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Alcohol: A Simple Nutrient with Complex Actions on Bone in the Adult Skeleton

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Abstract

Background—Alcohol is an important nonessential component of diet, but the overall impact of drinking on bone health, especially at moderate levels, is not well understood. Bone health is important because fractures greatly reduce quality of life and are a major cause of morbidity and mortality in the elderly. Regular alcohol consumption is most common following skeletal maturity, emphasizing the importance of understanding the skeletal consequences of drinking in adults.

Method—This review focuses on describing the complex effects of alcohol on the adult skeleton. Studies assessing the effects of alcohol on bone in adult humans as well as skeletally-mature animal models published since the year 2000 are emphasized.

Results—Light to moderate alcohol consumption is generally reported to be beneficial, resulting in higher bone mineral density (BMD) and reduced age-related bone loss, whereas heavy alcohol consumption is generally associated with decreased BMD, impaired bone quality and increased fracture risk. Bone remodeling is the principle mechanism for maintaining a healthy skeleton in adults and dysfunction in bone remodeling can lead to bone loss and/or decreased bone quality. Light to moderate alcohol may exert beneficial effects in older individuals by slowing the rate of bone remodeling but the impact of light to moderate alcohol on bone remodeling in younger individuals is less certain. The specific effects of alcohol on bone remodeling in heavy drinkers is even less certain because the effects are often obscured by unhealthy lifestyle choices, alcohol-associated disease, and altered endocrine signaling.

Conclusions—Although there have been advances in understanding the complex actions of alcohol on bone, much remains to be determined. Limited evidence implicates age, skeletal site evaluated, duration and pattern of drinking as important variables. Few studies systematically evaluating the impact of these factors have been conducted and should be made a priority for future research. In addition, studies performed in skeletally mature animals have potential to reveal

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mechanistic insights into the precise actions of alcohol and associated co-morbidity factors on bone remodeling.

Keywords

osteoporosis; bone remodeling; bone resorption; bone formation; bone mineral density

Introduction

Alcohol is an important nonessential component of diet with bioactivity transcending its nutrient value. Per capita alcohol consumption in many countries contributes 5–10% of daily energy requirements. Dietary alcohol is metabolized by a 2 step process in which alcohol is first metabolized to acetaldehyde and then to acetate (Cederbaum, 2012). Acetate is readily metabolized to acetyl CoA which can be used for immediate energy, converted to fat for storage or used in the synthesis of the neurotransmitter acetylcholine. Ethanol and its metabolites acetaldehyde and acetate also have bioactivities independent of their caloric value. Acetate is a short chain fatty acid which is important to gut health, is oxidized in the brain (Jiang et al., 2013) and regulates appetite via centrally-mediated pathways (Frost et al., 2014). However, excessive production of acetate leads to accumulation of NADH which inhibits gluconeogenesis and fatty acid oxidation leading to increased fatty acid synthesis and triglyceride deposition within liver. Alcohol can directly affect neurotransmitter systems (Brust, 2010) and acetaldehyde is a highly reactive molecule that has been implicated in alcohol-related disease (Eriksson, 2015). Thus, consumption of alcoholic beverages can influence organ systems via its impact on nutrition or by bioactivity of ethanol and its metabolites.

Frequency and volume of alcohol consumed increases following puberty with regular alcohol use becoming more prevalent during late adolescence (16–17 y) and peaking during early adulthood (21–29 y) (Figure 1). Alcohol has been implicated in influencing skeletal health and increasing fracture risk. Understanding the long-term impact of regular alcohol consumption on the skeleton is important because fractures represent a major cause for morbidity and mortality (Johnell and Kanis, 2006). This review focuses on the effects of alcohol consumption on bone in adult men and women. For additional perspectives the reader is directed to two recent reviews (Maurel et al., 2012a, Turner et al., 2012).

Bone Remodeling

Bone is continuously broken down and reformed in adults by a coupled process known as bone remodeling (Figure 2). At the initiation of bone remodeling, osteoclasts are recruited to quiescent bone surfaces under control of osteocytes where they degrade bone. Osteoblasts are then recruited to the resorption site where they secrete a collagenous matrix that undergoes mineralization to form bone. A bone remodeling cycle requires 4–6 months to complete. Superimposed upon the prevailing levels of bone remodeling are circadian fluctuations in the rates of bone formation and bone resorption. Normally, serum bone formation markers peak during the day and resorption markers peak at night (Eriksen, 2010).

A Basic Multicellular Unit, the functional unit of bone remodeling, includes not only bone cells (osteocytes, osteoblasts and osteoclasts) but cells that support bone functions, including endothelial and immune cells. An increased rate of bone resorption relative to formation will eventually result in lower bone mineral density (BMD) (Riis et al., 1996). Additionally, bone quality can be negatively impacted when bone remodeling becomes abnormally low or high. Excessive rates of bone remodeling will impair bone quality by increasing cortical porosity and/or decreasing trabecular connectivity. On the other hand, bone remodeling is necessary for repair of microfractures generated by activities of daily living. Reduced bone remodeling can lead to accumulation of microfractures, which can reduce bone strength (Burr et al., 1997).

It is important to distinguish remodeling from bone growth and modeling. Growth is the process by which bones increase in length (up to ~age 18y) through endochondral ossification. Modeling is a process by which bone is added to a preexisting cortical or cancellous bone surface without a requirement for prior bone resorption or by which bone is resorbed from a bone surface without initiating coupled bone formation. Modeling is very important for optimizing bone mass, BMD, microarchitecture and quality during growth but is superseded by remodeling in the adult skeleton.

Alcohol and Bone

Effects of Alcohol on Bone in Humans

The definition of low, moderate and heavy drinking varies among authors. For the purpose of this review, low refers to infrequent consumption of small quantities of alcohol, moderate is regular (3 d/w) consumption of 14 g/d ethanol for women and 28 g/d ethanol for men and heavy refers to consumption levels of alcohol substantially above moderate, either as chronic (most d/w) or binge (70 g/setting) or both (Table 1).

The effects of alcohol on the adult skeleton are influenced by age and drinking pattern. Moderate alcohol consumption is often associated with higher BMD (Figure 3) and lower fracture risk (Felson et al., 1995, Ganry et al., 2000, Holbrook and Barrett-Connor, 1993, Marrone et al., 2012, Mukamal et al., 2007, Rapuri et al., 2000, Sommer et al., 2013). However, in some studies no effects on BMD were detected (Laitinen and Valimaki, 1993, Wosje and Kalkwarf, 2007). There is no adequate explanation for this variability, although negative studies tended to focus on younger individuals at or near peak BMD and at low fracture risk. These findings suggest that moderate alcohol does not increase peak bone mass but may slow age-related bone loss. Estrogen deficiency in postmenopausal women results in increased bone remodeling where the increase in resorption outpaces formation (Turner and Sibonga, 2001). Moderate alcohol may partially counteract these negative skeletal effects by slowing bone remodeling. In support, moderate alcohol consumption in postmenopausal women is associated with decreased serum markers of bone resorption and formation (Rapuri et al., 2000). A prospective study by Marrone et al. provides additional evidence (Marrone et al., 2012); serum markers of bone remodeling were increased after two weeks of alcohol abstention in healthy postmenopausal women who had averaged 1.25 standard (American) drinks/d (19 ± 1 g alcohol/d), whereas return to a pretreatment alcohol intake level resulted in a rapid (overnight) decrease in these markers. In younger men and

women, moderate alcohol consumption resulted in acute (within hours) suppression of a serum marker of bone resorption but there was no change in a marker of bone formation (Sripanyakorn et al., 2009). The findings of these two studies support the concept that moderate alcohol consumption has the potential to slow bone resorption, a strategy often employed in the pharmacological treatment of osteoporosis.

The remarkably rapid changes observed in the studies described above suggest that alcohol can acutely reduce the activity of osteoblasts and osteoclasts. Because of the long length of the remodeling cycle, these changes are likely to reflect lowering of bone cell activity during the circadian cycle. This conclusion is consistent with Nielsen et al. who investigated the acute effects of alcohol on serum osteocalcin in young men and women (Williams et al., 2005). On the other hand, no changes were observed in markers of bone turnover when postmenopausal women were rotated through 3 treatment regimens (no alcohol, 15 g/d and 30 g/d) for 8 week treatment intervals separated by 5 week washout periods (Mahabir et al., 2014). Clearly, additional intervention studies are required to understand the specific effects and mechanisms of action of moderate alcohol on bone health.

In contrast to moderate drinking which appears to have neutral or beneficial effects, chronic heavy alcohol consumption is associated with decreased BMD (Diamond et al., 1989a, Hyeon et al., 2016, Kim et al., 2003, Kouda et al., 2011, Malik et al., 2009) and increased fracture risk (Gonzalez-Reimers et al., 2011, Hoidrup et al., 1999, Santori et al., 2008), but there have been notable discrepancies. Differences may be related to magnitude, pattern and duration of drinking and skeletal site(s) evaluated. In one of the few studies to address these issues, Pumarino et al. evaluated the skeleton in male continuous and intermittent heavy drinkers (Pumarino et al., 1996). Osteopenia was noted in femur neck but not spine, suggesting site specificity, and the type of alcohol abuse was found to be less important than duration.

The increased fracture risk often associated with chronic alcohol abuse in humans may be greater than what is explained by decreased BMD, implying a negative effect on bone quality (Figure 3) (Hui et al., 1989, Kanis et al., 2005, Riis et al., 1996). Unfortunately, no routine, noninvasive method for evaluating bone in humans provides insight into bone quality. However, a recent study evaluated the relationship between current alcohol consumption and bone in the distal radius and tibia in aged men and women using high resolution computed tomography (Paccou et al., 2015). Moderate to heavy alcohol consumption was associated with minimal changes in bone geometry, density and microarchitecture. Inexplicably, light drinking was associated with generally negative effects on indices of bone quality in males but not females.

Although invasive, histological evaluation of iliac crest bone biopsies is the “gold standard” for evaluating bone remodeling. Analyses performed to date indicate that chronic heavy alcohol consumption is consistently associated with reduced indices of bone formation in males (Bikle et al., 1985, Bikle et al., 1993, Chappard et al., 1991, Crilly et al., 1988, Diamond et al., 1989b, Schnitzler et al., 2010). In agreement, serum biomarkers of bone formation were also reduced in alcoholics (Diamond et al., 1989a, Diez et al., 1994, Gonzalez-Reimers et al., 2011, Labib et al., 1989, Laitinen et al., 1993, Nyquist et al., 1996).

On the other hand, the effects of alcohol on bone resorption parameters in biopsies were more variable as have been biochemical markers of bone resorption (Bikle et al., 1993, Chappard et al., 1991, Diamond et al., 1989a, Diez et al., 1994, Pepersack et al., 1992, Nyquist et al., 1996). However, evidence for decreased bone resorption in alcoholics was reported in the majority of studies, suggesting an overall reduction in rate of bone remodeling. It should be noted, alcoholics differed among studies in age and duration of alcohol abuse and ranged from healthy to having cirrhosis, pancreatitis, diabetes and other conditions that may influence bone metabolism, potentially confounding interpretation of results.

The contribution of comorbidities is illustrated by a study comparing patients having alcohol-related pancreatitis to ones having idiopathic pancreatitis. As a group, patients with pancreatitis had low BMD, but no independent effect of alcohol abuse was noted (Prabhakaran et al., 2014). The inclusion of individuals with alcohol-related secondary diseases may have contributed to variable results. Laitinen et al., for example, reported that an average alcohol consumption of 186 ± 85 g/d in women without cirrhosis had no effect on BMD (Laitinen et al., 1993). Similar findings have been reported in men (Santori et al., 2008). However, other studies report lower BMD in male chronic alcohol abusers without liver cirrhosis (Kim et al., 2003). As with moderate drinking, there is no adequate explanation for these discrepancies.

Bone loss in adults can be caused by (1) elevated bone remodeling, where increased resorption outpaces the increase in formation (high turnover), (2) uncoupled bone remodeling, where resorption increases and formation decreases, or (3) reduced bone remodeling where resorption, although reduced, outpaces formation (low turnover). Uncoupled bone remodeling leads to very rapid (e.g., months) bone loss (Seibel et al., 2013, Sibonga, 2013). In contrast, bone loss resulting from a remodeling imbalance associated with increased or decreased bone remodeling rates requires much longer intervals (years to decades) for comparable bone loss (Nordin et al., 1998). In the absence of pancreatitis or liver disease, the changes in chronic alcoholics generally exhibit a low turnover pattern of bone loss. For example, a group of 20 middle-aged men and women abusing alcohol (207 g/d) for a mean duration of 26 years had T-scores for total hip and lumbar spine significantly lower than controls but the majority remained within the normal range (Gonzalez-Reimers et al., 2013). Mechanistically, reduced bone turnover in alcoholics is associated with increased serum sclerostin, an osteocyte-derived inhibitor of bone formation, and increased osteoprotegerin, an inhibitor of osteoclast differentiation (Garcia-Valdecasas-Campelo et al., 2006, Gonzalez-Reimers et al., 2013).

Reversibility of the Skeletal Actions of Alcohol

A two-week abstinence in postmenopausal moderate drinkers resulted in a small but significant increase in biochemical markers of bone turnover (Marrone et al., 2012) while suppressed bone formation returned to normal in noncirrhotic male alcoholics (Laitinen et al., 1992). A bone formation marker was elevated in alcoholic women following only 5 days of abstinence (Clynes et al., 2015). In contrast to moderate postmenopausal drinkers, short duration abstinence did not lead to an increase in resorption markers in the alcohol abusers.

The positive effects of abstinence on bone health continue over longer time intervals. A 6-month period of abstinence in alcoholics resulted in increased BMD and biochemical markers of bone turnover (Alvisa-Negrin et al., 2009). In a longitudinal study, lumbar and femoral neck BMD was found to be lower in male alcoholics than in controls and increased during 2 years of abstinence. Interestingly, during the abstinence interval BMD decreased in the controls (Peris et al., 1994). In the same study, a bone formation marker increased following abstinence, suggesting increased bone formation contributed to the increase in BMD. Although the skeleton has demonstrated remarkable ability to respond positively, it is not yet clear whether BMD returns to normal in abstinent alcoholics.

Effects of Alcohol on Bone in Animal Models

Animal models have potential to further our understanding of the specific actions of alcohol on bone remodeling (Iwaniec and Turner, 2013a). However, in the vast majority of recent studies alcohol treatment was initiated when the animals were growing (Table 2). These studies address concerns such as the impact of heavy underage drinking on bone growth and repair. However, their use is contraindicated for evaluating the adult skeleton because the mechanisms that regulate bone mass and architecture during growth (longitudinal growth, cortical modeling, and cancellous modeling) are either greatly diminished (modeling) or absent (longitudinal growth) following skeletal maturity. Rats begin to exhibit bone remodeling (endocortical and cancellous) in their long bones at ~3 months of age and remodeling predominates after 6 months of age. In contrast to humans, small rodents do not fully resorb epiphyseal cartilage. However, the growth plate becomes perforated by bony bridges that restrict further growth. Complete closure (maximum number of bridges) in Sprague Dawley rats occurs in the proximal tibia at ~8 months of age in males and ~10 months of age in females (Martin et al., 2003), but longitudinal bone growth is negligible after 6 months of age in both genders. As in humans, the timing of epiphyseal closure in rodents varies among bones and growth plates. Longitudinal bone growth slows and onset of bone remodeling occurs earlier in vertebra than long bones. Nevertheless, modeling continues to play an important role in the increase in vertebral dimensions from puberty to skeletal maturity (Jee and Yao, 2001). Mice reach skeletal maturity at slightly earlier age (~6 months) than rats but the time course for progression from modeling to remodeling has not been established for this species.

There are no established criteria for light, moderate, or heavy drinking in animals, however it is reasonable to directly model human drinking. For the purpose of this review, heavy drinking, whether continuous or binge, requires delivering alcohol at levels resulting in blood alcohol concentrations equal to or exceeding 0.1% and/or contributing >10% of daily energy.

As indicated by Hogan et al. (Hogan et al., 2001), the impact of alcohol on bone mass and strength in skeletally-mature rats requires longer intervals of exposure and is mild compared effects attributable to growth inhibition in young animals, further emphasizing the importance of modeling adult-onset drinking using skeletally-mature animals. Specifically, administration of alcohol contributing 35% of total calories for 8 weeks to 9-month-old female rats, resulting in blood alcohol concentrations of ~0.12%, had minimal impact on the

skeleton. However, extending the treatment for longer intervals led to cortical and trabecular thinning (Sibonga et al., 2007, Turner et al., 2001b) and reduced mechanical strength (Hogan et al., 2001). At the cellular level, alcohol led to reduced histomorphometric indices of bone formation and resorption (Turner et al., 2001b).

Studies performed in older rats have focused on the skeletal effects of chronic heavy alcohol consumption. In an exception, bone formation in 8-month old female rats was reduced by administration of as little as 3% of energy as alcohol (Turner and Sibonga, 2001). In this dose-response study, alcohol reduced cancellous bone lined by osteoclasts as well as bone lined by osteoblasts. At higher dose rates of alcohol, the reduction in osteoblasts exceeded the reduction of osteoclasts resulting in bone loss when alcohol contributed 13% of kcal. In good agreement with results from human studies, the principal effect of chronic high alcohol consumption in skeletally-mature rats is a remodeling imbalance, leading to slow but progressive bone loss. In skeletally-mature female rats, suppressed bone formation normalized within 6 weeks of cessation of drinking, but the reduction in cancellous bone volume induced by 16 weeks of heavy alcohol consumption was not reversed (Sibonga et al., 2007). However, treatment of abstinent rats with pharmacological doses of parathyroid hormone (PTH) resulted in a further increase in bone formation and reversal of bone loss. These encouraging findings suggest that the skeletally-mature rat is an appropriate model to investigate the underlying molecular mechanisms mediating the effects of alcohol on cancellous and endocortical bone remodeling. However, to date this model has not been exploited for this purpose.

Studies in skeletally-mature rodents have utilized the Lieber-DeCarli diet (Lieber and DeCarli, 1989), which contains either ethanol or an isocaloric amount of simple carbohydrate. A limitation of this method is that including alcohol as an integral component of diet models the drinking pattern of a minority of alcohol consumers. Ethanol can be administered to skeletally-mature rodents using methods to model occasional or binge drinking. The strengths and weaknesses of available models have been discussed (Iwaniec and Turner, 2013a, Lieber and DeCarli, 1989, Turner et al., 2012). Key concerns when choosing a method to deliver alcohol are avoiding caloric restriction, dehydration, nutritional imbalance, excessive stress, and inflammation.

An important limitation of small rodents is that even as adults they do not exhibit intracortical remodeling (also referred to as osteonal or Haversian remodeling), the process through which cortical bone is remodeled in humans (Hillier and Bell, 2007, Lelovas et al., 2008). Since cortical bone comprises nearly 80% of skeletal mass in humans (Clarke, 2008), is a major determinant of BMD, and is crucial for structural support, alcohol-induced alterations in intracortical bone remodeling may play an important but largely uninvestigated role in fracture risk. This limitation can be addressed using larger animal models, such as non-human primates.

Many drinkers have irregular patterns of alcohol consumption that can range from occasional, to regular, to binge or change over time. A nonhuman primate model was used to assess the effects of voluntary alcohol self-administration on bone (Baker et al., 2014, Grant and Bennett, 2003, Grant et al., 2008, Kroenke et al., 2014, Gaddini et al., 2015). The

pattern of alcohol consumption and blood alcohol concentrations in the monkeys evaluated (Figure 4) are very similar to drinking patterns and blood alcohol concentrations of alcoholic men given 24 h/d free access to alcohol for 10–60 consecutive days (Mello and Mendelson, 1970, Mello and Mendelson, 1971). In a series of studies, male rhesus macaques (*Macaca mulatta*) were allowed to voluntarily self-administer water or alcohol (4% ethanol w/v) for 22 h/d, 7 d/wk for 12 months (Gaddini et al., 2015). Age-matched controls received water and an isocaloric maltose-dextrin solution. Differences in tibial BMC, BMD, and cortical bone architecture in the tibial diaphysis were not detected with treatment. However, both cortical porosity and osteon density were lower in alcohol-consuming monkeys, indicating a reduced rate of intracortical bone remodeling (Figure 4). These results suggest that, by suppressing intracortical bone turnover, chronic heavy alcohol consumption may negatively impact bone health independent of its actions on BMD.

Mechanisms by Which Alcohol May Impact Bone Metabolism

Understanding the precise mechanisms mediating the dose-dependent effects of alcohol on bone, including how drinking interacts with lifestyle factors and comorbidities, is important to better understand the long-term impact of moderate alcohol intake on bone and develop rational interventions to prevent or reverse the negative skeletal effects of excessive alcohol intake.

Nutrition

The effects of low to moderate alcohol consumption on nutritional status has received limited attention. To date, there is no clear indication that moderate alcohol results in changes in nutrition that would impact bone metabolism in humans. In contrast, malnutrition is frequently reported to be associated with chronic alcohol abuse (Lieber, 2003) and low body weight is a well-established risk factor for low BMD (Tomlinson and Morgan, 2013). Acute weight loss and weight cycling have been shown to be particularly detrimental to bone health and are associated with binge drinking (Shapses and Riedt, 2006). Therefore, inadequate energy availability to support bone metabolism likely contributes to the lower BMD frequently observed in underweight chronic alcohol abusers. This conclusion is supported by animal studies. A striking dose-response effect of alcohol on food consumption was reported in 8-month-old female rats. Animals fed liquid diets containing 0.5% (Turner and Iwaniec, 2010) and 3% (Turner et al., 2001b) of their energy as alcohol consumed more diet and gained more weight than controls but levels of alcohol 12% suppressed food consumption and weight gain. Other studies have reported that weight loss in rats results in bone loss due to a combination of increased bone resorption and decreased bone formation (Talbot et al., 2001).

While inadequate energy availability can negatively impact the skeleton, alcohol may also affect the intake, absorption, metabolism, and excretion of micronutrients. Alcoholics often voluntarily replace nutrient-dense foods with alcohol (Gruchow et al., 1985); thus, many alcoholics may suffer from deficient intakes of micronutrients, including calcium and vitamin D, that are critical to bone health. The skeleton plays an integral role in mineral homeostasis and alcohol abuse is associated with deranged mineral balance.

Hypomagnesemia is the most common deficiency, but hypocalcemia, hypercalciuria, hypophosphatemia, and hypokalemia occur frequently (De Marchi et al., 1993, Decaux et al., 1980, Elisaf et al., 1995). Furthermore, a wide range of defects in renal tubular handling of minerals are prevalent in alcoholics (De Marchi et al., 1993).

Magnesium deficiency in humans, a known risk factor for bone loss, can result in hypocalcemia, impaired PTH secretion, and low serum concentrations of 1,25-dihydroxyvitamin D, all of which are commonly observed in chronic alcohol abusers. Furthermore, magnesium repletion was shown to simultaneously correct hypomagnesemia and hypocalcemia (Medalle et al., 1976). However, no studies have been performed to determine the efficacy of magnesium supplementation as a strategy to prevent alcohol-associated bone loss.

Alterations in Hormone Levels and Signaling

Consuming alcohol is associated with alterations in levels and/or signaling by several classes of hormones known to influence bone metabolism. These include classes of hormones that regulate mineral homeostasis (e.g., vitamin D and PTH), hormones that couple bone remodeling to energy metabolism (e.g., growth hormone) and reproductive hormones (e.g., androgens and estrogens).

Many alcoholics have subnormal levels of 25-hydroxyvitamin D (Schnitzler et al., 2010). Very low vitamin D levels could contribute to the calcium and phosphate deficiencies in some alcohol abusers. Those with Laennec's cirrhosis also have low levels of vitamin D binding protein due to impaired hepatic protein synthesis and have low serum concentrations of total, but not free, 1,25-dihydroxyvitamin D. The low 25-hydroxyvitamin D in some alcoholics have been attributed to reduced hepatic 25-hydroxylase activity, lack of sun exposure, inadequate dietary intake, and malabsorption (Pitts and Van Thiel, 1986). Changes in vitamin D status in many alcohol abusers may not be entirely specific to alcohol; vitamin D deficiency associated with cirrhosis was found to relate to liver dysfunction (Malham et al., 2011). In addition, smoking, which is common in alcohol abusers, is associated with lower 25-hydroxyvitamin D, 1,25-dihydroxyvitamin D and PTH levels as well as lower levels of osteocalcin, a biochemical marker of bone formation (Brot et al., 1999). Irrespective of the underlying cause, inadequate 1,25 dihydroxyvitamin D (Lindholm et al., 1991) can result in impaired intestinal calcium absorption which, in turn, could negatively impact bone metabolism (Atkins et al., 2007). The effects of alcohol on vitamin D status in adult rodents has not been reported but increased 25-hydroxyvitamin D and decreased 1,25-dihydroxyvitamin D have been observed in nutritionally adequate, alcohol-fed growing rats and mice, suggesting alcohol disrupts normal vitamin D homeostasis (Mercer et al., 2012, Turner et al., 1988). However, abnormalities in bone metabolism preceded changes in levels of vitamin D metabolites in rats (Turner et al., 1988, Turner et al., 1987). Furthermore, the increased 25-hydroxyvitamin D levels observed in alcohol-fed rodents contrasts with the normal or decreased levels observed in chronic alcohol abusers. Therefore, age and species differences in vitamin D metabolism should be considered when interpreting results of animal studies.

Alcohol withdrawal from alcoholics with low 25-hydroxyvitamin D levels resulted in rapid increases in bone formation but had inconsistent effects on 25-hydroxyvitamin D. Bone formation increased within 5 days of abstinence in women and 2 weeks in men without changes in 25-hydroxyvitamin D levels (Clynes et al., 2015, Laitinen et al., 1992), suggesting that low 25-hydroxyvitamin D levels play a limited causal role in alcohol-associated reductions in bone formation. This conclusion is consistent with the concept that 25-hydroxyvitamin D is a prohormone that requires extreme changes in its levels to impact production of 1,25-dihydroxyvitamin D. Additionally, not all studies reporting negative effects of alcoholism on bone metabolism have detected alterations in vitamin D status (Bikle et al., 1993) and twelve months of voluntarily self-administration of alcohol did not alter 25-hydroxyvitamin D levels in male rhesus macaques fed a nutritionally adequate diet (Gaddini et al., 2015). Taken together, low 25-hydroxyvitamin D levels detected in some chronic alcohol abusers likely reflect inadequate nutrition but altered metabolism cannot be excluded.

Short-term alcohol administration was shown to cause transitory hypoparathyroidism. Specifically, the parathyroid glands did not respond to hypocalcemia by increasing PTH secretion in alcohol-intoxicated individuals (Laitinen et al., 1994). An inappropriate PTH response may account, at least in part, for the transient hypocalcemia, hypercalciuria, and hypermagnesuria that sometimes follow alcohol ingestion (Laitinen et al., 1991).

Growth hormone plays a seminal role in bone acquisition during growth and in bone remodeling in adults. Alcoholism was shown to be associated with an impairment in the serotonergic-stimulatory regulation of growth hormone secretion as well as the growth hormone response to insulin-induced hypoglycemia (Coiro and Vescovi, 1995, Coiro and Vescovi, 1998)). Many of the actions of growth hormone are mediated through circulating and/or locally produced IGF-I and acute as well as chronic alcohol consumption is associated with reduced serum IGF-I. Low IGF-I levels are associated with malnutrition but ingestion of 1.35 g/kg of ethanol by healthy individuals resulted in a protracted decline in IGF-I (Rojdmark et al., 2000), and serum IGF-I levels were lower in skeletally-mature rats fed high levels of alcohol for 5.5 months (Sibonga et al., 2007). Serum IGF-I levels are reduced in men with idiopathic osteoporosis and low IGF-I may contribute to low BMD and bone formation rate in alcohol abusers (Kurland et al., 1997).

Moderate alcohol consumption is associated with decreased plasma total and free testosterone (Cigolini et al., 1996). The effects of chronic heavy alcohol consumption on serum testosterone levels are conflicting, with some studies reporting decreases in humans (Venkat et al., 2009) and rats (Sibonga et al., 2007) and others finding no effect (Onland-Moret et al., 2005, Wezeman et al., 1999). The discrepancy may be because serum testosterone levels are diminished immediately following acute bouts of alcohol intake in humans (Maneesh et al., 2006, Mendelson et al., 1977), but quickly return to normal. Testosterone deficiency is associated with low BMD in humans (Amory et al., 2004, Arisaka et al., 1995, Isaia et al., 1992, Kenny et al., 2010) and orchiectomy results in increased bone turnover and bone loss in skeletally-mature rats (Erben et al., 2000, Reim et al., 2008). Thus, the pronounced dose-related differences in skeletal response to alcohol consumption may be due, in part, to changes in testosterone levels. However, there have been no intervention

studies that have carefully evaluated the precise role of transient reductions in serum testosterone with bone loss. This may not be feasible in humans but could be accomplished using orchiectomized skeletally-mature animals.

The effects of alcohol consumption on estrogen levels have also been investigated. Estrogens influence bone metabolism throughout life (Turner et al., 1994), including regulation of activation of bone remodeling units and the maintenance of proper balance between osteoblast and osteoclast activity. In adults, estrogen deficiency results in increased bone remodeling and a remodeling imbalance where resorption outpaces formation (Turner and Sibonga, 2001). Lower serum estrogen is associated with lower BMD in elderly men and women (Slemenda et al., 1997, Wosje and Kalkwarf, 2007), and estrogen replacement therapy slows bone loss (Felson et al., 1993) and decreases fracture risk (Cauley et al., 1995). There is evidence that moderate alcohol may modulate the effects of estrogen through increased estrogen receptor alpha expression (Colantoni et al., 2002, Singletary et al., 2001), increased aromatization of testosterone to estrogens in liver (Cheung et al., 1995, Gordon et al., 1979) and decreased clearance of estrogen, potentially via inhibition of estradiol catabolism (Ginsburg et al., 1995, Hoffman et al., 1981). In humans, moderate alcohol intake is associated with increased serum estrogen in postmenopausal women (Nagata et al., 1997, Onland-Moret et al., 2005, Reichman et al., 1993). Some studies report that moderate alcohol intake is linked to increased serum estrogens in men and premenopausal women (Rinaldi et al., 2006, Venkat et al., 2009). Acute alcohol administration was reported to result in increased plasma estradiol within 25 minutes of administration but not later (Mendelson et al., 1988). This time dependence may explain why some studies report no differences in estrogen levels in either male or female moderate drinkers (Dorgan et al., 1994, Sierksma et al., 2004). It is not clear whether brief transitory increases in estrogen levels induced by alcohol consumption would affect bone metabolism. To test this possibility, 6-month-old ovariectomized rats were fed diets with varying levels of alcohol. Slightly higher uterine weight, a sensitive measure of estrogen status, was observed when alcohol contributed 12% of daily energy. However, neither this level nor 36% of total energy impacted ovariectomy-induced cancellous bone loss (Kidder and Turner, 1998). Thus it is not yet clear whether changes in estrogen levels associated with regular alcohol consumption are sufficient to impact bone metabolism.

Molecular Mechanisms by Which Alcohol May Impact Bone Metabolism

Chronic administration of ethanol to rodents can reduce differentiation of osteoblasts from progenitor cells in culture (Rosa et al., 2008, Suh et al., 2005) and high levels of ethanol can act directly on cultured osteoblasts to decrease their activity (Friday and Howard, 1991), induce senescence pathways (Chen et al., 2009), and stimulate expression of receptor activator of nuclear factor kappa-B ligand (Chen et al., 2008). Additionally, high levels of ethanol can act directly on osteoclasts to increase their activity and differentiation (Cheung et al., 1995). Ethanol has also been shown to promote the differentiation of mesenchymal stromal cells into adipocytes rather than osteoblasts (Cui et al., 2006, Wezeman and Gong, 2004). This latter action could explain the increased marrow adiposity and decreased bone mass observed following chronic heavy ethanol consumption in rodents (Maddalozzo et al., 2009). In general, cultured osteoblasts are very resistant to direct toxic effects of ethanol.

Acetaldehyde has been shown to be a much more potent inhibitor of osteoblast differentiation and activity in culture (Giuliani et al., 1999). However, the major site for alcohol metabolism is liver and it is not clear whether free acetaldehyde is present in blood (Eriksson, 2007). Some *in vitro* studies have interpreted time-dependent decreases in the concentration of ethanol in cell culture media as evidence for local metabolism of ethanol to acetaldehyde. However, Maran *et al.* reported similar time course reductions in ethanol in culture media with and without bone cells, emphasizing the need to measure the actual concentration of metabolites (Maran et al., 2001).

The effects of ethanol on bone resorption and formation *in vitro* were replicated by addition of dimethyl sulfoxide, ethylene glycol, and lecithin and opposed by cholesterol (Farley et al., 1985). This finding suggests that, by altering the organization of cell membranes, ethanol may non-selectively disrupt a multitude of regulatory pathways in bone cells. This hypothesis is supported by the intriguing diversity of actions that have been reported *in vitro*, including altered Wnt/ β -catenin signaling, PTH signaling, estrogen receptor signaling, IGF-II signaling, oxidative stress, TGF- β 1 and TGF- β 2 gene expression, and cAMP and prostaglandin E₂ production (Chen et al., 2009, Chen et al., 2010, Farley et al., 1985, Maran et al., 2001). Furthermore, animal studies implicate several additional pathways, including ethanol-induced expression of TNF α and IL-6, and reduced growth hormone signaling (Irie et al., 2008, Turner et al., 2010, Wahl et al., 2007). Thus, there is no lack of putative molecular mechanisms mediating the actions of high concentrations of ethanol on cultured bone cells or bone growth and modeling. However, mechanistic studies to date fail to address the impact of alcohol consumption on the bone remodeling cycle, the principal mechanism for maintaining bone balance in adults. There is a compelling need to perform mechanistic studies using models that more closely replicate bone physiology and drinking patterns in adult human alcohol consumers.

Conclusions

Although there have been advances in understanding the complex actions of alcohol on bone, much remains to be determined. Light to moderate alcohol consumption appears to slow age-related bone loss by decreasing the overall rate of bone remodeling. However, in spite of alcohol typically contributing 5% of total dietary energy in a moderate drinker, only a handful of intervention studies exist investigating the actions of moderate alcohol intake on bone metabolism. As such, the aforementioned conclusion must remain tentative. The reported effects of heavy drinking on bone health are highly variable, ranging from negligible to severe. Limited evidence implicates age, skeletal site evaluated, duration and pattern of drinking as important variables, but few studies systematically evaluating the impact of these have been conducted. Additionally, much of the individual variation in skeletal response to alcohol intake may be explained by the presence or absence of alcohol-associated comorbidities, including liver disease, pancreatitis, disrupted endocrine signaling, and unhealthy lifestyle choices common to alcoholics. However, the specific contribution of each remains to be established. It is reasonable to ask whether comorbidities are entirely responsible for the negative skeletal consequences associated with alcoholism. Animal studies suggest that they are not; controlled feeding studies performed in healthy rodents and non-human primates demonstrate that chronic heavy alcohol consumption suppresses

intracortical and cancellous bone remodeling. Future studies performed in skeletally-mature animal models could provide important insight into the molecular mechanisms mediating the effect of alcohol on bone remodeling, assess how comorbidities interact with alcohol in affecting bone metabolism, and model the efficacy of pharmacological interventions in restoring bone in a recovering alcoholic.

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Figure 1.

Results from the 2013 National Survey on Drug Use and Health (NSDUH) on current, binge, and heavy alcohol use among persons aged 12 or older, by age. For the NSDUH report a "drink" was defined as a can or bottle of beer, a glass of wine or a wine cooler, a shot of liquor, or a mixed drink with liquor in it. As described in the survey, estimates for the prevalence of alcohol use are reported at three levels defined for both males and females and for all ages as follows: Current (past month) use - at least one drink in the past 30 days. Binge use - five or more drinks on the same occasion (i.e., at the same time or within a couple of hours of each other) on at least 1 day in the past 30 days. Heavy use - five or more drinks on the same occasion on each of 5 or more days in the past 30 days. Please see (SAMHSA, 2014) for details.

Figure 2.

Trabecular (cancellous) and cortical Basic Multicellular Units (BMUs). Bone remodeling (resorption followed by formation) occurs throughout adult life and is a highly orchestrated process. BMUs include cells (osteoclasts, osteoblasts, and osteocytes) responsible for remodeling bone on bone surfaces (common on trabecular and endocortical but uncommon on periosteal surfaces) and within cortical bone. An imbalance between bone resorption and formation is the principal cause for osteoporosis. See (Sims and Martin, 2014) for details.

Figure 3. Relationship between alcohol consumption, BMD and fracture risk. A linear relationship existed between alcohol consumption and femoral neck BMD (A). However, compared with abstainers, persons consuming 0.5–1 drink/day have a lower risk for having a hip fracture whereas persons consuming >2 drinks/day are at greater risk (B). These findings suggest that BMD has limited value in predicting fracture risk in alcohol consumers. See (Berg et al., 2008) for details.

Figure 4.

Pattern of voluntary alcohol consumption in rhesus macaques. Variation in daily alcohol consumption (A) and corresponding blood alcohol concentrations taken every fifth day (B) over the 12-month duration of study in four representative male rhesus macaques. This drinking pattern is similar to that observed in human alcoholics. Photomicrographs depicting intracortical remodeling are shown in a control rhesus macaque (C) and an alcohol-consuming rhesus macaque (D). The photos illustrate the reduction in tetracycline labeled

osteons and in porosity indicating reduced intracortical bone remodeling in the monkey voluntarily consuming alcohol. See (Gaddini et al., 2015) for details.

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Table 1

Definition of low, moderate, and high alcohol consumption. In the U.S., a standard alcoholic drink is defined as 14 g (or 17.7 ml) of pure ethanol (cdc, 2012). This is roughly equivalent to 12 fl oz of beer, 8 fl oz of malt liquor, 5 fl oz of wine, or 1.5 fl oz of 80-proof distilled spirits.

Low	Infrequent consumption of small quantities of alcohol
Moderate	Regular (3 d/w) consumption of 14 g/d ethanol for women and 28g/d ethanol for men
High	Consumption levels of alcohol substantially above moderate, either as chronic (most d/w) or binge (70 g/setting)

Table 2

Summary of representative animal studies (2000-present) investigating the effects of ethanol on bone in skeletally immature and skeletally mature rodents. See (Turner 2000) for review of earlier studies.

Model	Gender	Age at Start of EtOH Treatment (months)*	Duration of EtOH Treatment (months)*	Method of EtOH Administration	Quantity of EtOH Administered	Reference
<u>Skeletally immature</u>						
Rat (Sprague-Dawley)	M/F	1.0	1.0	Liquid diet	36% of calories	Wezeman et al., 2003
Rat (Sprague-Dawley)	M	1.0	1.4	Liquid diet	35% of calories	Maddalozzo et al., 2009
Rat (Wistar)	M	1.4	0.7	IP injection	3 g/kg/d for 4 consec d/wk	de Araujo et al., 2014
Rat (Wistar)	M	1.5	3.0	Drinking water	30.3±1.5 mL/d of 15% (v/v)	Lima et al., 2011
Rat (Sprague-Dawley)	M	1.6	0.3 or 1	IP injection	3 g/kg/d for 3 consec d/wk	Callaci et al., 2010
Rat (Sprague-Dawley)	M	1.6	0.3 or 1	IP injection	3 g/kg/d for 3 consec d/wk	Lauing et al., 2008
Mouse (C57BL/6)	M	1.9	4.0	Liquid diet	26% of calories	Zhang et al., 2002
Mouse (C57BL/6)	M	1.9	4.0	Liquid diet	26% of calories	Dai et al., 2000
Rat (Wistar)	M	2.0	3.0	Drinking water	7.6 g/kg/day	Broutik et al., 2010
Mouse (C57BL/6)	M	2.0	1.7	Liquid diet	36% of calories	Wahl et al., 2007
Rat (Wistar)	M	2.1	0.7	Liquid diet	35% of calories	Iitsuka et al., 2012
Mouse (C57BL/6)	F	1.4	2.6	Liquid diet	10% or 36% of calories	Mercer et al., 2012
Mouse (C57BL/6)	F	1.4	1.3	Liquid diet	30% of calories	Mercer et al., 2012
Mouse (C57BL/6)	M	1.4	2.0	Liquid diet	28% of calories	Yang et al., 2014
Mouse (C57BL/6)	F	1.4	1.3	Liquid diet	30% of calories	Mercer et al., 2014
Rat (Wistar)	M	1.9	4.0	Drinking water	35% (v/v)	Maurel et al., 2011
Rat (Wistar)	M	1.9	4.0	Drinking water	35% (v/v)	Maurel et al., 2012
Rat (Wistar)	M	1.9	4.0	Drinking water	35% (v/v)	Maurel et al., 2013a
Rat (Wistar)	M	1.9	2.6	Ethanol vapor		Maurel et al., 2013b
Rat (Wistar)	M	1.9	4.0	Drinking water	25% or 35% (v/v)	Maurel et al., 2014
Rat (Sprague-Dawley)	F	2.0	1.0	Intragastric infusion	12 g/kg/day	Chen et al., 2011
Rat (Sprague-Dawley)	M	2.1 (mean)	3.0	Liquid diet	36% of calories	Chakkalakal et al., 2002
Rat (Sprague-Dawley)	M	2.3	1.5	Liquid diet	15% (v/v)	Nyquist et al., 2002
Rat (Wistar)	M	2.3	1.0	Liquid diet	36% of calories	Irie et al., 2008
Rat (Sprague-Dawley)	M	2.3	1.4	Liquid diet	35% of calories	Maddalozzo et al., 2009

Model	Gender	Age at Start of EtOH Treatment (months) *	Duration of EtOH Treatment (months) *	Method of EtOH Administration	Quantity of EtOH Administered	Reference
Rat (Sprague-Dawley)	M	2.6	2.2	Intragastric infusion	12–13 g/kg/d	Ronis et al., 2014
Rat (Wistar)	M	2.7 (mean)	3.0	Liquid diet	26 or 36% of calories	Chakkalakal et al., 2005
Rat (Sprague-Dawley)	M	3.0	1.4	Liquid diet	35% of calories	Howe et al., 2011
Rat (Sprague-Dawley)	M	3.0	1.4	Liquid diet	35% of calories	Iwaniec et al., 2008
Rat (Sprague-Dawley)	M	3.0	0.5	Liquid diet	12–35% of calories	Turner et al., 2010
Rat (Sprague-Dawley)	M	3.0	0.5	Liquid diet	35% of calories	Turner et al., 2001a
Rat (Sprague-Dawley)	M	3.0	1.4	Total enteric nutrition	12 g/kg/d	Perrien et al., 2004
Rat (Sprague-Dawley)	M/F	3.0	1.4	Total enteric nutrition	12 g/kg/d	Perrien et al., 2002
Rat (Wistar)	F	3.0	1.9	Drinking water	20% solution	Marchini et al., 2014
Rat (Sprague-Dawley)	M	3.0	1.4	Liquid diet	35% of calories	Trevisol et al., 2007
Rat (Sprague-Dawley)	M	3.7	0.3 or 1	IP injection	3 g/kg/d for 3 consec d/wk	Callaci et al., 2009
Rat (Sprague-Dawley)	M	3.7	0.3 or 1	IP injection	3 g/kg/d for 3 consec d/wk	Himes et al., 2008
Rat (Sprague-Dawley)	M	4.0	0.3	IP injection & gavage	1.2 g/kg/d	Iwaniec and Turner, 2013
<u>Nearing skeletal maturity</u>						
Rat (Sprague-Dawley)	M	6.0	3.7	Liquid diet	35% of calories	Reed et al., 2002
Rat (Sprague-Dawley)	M	6.0	3.7	Liquid diet	35% of calories	Johnson et al., 2014
Rat (Sprague-Dawley)	M	6.0	1.0	Liquid diet	35% of calories	Hefferan et al., 2003
<u>Skeletally mature</u>						
Rat (Sprague-Dawley)	M	8.0	3.7	Liquid diet	35% of calories	Sibonga et al., 2007
Rat (Sprague-Dawley)	F	8.0	4.0	Liquid diet	3,6,13, or 35% of calories	Turner et al., 2001b
Rat (Sprague-Dawley)	F	9.0	1.9 or 3.3	Liquid diet	35% of calories	Hogan et al., 2001
Mouse (C57BL/6)	M	12.0	3.0	Liquid diet	36% of calories	Wahl et al., 2006
<u>Unknown skeletal age</u>						
Rat (Sprague-Dawley)	F	Unspecified	0.5 or 1	IP injection	3 g/kg/d for 3 consec d/wk	Callaci et al., 2006
Rat (Wistar)	M	Unspecified	1.7	Drinking water?	20%	Correa et al., 2015
Rat (Sprague-Dawley)	F	Unspecified	0.7	Intragastric infusion	12g/kg/d	Chen et al., 2006
Rat (Sprague-Dawley)	F	Unspecified	0.5	Intragastric infusion	8.5–14 g/kg/d	Shanker et al., 2006
Rat (Sprague-Dawley)	F	Unspecified	0.3, 0.5, or 1	Intragastric infusion	13g/kg/d	Shanker et al., 2008
Rat (Wistar)	M	Unspecified	1.9	Liquid diet	17 g/kg/day	Elmali et al., 2002

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Model	Gender	Age at Start of EtOH Treatment (months) *	Duration of EtOH Treatment (months) *	Method of EtOH Administration	Quantity of EtOH Administered	Reference
Rat (Sprague-Dawley)	M	Unspecified	0.2 or 1.4	IP injection	20 or 30% (v/v)	Rai et al., 2008

* Converted from days or weeks to months based on a 30-day month
Abbreviations: EtOH, ethanol; M/F, male/female; IP, intraperitoneal; consec, consecutive; vol, volume.