurement cannot be used in the diagnosis of osteoporosis or other diseases of bone fragility, but only in monitoring the response to treatment or in population-based studies. Nonetheless, Garnero et al. (2003b) have recently shown that CTx and osteocalcin levels can be used to classify subjects as having high or low bone turnover over a 4-year period, with <10% being misclassified.

The organic matrix, more specifically type I collagen (which constitutes 90% of the bone organic matrix), is an important component of bone quality as it contributes to the micro-architecture and mechanical properties (i.e. flexibility and toughness) of bone (Ebeling & Akesson, 2001; Felsenberg & Boonen, 2005). A 35% decrease in the strength of the collagen network and 50% decrease in its toughness are associated with an age-related decline in bone strength (Burr, 2002). A decrease in overall collagen content is reported in osteoporotic bones, as is an abnormal value for reducible collagen cross-links: non-reducible collagen cross-links in bones of subjects prone to fractures (even in some subjects with normal BMD), suggesting that an abnormal bone collagen quality contributes to bone fragility (Banse et al. 2002; Paschalis et al. 2004). Thus, bone markers, particularly the resorption marker CTx, but also osteocalcin, which up to now have been used to monitor bone turnover or bone cell activity during therapy, have also been found to be useful in predicting fracture risk (Eastell et al. 2003; Khosla, 2003; P Delmas, unpublished results). Increased levels of bone-resorption markers predict hip, vertebral and non-vertebral fractures independently of BMD (Garnero et al. 2003b). Similarly, the reduction in vertebral fracture over 4 years is correlated with the 1-year decrease in bone turnover (Roux et al. 2005). At 6 months following commencement of bisphosphonate treatment, changes in urinary CTx and N-terminal telopeptide can explain 50–70% of the reduction in fracture risk (Roux et al. 2005). Likewise, osteocalcin levels at 2 years can account for 34% of the reduction in total vertebral risk fracture, greater than can be predicted from BMD measurement (Sarkar et al. 2004). Under-carboxylated osteocalcin is associated with increased risk of low BMD and increased risk of hip fracture (relative risk 5.3; Ebeling & Akesson, 2001).

Thus, together bone-turnover markers and BMD greatly improve fracture-risk prediction as compared with their individual use (Eastell et al. 2003; Garnero & Delmas, 2004; P Delmas, unpublished results). This finding is not surprising as BMD and bone markers are two separate and, at least partially, independent measures of bone quality (Felsenberg & Boonen, 2005) that may be independently
affected by therapy. Indeed, bone-turnover markers are only weakly correlated with BMD (Miller et al. 2005).

Although many of the studies on moderate alcohol intake and bone health that are outlined later have referred to measures of bone health assessed by BMD, some of them have also looked at bone markers. Together these assessments allow better conclusions to be drawn on the true association between alcohol intake and bone health at the population level.

Moderate ingestion of alcoholic beverages and bone health

Reports showing a beneficial effect of moderate alcohol intake on bone health are increasing. A small cross-sectional study conducted in the early 1990s by Laitinen et al. (1991) has shown a positive correlation between alcohol intake and BMD in post-menopausal women at the lumbar spine (r 0.44, P<0.01) and Ward’s triangle (an area of the hip; r 0.34, P<0.05). Post-menopausal women who drink alcohol (approximately 11–77 g/week) were found to have a higher BMD (g/m²) at the lumbar spine and Ward’s triangle compared with non-drinkers; i.e. 1.094 (SD 0.080) v. 0.978 (SD 0.133) (P<0.001) and 0.793 (SD 0.164) v. 0.690 (SD 0.138) (P<0.05) respectively. A subsequent study, which made adjustment for confounding factors such as age, BMI, smoking, regular exercise and use of osteoestrogen supplements in women, has also shown an alcohol-related increase in femoral neck BMD in men (P<0.01) and lumbar spine BMD in women (P<0.01) aged ≥45 years (Hollbrook & Barrett-Connor, 1993). In addition, May et al. (1995) have confirmed that older men (aged 64–76 years) who consume alcohol have a higher trochanter (site of the femur that is rich in trabecular bone) BMD than non-drinkers after adjusting for age, weight, smoking, caffeine intake and physical activity. This positive effect of alcohol is supported by a population-based epidemiological study by Kroger et al. (1994), which has reported that moderate intake of alcohol significantly protects against the reduction in lumbar spine BMD peri-menopausally (odds ratio (OR) 0.6, P<0.05). Similarly, Smeets-Goevaers et al. (1998) have also found a protective effect of current alcohol intake (T score <-1 SD) in peri-menopausal women with low bone mass (OR 0.71 (95% CI 0.61, 0.83)).

A bigger study by Felson et al. (1995) has investigated the association between BMD and average alcohol intake over the previous 20 years in 1154 men and women (aged between 68 and 96 years) in the Framingham Cohort Study. Their results show that women with an average intake of ≥199 g alcohol/week (29 g/d) have a higher BMD at the ultra-radius, proximal femur and lumbar spine, with the mean differences ranging between 4.2 and 13% compared with those consuming less than this amount after adjusting for age, weight, height, smoking and, in women, age at menopause and number of years of oestrogen use. The same pattern is also observed after additionally adjusting for caffeine intake, physical activity and vitamin D levels. Similarly, a prospective study from the Nurses’ Health Study (Feskantich et al. 1999) has reported that Caucasian post-menopausal women consuming ≥75 g alcohol/week (11 g/d) have a significantly greater lumbar spine BMD compared with abstainers (P=0.001), accounting for a 10–12% difference in BMD at this site. This finding is not related to the use of hormone-replacement therapy (HRT; being 1.074 v. 0.965 g/cm² (P=0.02) for current users of HRT and 0.894 v. 0.771 g/cm² (P=0.02) for non-users of HRT). A similar pattern was also found after additionally adjusting for physical activity, daily intake of Ca, vitamin D, protein and caffeine. As discussed later (p. 298) these data may provide important clues in delineating the true mechanism of action of moderate alcohol consumption on bone health.

In a cross-sectional study of 455 elderly women (aged 65–77 years) who were recruited for a multi-centre osteoporosis study, Rapuri et al. (2000) have reported a significantly higher BMD at the lumbar spine (7–16%), mid-radius (8–14%) and total body (5–12%) in alcohol drinkers (consuming ≥28.6 g alcohol/week or 4.1 g/d) compared with non-drinkers after adjusting for height, weight, smoking, oestrogen use and other significant covariates. The most marked difference in BMD was found in moderate drinkers consuming ≥28.6–≤57.2 g alcohol/week. This finding is consistent with the ‘U’ or ‘J’-shaped curve associated with health outcome and alcohol consumption, where the positive effect of alcohol on health is seen over a short consumption range (moderate intake; one to four servings per d) and the detrimental (or negative) effects with higher consumption (de Lorimier, 2000). Importantly, bone-marker analysis also undertaken in this study suggests that the higher BMD in moderate alcohol drinkers is chiefly associated with a reduction in the bone remodelling effects of alcohol. It should be noted that the apparent discrepancy between studies in absolute intakes of alcohol that are associated with positive effects on bone health are most likely a result of differing methodologies, as detailed earlier.

A larger French epidemiological study also published in 2000 (Ganry et al. 2000) has investigated the effect of alcohol on BMD after adjusting for potential confounders in 7598 woman aged ≥75 years who were participants in the Épidemiologie de L’Osteoporose study. The results show a positive effect of moderate alcohol intake on trochanter (P=0.03) and total body (P=0.05) BMD after adjustment for age and weight. Lumbar-spine BMD was not reported in this work. Interestingly, the significance of the effect was found to be particularly notable when using the Z score (P=0.0004 for trochanter Z score and P=0.04 for total body Z score when adjusting for oestrogen-replacement therapy, current smoking status, usual physical activity, dietary Ca, educational attainment, family monthly income and general health status). More rigorous adjustment for multiple comparisons shows that only moderate alcohol consumption (i.e. 11–29 g/d) is associated with a higher adjusted Z score BMD at the trochanter, which suggests a protective effect of moderate alcohol intake on trabecular bone loss. A study (Ilich et al. 2002) in 136 Caucasian post-menopausal women with a mean age of 68.6 years has made adjustment for even more potentially-confounding factors, including hormone status (serum PTH, vitamin D), body composition, age, dietary Ca intake, past smoking, caffeine and past physical activity. Again, for moderate alcohol drinkers (approximately...
8 g alcohol/d on this occasion) an association with higher lumbar spine (P<0.001) and total body BMD (P = 0.04) was still found. A recent small but tightly-controlled study (Williams et al. 2004) that used forty-two female monzygotic twin pairs who were classified as discordant for alcohol intakes again confirms a positive effect of moderate alcohol ingestion on adjusted BMD. Such studies are important as they control for genetic influences and allow the effect of independent environmental factors, in this case moderate alcohol ingestion, to be observed. In this study a positive association between alcohol intake and adjusted BMD was again found at the total spine and total hip sites (P ≤ 0.02) but no interaction was noted with either bone-formation markers (osteocalcin and alkaline phosphatase) or bone-resorption markers (urinary CTx). This result is in contrast to the findings of Rapuri et al. (2000) but, given the inter- and intra-individual variance in bone markers, it may simply be a result of low study numbers in the former or, possibly, an uncontrolled-for genetic influence in the latter.

A current longitudinal study is investigating the associations between nutrient intakes and bone loss during the menopausal transition in 891 subjects who took part in the Aberdeen Prospective Osteoporosis Screening Study. Using relatively robust energy-adjusted dietary intake measures (Macdonald et al. 2004), surprisingly, only moderate alcohol intake, but not other nutrient intakes, has been found to be a significant predictor of changes in lumbar spine BMD when adjusting for other important confounders (P = 0.0002). These findings contrast with those of Bainbridge et al. (2004), who have reported higher femoral neck BMD in moderate alcohol drinkers aged 24–44 years (P = 0.0002) compared with abstainers but no effect at the lumbar spine. As noted earlier, this disparity highlights the need to be conservative in extrapolating findings from one population (in this case perimenopausal women) to another (in this case premenopausal women) and from different skeletal sites.

Although most studies have shown a positive effect of moderate alcohol intake on bone health in the elderly and in peri-menopausal and post-menopausal women, a few studies have reported no effect in these groups (Bauer et al. 1999; Teegarden et al. 1999; Tucker et al. 1999; Macdonald et al. 2004). The most important question, therefore, is whether such an association with alcohol carries through to a reduced risk of osteoporotic fracture.

Cumming & Klineberg (1994) have reported that alcohol intake is not associated with the risk of hip fracture in men and women aged ≥65 years, and similar findings have been reported by Huang et al. (1996) using prospective data from the National Health and Nutrition Examination Survey I that included 2513 caucasian women aged ≥45 years. However, the Dubbo Osteoporosis Epidemiology Study (Nguyen et al. 1996) in Australia has shown a lower risk of atraumatic fractures in 820 elderly men aged ≥60 years who drank alcohol in moderation (OR 0.70 (95% CI 0.66, 0.81)). Multiple regression analysis has suggested that the protective effect of moderate alcohol intake on the risk of fracture is mediated through its effect on BMD, as the relative risk is attenuated after adjusting for BMD. Subsequently, however, analysis of longitudinal studies in Copenhagen (Denmark) that included 17868 men and 13917 women (Hoidrup et al. 1999) has shown that moderate alcohol consumption of less than fourteen drinks per week in women and less than twenty-eight drinks per week in men is not associated with hip fracture compared with abstainers after adjusting for age, study of origin, smoking, physical activity, BMI and school education. There is some suggestion that those who prefer wine tend to have a lower risk of hip fracture (OR 0.77), although a more comprehensive analysis of potential confounders would be required to reach this conclusion. Similarly, Huopio et al. (2000) have reported no association between alcohol intake and the development of any particular type of osteoporotic fracture. However, a large population-based case-control study in Sweden, with a high rate of osteoporotic fracture, has reported an inverse association between moderate alcohol intake and risk of hip fracture in post-menopausal women (Baron et al. 2001). Subjects who drink alcohol in moderation were found to have a 20% lower age-adjusted risk of hip fracture (OR 0.80 (95% CI 0.69, 0.93)), which was shown to be even lower (OR 0.70 (95% CI 0.60, 0.82)) after adjusting for other confounders (i.e. age, HRT, BMI and cigarette smoking). Interestingly, after classifying the types of alcoholic beverage, beer (4.5% alcohol) was found to provide the lowest risk of hip fracture after adjusting for confounders (OR 0.52) followed by wine (OR 0.67). These findings contrast with those of Hoidrup et al. (1999), who have suggested that a higher risk of hip fracture may occur in beer drinkers, perhaps because some heavy drinkers were included in this group. Kanis et al. (2005) have examined the risk of fracture in 16 971 men and women from three prospective cohorts (The Canadian Multi-center Osteoporosis Study, The Dubbo Osteoporosis Epidemiology Study and The Rotterdam Study) and have found that
the risk of osteoporotic fracture is not significantly increased in those who drink no more than two units/day. Excessive alcohol consumption or alcohol abuse is associated with increased risk of morbidity and mortality, including liver disease, CVD, low bone density, hypertension and breast cancer (de Lorimier, 2000). There is also an increased risk of fractures due to increased risk of falls with alcohol consumption. Thus, unlike with excessive consumption of alcoholic beverages, there is no evidence that moderate consumption of alcohol leads to an increased risk of fracture. There are some limited data suggesting a decreased risk of fracture but, predominantly, the data suggest no overall risk. Nonetheless, the rather limited number of moderate-alcohol-focussed studies, coupled with the potential for confounding factors, means that further work is still required in delineating the association between fracture risk and qualitative or quantitative aspects of moderate alcohol intake. Clearly, it is important to determine whether the generally positive association that is observed for moderate alcohol ingestion and BMD (especially in older individuals) is reflected in a reduced risk of fracture and how this reduction relates to total ethanol intake and to the different types of alcoholic beverage.

**Potential mechanisms**

A number of mechanisms have been proposed for the positive association between moderate alcohol consumption and BMD. Generally, it is considered that alcohol inhibits bone turnover (Rapuri et al. 2000; Turner et al. 2001), and the stimulation of calcitonin production (Rico, 1990) and/or a reduction in serum PTH (Laipinen et al. 1992; Rapuri et al. 2000; Ilich et al. 2002) have been proposed. However, studies on the effects of acute alcohol ingestion on circulating PTH levels in healthy volunteers have used 35–90 g ethanol in a short period of time, which is not generally in line with moderate consumption (Laipinen et al. 1992; Garcia-Sanchez et al. 1995). Similar concerns can be voiced over work showing ethanol-induced changes to calcitonin concentrations. In fact, in preliminary volunteer studies (Powell et al. 2005b) it has recently been shown that serum PTH and calcitonin levels are not significantly changed following moderate ethanol ingestion. Serum levels of intact PTH were found to decline following Ca ingestion (positive control) but to remain unchanged compared with normal diurnal variation following ingestion of <28 g ethanol. Similarly, serum calcitonin levels were found to be unchanged in response to ethanol consumption. Nonetheless, it has been possible to show that moderate ethanol ingestion does appear to acutely inhibit bone resorption, based on suppression of circulating CTXs in volunteers (Powell et al. 2005b). Although unrelated to calcitonin and PTH, mechanisms that could, in part, be related to the energy content of ethanol continue to be investigated. Since no other data are currently available on acute mechanisms of ethanol, the remaining text will focus on the alcohol-oestrogen–bone relationship and on the non-ethanol components of alcoholic beverages that could be related to bone health.

**Oestrogenic effects of alcohol: any relevance to bone mineral density?**

Oestrogens play a key role in skeletal protection, as emphasised by bone loss in oestrogen-deficient states such as post-menopausally and following ovariectomy. Cellular mechanisms appear to be mediated through oestrogen receptors (ER) α and β, both of which are expressed in bone, as well as through non-ERα and β pathways (Ioannidis et al. 2002; Shearman et al. 2004). Oestrogenic effects of alcohol are well recognised, and while there is no suggestion that ethanol or its metabolites (e.g. acetaldehyde) have oestrogen-mimetic properties, three other possibilities have been widely considered.

First, ethanol consumption may increase ER activity, especially ERα, in a number of tissues. However, while this effect is well recognised with abusive alcohol consumption (Colantoni et al. 2002), its extension to moderate consumption is not so clear, at least in vivo. Nonetheless, in in vitro studies in human breast cancer cell lines (Fan et al. 2000; Singletary et al. 2001) moderate to high levels of ethanol stimulate ERα activity, which is in part driven by increased ERα expression (Fan et al. 2000; Singletary et al. 2001), but suppression of the breast cancer susceptibility gene BRCA1, which is an inhibitor of ERα activity, also occurs (Fan et al. 2000). Whether these observations can be extended to moderate alcohol intake and effects on bone cells in vivo remain to be determined. Second, tissue exposure to oestrogens in part occurs through aromatisation of androgens to oestrogens (and in normal males and post-menopausal females exclusively through this pathway). A number of cellular and animal studies have indicated that alcohol can promote such aromatisation (Purohit, 2000). Again, however, in vivo studies suggest that only heavy chronic alcohol intake can increase circulating oestrogen in this fashion (Purohit, 2000). This finding is consistent with the observation that alcohol ingestion only notably increases circulating oestradiol in post-menopausal women taking oestrogen-replacement therapy (Purohit, 1998), indicating a direct oestrogenic effect rather than an aromatisation effect. Indeed, it is direct inhibition of oestradiol catabolism that is the third and likely dominant influence of moderate ethanol consumption on oestrogens in vivo. Premenopausal women taking oral contraceptives exhibit alcohol-induced (0.5 g/kg) increases in oestradiol (Sarkola et al. 1999), while the majority of studies indicate that even in the absence of supplemental oestrogens there is a slight but significant effect of moderate alcohol ingestion on circulating oestradiol levels in this population (Muti et al. 1998; Ginsburg, 1999; Martin et al. 1999). The influence of genotype on alcohol metabolism (Coutelle et al. 2004) and the difficulties in capturing a true record of alcoholic beverage intake (Gavalier & Love, 1992) and then being able to relate such factors to oestradiol levels that naturally cycle means that further work is required to delineate the true influence of moderate alcohol ingestion on oestradiol levels in premenopausal women. This approach may help in understanding bone or cardiovascular health, but it is especially important in determining the link between alcohol intake, oestrogens and female cancers (Coutelle...
alcohol intake promotes some oestrogen activity (i.e. anabolic instead of anti-catabolic), then the same data (Feskanich et al. 1999; Ganry et al. 2000) do not support a marked influence on bone because oestrogen-replacement therapy would be expected to overwhelm the ethanol effect, which is clearly not observed. Indeed, any notion that the moderate effect of alcohol on bone is best observed under conditions of low oestradiol status is probably misplaced and, as noted earlier, is more likely to be related to the fact that post-menopausal women and/or the elderly have simply been the best-studied groups for diet–BMD interactions because they are the main targets for assessment and treatment of osteopenia and osteoporosis. Finally, it is possible that alcohol has differential effects on the genotoxic and hormonal pathways of oestrogen, so extrapolation between pro-carcinogenic activity and skeletal or cardiovascular protection may not be valid (Feskanich et al. 1999). Thus, in summary, it seems probable that moderate alcohol consumption can to some extent influence circulating oestrogens, or even androgens (Gavaler et al. 1991; Katsouyanni et al. 1991; Hankinson et al. 1995), but critical review of existing data does not then support a knock-on effect in terms of bone health.

Phenolic and antioxidant aspects of alcoholic beverages

Alcoholic beverages contain a number of phenolic constituents (many of which are also found in some fruit and vegetables) that may impact on bone health. Wine contains several polyphenols that are derived from the grapes or from the wood or yeast during vinification. Although wine, particularly red wine, is often the principal alcoholic beverage discussed in relation to phenolics, beer is also a rich source of polyphenols, several of which are derived from the hops added during the brewing process. Typically, polyphenols are understood to be antioxidants, although some may also have oestrogen-mimetic properties, and their effects have principally been studied in relation to CVD and cancer, but emerging evidence from in vitro studies suggests that they may also affect bone.

Several classes of phenolic compounds have been identified in alcoholic beverages, and they can be broadly classified into different groups (for examples, see Table 2). Resveratrol, a non-flavonoid stilbene, is probably the best-known red wine polyphenol. However, other phenolic compounds are present in higher concentrations, including conjugates of flavonols (rutin, quercitin, myricetin and others), flavanols (catechin and related compounds), anthocyanins (delphinidin and cyanidin), hydroxycinnamic acids (caffeic acid, coumaric acid) and gallic acid (Burns et al. 2001; Zafrilla et al. 2003). White and/or rose wines also contain several of these compounds, although generally at lower concentrations when compared with red wine, with the possible exception of hydroxycinnamic acids (Carando et al. 1999; Dugo et al. 2003; Lugasi & Hovari, 2003; Zafrilla et al. 2003). Concentrations of phenolic compounds vary widely, even within a similar type of wine, because of differences in grape varieties and storage time and different approaches to the vinification process.
Beer contains several classes of phenolic compounds, including flavonols (such as quercetin, kaempferol and myricetin), proanthocyanidins, flavonoids (including catechins) and various phenolic acids (Stevens & Page, 2004; Tedesco et al. 2005; Nardini et al. 2006). Beer is also the most important dietary source of xanthohumol (XH) and its related prenylflavonoids (Stevens & Page, 2004; Gerhauser & Frank, 2005). XH exerts anti-carcinogenic effects in vitro and in vivo. In addition in vitro, it has anti-microbial properties and inhibits the formation of triacylglycerols (reviewed in Gerhauser & Frank, 2005). It is the major prenylflavonoid found in hops, but several others exist, e.g. desmethoxyxanthohumol, humulone and 3’-geranylnaringenine. These compounds are usually converted into their isomeric flavanones, isoXH (the principal dietary prenylflavonoid), prenylnaringenins and geranylflavonoids by thermal isomerisation during the brewing process (Stevens et al. 1999). As with wine, the phenolic content of different beers varies widely, probably depending on the type of beer, source and concentration of hops and the brewing process (Stevens & Page, 2004; Tedesco et al. 2005).

Although little is known about the action of phenolic compounds on the skeleton, recent evidence, mainly from in vitro studies, suggests that they are worth discussing in relation to bone health. Muhlbauer studies, suggests that they are worth discussing in vitro compounds on the skeleton, recent evidence, mainly from

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Several flavonols found in alcoholic beverages have been reported to modulate bone cell activity in vitro, although very little is known about their effects in vivo. In ovariectomised rats rutin supplementation for 3 months prevents the loss of femoral BMD induced by ovariectomy. In addition, following supplementation plasma osteocalcin concentration is higher and urinary excretion of deoxypyridinoline and Ca is lower in the rutin-supplemented group than in the control ovariectomised rats (Horcajada-Molteni et al. 2000). These in vivo effects suggest that either rutin, or its active metabolites quercetin and isorhamnetin, may exert modulatory effects on bone cells. Indeed, results from in vitro studies (Wattel et al. 2003, 2004; Woo et al. 2004;
Rassi et al. (2005) suggest that both rutin and quercetin may directly affect bone cells, particularly osteoclasts. Quercetin has been reported to induce apoptosis in primary rabbit or porcine osteoclasts (Wattel et al. 2003; Rassi et al. 2005). In addition, quercetin (1–10 μM) decreases both osteoclast formation and activity in murine primary macrophage colony-stimulating factor-dependent osteoclast progenitors and in RAW 264.7 macrophages treated with receptor activator of NF-κB (RANK) ligand, as well as human peripheral blood mononuclear cells differentiated with macrophage colony-stimulating factor and RANK ligand (Wattel et al. 2004; Woo et al. 2004). Moreover, Rassi et al. (2005) have recently demonstrated in a porcine bone-marrow model that even low nanomolar concentrations of quercetin or rutin significantly inhibit osteoclast formation and activity similar to 17β oestradiol. Kaempferol (1–6 μM), another flavonol found in alcoholic beverages, also induces apoptosis and inhibits bone resorption activity in primary rabbit osteoclasts even at concentrations of less than 1 μM (Wattel et al. 2003). The action of the flavonols on bone is not limited to effects on osteoclasts. Both kaempferol and quercetin have also been reported to stimulate alkaline phosphatase activity in human MG63 osteoblasts at 50 μM for 48 h this results in a 1.5- and 1.7-fold increase, respectively (Prouillet et al. 2004). In contrast, Yamaguchi & Jie (2001), who found no effects with EGCG or catechin in their bone model, have also reported that quercetin (0.1–100 μM) has no effects on either bone calcification or alkaline phosphatase activity in femoral diaphyseal and metaphyseal tissues of rats in vitro. However, overall, these in vitro studies suggest that some of the flavonols found in wine and beer may exert effects on bone; in vivo studies are required for confirmation.

**Resveratrol**

In animal studies supplementation with resveratrol leads to varying effects on bone. In a recent study (Liu et al. 2005) ovariectomised rats supplemented with 0.7 mg resveratrol per kg body weight for 3 months were found to have a higher epiphysis BMD and femoral bone Ca content compared with ovariectomised controls. Resveratrol supplementation also inhibits bone loss, increases hydroxyproline content and femur bone strength in ovariectomised stroke-prone spontaneously-hypertensive rats compared with ovariectomised controls (Mizutani et al. 2000). In contrast to the findings of these studies, resveratrol has been shown to have no effect on radial bone growth (periosteal bone formation and mineral apposition rates) at the tibia–fibula synostosis in weanling rats (Turner et al. 1999). In vitro rat and chicken osteogenesis models resveratrol has been reported to reverse the effects of arylhydrocarbons, which inhibit osteoblast differentiation and markers of differentiation (including alkaline phosphatase, collagen type 1, osteopontin and bone sialoprotein) and reduce mineralisation (Singh et al. 2000; Andreou et al. 2004). In addition, resveratrol (1–100 nM) increases DNA synthesis, alkaline phosphatase and prolyl hydroxylase activity in murine MC3T3-E1 osteoblasts, suggesting that it stimulates osteoblast proliferation and differentiation (Mizutani et al. 1998). In these cells and in human MG63 cells resveratrol also inhibits prostaglandin E2 production, which decreases alkaline phosphatase activity and, concomitantly, increases acid phosphatase activity and enhances osteoclast differentiation from stem cells (Boyan et al. 2001). Overall, the in vitro studies to date support a role for resveratrol in modulation of bone cell activity.

**Prenylated flavonoids**

Little is known about the effects on bone health of prenylated flavonoids, which are found in beer. 8-Prenylnaringenin (PN) is one of the most abundant prenylflavonoids in beer and has been reported to be a potent phyto-oestrogen in vitro (Stevens & Page, 2004). In a rat model 8-PN has been shown to inhibit ovariectomy-induced loss of BMD in the proximal tibia and suppress urinary excretion of the bone markers hydroxyproline, pyridinoline and deoxypyridinoline (Miyamoto et al. 1998). 8-PN increases alkaline phosphatase activity in vitro in normal human fetal osteoblasts (0.5 μM results in a 2.5-fold increase) (Effenberger et al. 2005). In addition, 8-PN and, to a lesser extent, 6-PN, XH and isoXH increase alkaline phosphatase gene expression in U2-human osteosarcoma cells. IL-6, a marker of bone resorption, is also down regulated in these cells by 8-PN and 6-PN; a process that is ERα dependent. In contrast, 8-PN may not affect osteoclast activity, since it has no effect on bone resorption in a pit-formation assay (Tobe et al. 1997). Other constituents of hops, including XH and humulone, are inhibitory in this bone-resorption model (with half maximal inhibitory concentrations of approximately 1 μM and 6 μM respectively). Humulone also exerts effects in osteoblasts; for example, in MC3T3-E1 cells humulone suppresses TNFα-dependent cyclooxygenase-2 transcription, which plays an important role in prostaglandin E2 production (Yamamoto et al. 2000). These limited in vitro studies suggest that prenylflavonoids also exert effects on bone cells.

**Potential mechanisms of action of polyphenols in bone**

Phenolic compounds may act via several mechanisms to exert effects on bone (Fig. 1). Several polyphenols found in alcoholic beverages, e.g. EGCG, resveratrol, quercetin, kaempferol and the prenylflavonoids discussed earlier, bind to ERα and ERβ (Bowers et al. 2000; Goodin et al. 2002; Wattel et al. 2003; Prouillet et al. 2004; Rassi et al. 2005) and, therefore, may directly modulate gene transcription through these receptors. However, the mechanisms are complex as, depending on the environment, the type of ER (α or β) that they engage or the concentration or type of phenolic compound used, they may act either as oestrogen agonists or antagonists and may exert different oestrogenic activities. For example, although resveratrol binds ERα and ERβ with comparable affinity in CHO-K1 cells, resveratrol exerts oestrogenic activity through ERβ but both agonist and antagonist activities through ERα (Bowers et al. 2000; Goodin et al. 2002; Wattel et al. 2003; Prouillet et al. 2004; Rassi et al. 2005). In the human U2-osteosarcoma cell line, although XH, isoXH, 8-PN and 6-PN all bind ERα and ERβ, 8-PN shows no preferential affinity for either receptor while all the...
other compounds preferentially bind ERβ (Effenberger et al. 2005). In addition, binding to the different ER does not necessarily lead to oestrogenic activity. For example, although both EGCG and epicatechin gallate bind to ERα and ERβ in MCF-7 breast carcinoma cells, only EGCG exerts ER-mediated gene expression in these cells (Goodin et al. 2002).

As well as direct ER binding, several polyphenols exert non-ER-mediated cellular activities. Redox signalling pathways in osteoblasts and osteoclasts may be regulated by polyphenols, as the majority of these compounds display antioxidant activities. Catechins, for example, have been reported to inhibit oxidative stress in cultured rat calvarial osteoblasts (Park et al. 2003). In addition, reactive oxygen species are important secondary messengers in RANK ligand activation of the transcription factor NF-κB in osteoclasts (Ha et al. 2004). NF-κB plays a key role in inflammation, proliferation and inhibition of apoptosis. It regulates the production of osteoclastogenic cytokines and prostaglandins in osteoblasts and stimulates osteoclast activation and differentiation (Agarwal et al. 2003; Chae et al. 2004; Boyce et al. 2005). Several polyphenols found in alcoholic beverages, including EGCG, resveratrol, quercetin and humulon, inhibit NF-κB (Yamamoto et al. 2000; Yang et al. 2001; Estrov et al. 2003; Wattel et al. 2004), although few studies have examined these effects in bone cells. Yamamoto et al. (2000), however, have demonstrated that humulon suppresses TNF-dependent cyclooxygenase-2 transcription in MC3T3-E1 cells via the transcription factor NF-κB and another transcription factor, NF-IL6. In RAW cells pretreatment with quercetin inhibits RANK ligand-induced osteoclast formation and NF-κB and activator protein 1 activation (Wattel et al. 2004). In addition, Rassi et al. (2005) have observed that quercetin induces apoptosis in osteoclasts by caspase activation. These effects may be mediated via NF-κB, although this aspect was not investigated. It should be noted that even within the same bone cell type different polyphenols of the same class can exert varying mechanisms of action. For example, quercetin and rutin (a glycoside of quercetin) have both been shown to inhibit osteoclast activation, yet rutin acts by reducing RANK protein while quercetin induces apoptosis in the same cell model (Rassi et al. 2005). Recent research also suggests that many of these polyphenolic compounds activate intracellular antioxidants and cyto-protective enzymes, which may result in anti-inflammatory effects in bone leading to prevention of bone loss. For example, resveratrol and EGCG have been reported to induce the cellular antioxidant haem oxygenase-1 via the activation of the transcription factor NF-E2-related factor-2 (Chen et al. 2005). In addition, several prenylated flavonoids have been reported to activate NADPH quinone reductase and other phase II cyto-protective enzymes (Stevens & Page, 2004). Several of these enzymes are regulated by NF-E2-related factor-2 (Chen & Kusch, 2004). Activation of NF-E2-related factor-2 and haem oxygenase-1 leads to anti-inflammatory effects in vitro and in vivo in vascular models (Soares et al. 2005; Chen et al. 2006). Inflammation is an important trigger of osteoclastogenesis and, recently, Zwerina et al. (2005) have demonstrated the anti-inflammatory effects of haem oxygenase-1 in bone, as haem oxygenase-1 expression suppresses osteoclastogenesis in vitro and in vivo. Furthermore, NF-E2-related factor-2 is activated during osteoblast differentiation (Beck et al. 2001, 2003; Wu et al. 2005). Finally, some of these compounds may exert other effects independent of gene transcription; catechins, for example, have been found to increase collagen stability, although the mechanism is unclear (Madhan et al. 2005).
Intakes of phenolic compounds with moderate alcohol consumption: are they sufficient to induce effects in bone?

Virtually no studies have reported effects of either alcoholic beverage extracts or the individual phenolic components on bone health in human subjects. Although the studies discussed earlier suggest that phenolic compounds from wine and beer can affect bone cells in vitro, it is difficult to determine if moderate alcohol consumption provides sufficient concentrations of these compounds to affect BMD and metabolism in vivo. As previously discussed, there are difficulties in assessing moderate alcohol intake, and the concentrations of phenolic compounds vary considerably among alcoholic beverages, even when comparing similar wines from the same region or beers from the same country (Carando et al. 1999; Andrade et al. 2001; Stevens & Page, 2004; Tedesco et al. 2005). As with all dietary studies that relate to polyphenols, bioavailability remains an issue (for reviews of bioavailability and bioefficacy of polyphenols described in human subjects, see Manach et al. 2005; Williamson & Manach, 2005). The bioavailability of phenolic compounds is generally considered to be low. Several phenolic compounds are metabolised in the gut and potentially the liver, resulting in methyl, sulfate or glucuronic acid conjugates, and they are rapidly excreted (Ruefer et al. 2005; Williamson & Manach, 2005). Following oral administration, plasma concentrations of polyphenols are generally ≤5 μM. Among the few studies examining effects on bone in vivo, Horcajada-Molteni et al. (2000) have reported that rats fed high concentrations of rutin (2.5 g rutin per kg diet) achieve a mean plasma concentration of 9.5 μM-rutin metabolites, with isorhamnetin the highest at 6.8 μM. The bioavailability of trans-resveratrol is also relatively low; a 25 mg oral dose given to healthy men and women results in trace amounts in plasma, and metabolites peak 30–60 min later at concentrations of about 2 μM (Goldberg et al. 2003; Walle et al. 2004). There is a lack of studies on the absorption, metabolism and excretion of XH and its metabolites in human subjects. When XH is fed to rats twenty-two metabolites are isolated from faeces (including isoXH) and most of the parent XH is recovered (Nookandeh et al. 2004), suggesting that bioavailability is low. Nonetheless, low levels of XH monoglucurononides have been detected in plasma after administration of XH in male rats (Stevens & Page, 2004) and the bioavailability of other prenylflavanoids is unclear. Several questions remain to be answered in relation to the bioavailability of phenolic compounds found in alcoholic beverages. Further studies are required to examine their metabolism in the gut and the kinetics of metabolism. Although plasma levels may be low following oral administration, few studies have investigated whether they accumulate in tissues or whether their metabolites may be active, and research in the bone field is in the early stages. Finally, it should also be remembered that studies carried out with single doses may not be representative of dietary exposure where tissue accumulation could be postulated through gradual chronic exposure. Indeed, Suganuma et al. (1998) have demonstrated that after a single dose of EGCG low level accumulation occurs in several tissues, but after a second dose a 4–6-fold increase above the single dose is detected in tissues.

Higher intakes of polyphenol-rich foods have been associated with protective effects in CVD and cancer. Indeed, moderate alcohol consumption has been consistently associated with 20–30% reductions in risk for coronary artery disease (Klatsky, 2003), although, as with bone, it is unclear whether red wine or beer polyphenols confer any additional effect to that of ethanol. As moderate red wine consumption (375 ml red wine/d for 2 weeks) inhibits oxidative stress in normal healthy volunteers (Tsang et al. 2005), and this outcome is likely to be associated with the polyphenol content, it is possible that there may be additional effects. Several research groups (New et al. 1997; Tucker et al. 1999; McGartland et al. 2004; Prynne et al. 2006) have observed that higher intakes of fruit and vegetables, which are also a rich source of phenolic compounds, are positively associated with BMD. Clearly, more work is required with specific polyphenolic compounds.

Thus, in summary, phenolic compounds that are present in alcoholic beverages appear to have the potential to affect bone, as indicated by evidence from the in vitro studies described earlier. These effects may occur through various signalling pathways, some of which may be oestrogen dependent. Further studies are required to clarify the issues of bioavailability and metabolism of these compounds, and more analysis needs to be carried out to determine concentrations in different wines and beers. In addition, further in vitro studies are required using metabolites of these compounds at concentrations found in plasma and/or tissues, as well as in vivo studies to determine their effects on bone. Thus, it is currently unclear whether polyphenolic compounds contribute to the increase in BMD that is associated with moderate alcohol consumption.

Silicon

Dietary Si, which is absorbed as orthosilicic acid (Si(OH)₄), may be beneficial for growth and development of bone and other connective tissues (Jugdaohsingh et al. 2004). The role of Si in bone health has recently been reviewed (Sripanyakorn et al. 2005), and will be only briefly summarised here. However, as it has also been shown that certain alcoholic beverages, especially all beers, are high in Si (Sripanyakorn et al. 2004; Powell et al. 2005a), the potential for alcohol-BMD associations to be partly mediated through Si intakes is considered.

Alcoholic beverages as dietary silicon sources

Typically, between 20 and 40 mg Si per adult per d is ingested from Western diets (Pennington, 1991; Jugdaohsingh et al. 2002). Foods derived from plants rather than animals provide the highest sources of dietary Si (Varo & Koivistoinen, 1980; Pennington, 1991; Powell et al. 2005a), because certain plants, especially cereals, take up and accumulate Si at high levels (Sangster et al. 1986; Epstein, 1999). Amongst plant-based foods, whole grains have the highest level of Si, especially oats and barley, and rice bran and wheat bran and, not surprisingly, products...
made from the grains are also high in Si (Powell et al. 2005a; Pennington 1991). In particular, very high levels of Si are found in beer, which is made from macerated wholegrain barley (Sripanyakorn et al. 2004; Powell et al. 2005a). Recently, a mean concentration of 19-2 (so 0-6) mg Si/l was reported from an analysis of seventy-six different beers (Sripanyakorn et al. 2004). Si levels in the different beers were not found to be significantly different by alcohol content, geographical origin or type of beer (Sripanyakorn et al. 2004; Powell et al. 2005a). Not surprisingly, therefore, beer can be a major contributor to Si intake in the Western diet, especially for men (Pennington, 1991; Jugdaohsingh et al. 2002), contributing, on average, between 11 and 24% total daily Si intake (Jugdaohsingh et al. 2002, 2004). In contrast, levels of Si in wines are more varied and, although they can be as high as some beers (7-23 mg/l in a recent small analysis; Powell et al. 2005a), actual intakes of Si in terms of servings are 2-5 fold lower (0-62-2-89 mg Si per serving for wine compared with 3-8-16-3 mg Si per serving for beers, depending on the exact choice of beverage and serving size; Powell et al. 2005a). Moreover, the wines with the higher levels of Si may have achieved this level as part of the beverage processing, in which case chemical speciation and bioavailability of the Si is unclear (Powell et al. 2005a). Si levels in spirits are much lower (1-3 (so 0-04) mg/l; Jugdaohsingh et al. 2004) and intakes of these beverages are totally irrelevant in terms of dietary Si exposure.

Si bioavailability is generally high from fluids, including beer; ≥55% is absorbed, much of which is then rapidly excreted by the kidneys (Bellia et al. 1994; Sripanyakorn et al. 2004). Coupled with basic chemical speciation data, this finding suggests that Si is present in beer as the soluble and readily-absorbable form Si(OH)4 (Sripanyakorn et al. 2004).

**Dietary silicon and bone**

Mounting evidence over the last 30 years supports a biological role for Si in bone and connective tissue health, and includes evidence derived from cellular, animal and human studies (for review, see Sripanyakorn et al. 2005). More recent studies have shown that dietary Si, as soluble orthosilicic acid, which is in equilibrium with a very small amount of the orthosilicate anion (Si(OH)3O−), has anabolic effects on bone cells (Reffitt et al. 2003) and in animal models of post-menopausal osteoporosis (Hott et al. 1993; Rico et al. 2000). In the animal studies it has been shown that supplemental Si not only inhibits bone loss but also increases femoral BMD above that of controls, suggesting that Si may have both anti-resorptive and anabolic properties (Hott et al. 1993; Rico et al. 2000). There are few studies of Si in human subjects, but they have also shown that supplemental Si (6-14 mg/d) not only reduces bone loss but it may increase BMD by 1-5% in post-menopausal women (8-28 subjects per group) with low bone mass (Schiano et al. 1979; Eisinger & Clairet, 1993; Spector et al. 2005). Additionally, in a recent cross-sectional study in the Framingham Offspring Cohort it has been found that dietary Si intake is positively associated with BMD in premenopausal women, less so in men and not at all in post-menopausal women (Jugdaohsingh et al. 2004). These results, in pre- and post-menopausal women, have recently been confirmed in a cohort of the UK population (Macdonald et al. 2005). Altogether, Si does seem to play an important, if not essential, role in bone and connective tissue health and, although a number of possible mechanisms have been proposed, the exact role or biochemical function of Si remains enigmatic.

**Beer, silicon and bone**

Since beer is a major source of well-absorbed Si, an investigation has been undertaken into whether the contribution of Si from beer ingestion could contribute to the positive effects of moderate alcohol consumption on bone. First, as noted earlier, one 300-574 ml serving of beer would contribute 4-16 mg dietary Si/d (or 2-9 mg Si absorbed/d if bioavailability is taken into consideration), which could be enough to detect an association with BMD based on previous Si intake and BMD data (Jugdaohsingh et al. 2004). Indeed, in this previous work an increase in Si intake of 5-6 mg Si/d was shown to be associated with a 2-5% increase in BMD at the trochanter in men and in premenopausal women (see Fig. 2). Second, also in this previous work, the association between beer intake and adjusted BMD, with and without a mathematical correction for Si intake, was investigated. Correcting for the Si contribution from beer was found to attenuate the positive association and reduce or negate the significance of the association between beer intake and BMD (Jugdaohsingh et al. 2004). Similar investigations on the intake of non-beer alcohols have shown no effect of Si correction on the alcohol–BMD association, because of the small contribution of Si intake from non-beer alcohols (Jugdaohsingh et al. 2004).
et al., 2004). Thus, these findings suggest that the Si component of beer may explain or contribute to some of the positive effects of moderate alcohol consumption on bone. However, as noted previously, these findings are based on basic regression models as an initial ‘global overview’ and further, more detailed, studies are required using categorical data to really distinguish the role of Si from that of ethanol and/or other components in the association of the intakes of different alcoholic beverages with bone health.

Conclusion

Thus, in conclusion, it is becoming well established that moderate ingestion of alcoholic beverages is associated with increased BMD, a marker of bone health. However, the mechanisms are unknown. In the present review the current evidence has been summarised and potential mechanisms discussed, and a case has been made for moderate ethanol ingestion as the primary factor, through acute suppression of bone resorption. Si has also been indicated as a major constituent of beer that may additionally promote bone formation. The effects on bone health of other dietary constituents that are obtained through moderate ingestion of alcoholic beverages are probably not large, although further work is needed in human subjects. Thus, there may be differences between the three major classes of alcoholic beverages (wines, spirits and beers) in their associations with BMD as a result of differing patterns of alcohol exposure, differing Si intakes and, potentially, differing exposures to phytochemicals.

Further epidemiological studies incorporating measures of markers of bone turnover as well as BMD are thus required to dissect out the role of Si v. that of ethanol and/or other components in alcoholic beverages and to further elucidate mechanisms underlying the relationships between moderate drinking and bone health. Ultimately, the data provided from acute metabolism studies (e.g. of ethanol-induced suppression of bone resorption) and those obtained from epidemiological studies for the effects of Si and/or other beverage-related phytochemicals on bone, could combine in a model that quantitatively explains the influence of moderate ingestion of alcoholic beverages on bone health in different populations. This outcome should inform the debate on alcohol and public health as well as defining and communicating moderation.

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References


