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Magnesium homeostasis and alcohol consumption

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Abstract. Clinical and experimental evidence indicates alcohol consumption as one of the major causes of magnesium loss from several tissues. As a result of this loss, serum magnesium tends to decrease while urinary magnesium excretion increases 2-3 fold. Experimental data confirm that chronic consumption of 6% ethanol in the Lieber De-Carli diet for 3 weeks results in a marked decrease in total tissue magnesium content in rats. This decrease affects brain, liver and all skeletal muscle, including heart, to a varying extent. While a full picture of the implications of magnesium loss in these tissues is still lacking, it is becoming progressively clear that magnesium loss affects energy production, protein synthesis, cell cycle, and specific functions in the various organs affected. In addition, as magnesium regulated cytokine production and secretion, especially in macrophages and leukocytes, a major role of magnesium deficiency in alcohol-induced inflammatory processes can be envisioned. Considering all these various aspects together, it becomes apparent that magnesium loss may represent a predisposing factor to the onset of alcohol-induced pathologies including brain stroke, sarcopenia, cardiomyopathy, steatohepatitis and cirrhosis. The present review will attempt to clarify some of the mechanisms by which ethanol impairs magnesium transport and homeostasis in brain, brain vasculature, skeletal muscle, heart and liver cells, as a first step towards more mechanistic studies aimed at relating magnesium loss with the incurrence of short- and long-term ethanol-induced complications in these organs.

Key words: ethanol, Mg^{2+} homeostasis, Mg^{2+} deficit, liver, brain, vasculature, skeletal muscle, hearth

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Mg^{2+} homeostasis and transport

Magnesium (Mg^{2+}) is the second most abundant cellular cation after potassium [1]. Due to technical limitations, the cellular partitioning of Mg^{2+} remains incomplete and fragmentary. Based upon cellular fractionation and pharmacological or instrumental approaches, a picture emerges whereby Mg^{2+} is highly compartmentalized within nucleus, mitochondria, endo-(sarco)-plasmic reticulum, and cytoplasm [1, 2]. Within each of the three cellular compartments, concentrations of total Mg^{2+} ranging between 16 to 20 mM have been measured or estimated [1, 2]. In cytoplasm, Mg^{2+} predominantly forms a complex with ATP (4 to 5 mM) or other phosphonucleotides [3], while the free Mg^{2+} concentration ranges between 0.7 and 1 mM [1, 2]. A similar range of free Mg^{2+} concentration has been reported to be present in the mitochondrial matrix [4, 5] as well as outside the cell [1, 2], indicating that very little or no gradient exists among the free Mg^{2+} concentration in the extracellular space, the cytoplasm, and the mitochondria matrix. This near *zero trans* condition, however, does not prevent Mg^{2+} from moving across the biological membrane of the cell or cellular organelles upon specific hormonal or metabolic stimuli (reviewed in [1, 2]). During the last 20 years, compelling evidence has accumulated

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which indicates that mammalian cells tightly control Mg^{2+} transport across the plasma membrane under a variety of physiological conditions. All the eukaryotic cell types so far investigated show the operation of two distinct Mg^{2+} extrusion mechanisms in the plasma membrane, identifiable as a Na^+-dependent and a Na^+-independent transporters (reviewed in [1, 2]). Because neither of these transporters has been cloned, their operation is largely surmised, based upon experimental data either in intact cells [6, 7] or purified plasma membrane vesicles [8, 9]. Both experimental models consistently indicate that the Na^+-dependent Mg^{2+} extrusion mechanism, tentatively indicated as a Na^+/Mg^{2+} exchanger [10], is activated *via* cAMP-dependent phosphorylation [11, 12]. Mammalian cells are also able to accumulate Mg^{2+} from the extracellular space upon stimulation by hormones or agents that either decrease cellular cAMP [7] or activate PKC signaling [13, 14]. Several Mg^{2+} entry mechanisms have been identified. These mechanisms include channels (TRPM6 and TRPM7), solute transport carriers (SLC41-A1 and A2), as well as several other proteins (e.g. ACDP2, MagT1, MMgT1 and MMgT2), that can favor Mg^{2+} entry at least under well defined conditions. The modality of operation of these transporters has recently been reviewed by Schmitz *et al.* [15], and we refer the interested reader to that publication. However, the relative role of these transporters in Mg^{2+} accumulation and their modality of activation, especially upon hormonal stimulation, still remain to be fully elucidated. Lastly, our laboratory has reported that, at least in plasma membrane vesicles, the putative Na^+/Mg^{2+} exchanger present therein can operate in the reverse mode and favor Mg^{2+} accumulation in the vesicles in exchange for entrapped Na^+ [8].

Mg^{2+} homeostasis and transport in disease

As our understanding of the general mechanisms regulating Mg^{2+} homeostasis and content within tissues and in the blood has improved, so has the clinical evidence that both cellular and serum Mg^{2+} levels are significantly affected under a variety of hormonal or metabolic dysfunctions, including hyperaldosteronism [16], diabetes [17], hypertension [18] or alcoholism [19]. It is important to note that in all these conditions the decrease in Mg^{2+} content within tissues is associated to a decrease in K^+ content and an increase in Na^+ and eventually Ca^{2+} levels.

In the particular case of alcoholism and alcohol consumption, a growing body of evidence indicates ethanol as one of the most common causes of Mg^{2+} deficit in humans. The process is clearly multifactorial as diet, ethanol-dependent malnutrition and/or increased diuresis can all play a role in decreasing tissue and serum Mg^{2+} levels.

The present review will revised the state of our knowledge about the effect of ethanol (EtOH) on specific tissues in which dysregulation of Mg^{2+} homeostasis or transport by alcohol consumption has been studied more extensively.

Effect of ethanol on Mg^{2+} homeostasis in brain and brain vasculature

Chronic or "binge" EtOH consumption is associated to a series of neuronal complications ranging from Korsakoff's syndrome [20] to neurological deficits [21] and stroke [22, 23]. The majority of these complications have been attributed to the EtOH-induced vasospasm that affects arterioles and venules, which can then result in the rupture of cerebral microvessels, especially at the postcapillary level [24, 25]. A report by Altura's group [26] clearly indicates that a reduction in extracellular Mg^{2+} elicits arteriolar and venular vasospasms similar to those observed in the same vessels following EtOH administration. This observation, together with the report of an increase in cytosolic Ca^{2+} under basal conditions and following IP_3-induced reticular Ca^{2+} release [27] within endothelial cells, can easily explain the increased pulsatile vasoconstriction the brain vasculature experiences following EtOH consumption, and the consequent elevated incidence of brain hemorrhage and stroke observed in ethylists [28]. ^{31}P-NMR [29] and fluorescent [30] spectroscopy studies indicate a rapid loss of Mg^{2+} in the whole brain *in situ*, and in astrocytes in particular. The latter observation is particular important in that these glial cells are reputed to be essential to maintain metabolic homeostasis in the brain [31].

Aside from modification of Mg^{2+} content in astrocytes or other neuronal components (see [32] as a review], the major EtOH-induced modifications of Mg^{2+} homeostasis occur at the level of the endothelial cells of the cerebral vasculature. In fact, acute and chronic exposure of these cells to EtOH resulted in a decrease in cellular Mg^{2+} content [33], associated to changes in the function or expression of protein kinase C (PKC) isoforms [33, 34], in the level of tyrosine phosphorylation [34], and in oxidative processes [35]. Ethanol administration also decreases Mg^{2+} content inside cerebral

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vascular smooth muscle cells as well as in the extracellular milieu *in vivo* [36]. The decrease in extracellular Mg^{2+} concentration affects the activity of these cells in that it results in an upregulation of proto-oncogenes *c*-fos and *c*-jun, and in the expression of NF-kappaB [36], with potential important consequences for the development of hypertension, vascular disease, or stroke. On the other hand, Mg^{2+} supplementation exerts multiple beneficial effects in decreasing the intensity or the duration of clinical symptoms associated with excessive EtOH consumption or EtOH withdrawal [32].

Effect of ethanol administration on skeletal muscles: is there a role for Mg^{2+}?

Ethanol administration results in atrophy of skeletal muscles and in alcoholic cardio-myopathy. The group of Peters and Preedy has extensively investigated the mechanisms underlying skeletal muscle atrophy and decline in contractile strength, and provided strong, compelling evidence for the occurrence of both transcriptional and translational alterations [37]. These alterations involve a decrease in protein synthesis [38] in the absence of increased proteases activity [39] or ameliorating effect by antioxidant supplementation [38]. Wassif *et al.* [40] have shown the concomitant decrease in total mRNA and protein content in muscle biopsies from rats fed EtOH in their diet for 2 weeks. In contrast, acute ethanol administration results in a reduced protein translational rate in skeletal muscles [41] but not in liver or diaphragm [37, 42] in the absence of mRNA changes. The fiber composition appears to play a key role in the muscle response to EtOH consumption, in that the decrease in the rate of protein synthesis is greater in type II than in type-I fiber-predominant muscles (*i.e.* plantaris *vs* soleus). In mixed fiber muscle (*e.g.* gastrocnemius), EtOH administration reduced the protein synthesis in both fiber types to a comparable extent. Irrespective of acute or chronic EtOH administration, studies carried out with 4-methylpyrazole and cyanamide to inhibit ethanol conversion to acetaldehyde and acetate, respectively, indicate that the effect of ethanol administration on muscle fibers is exerted by ethanol *per se* and *via* acetaldehyde formation [37]. Aside from an early study by Gonzales-Reimers *et al.* [43], which reported no changes in muscle Mg^{2+} content upon chronic EtOH administration, no other study to our knowledge has addressed the possible involvement of EtOH-induced Mg^{2+} loss in some of the modifications induced by ethanol in skeletal muscle, despite the clear evidence of direct and indirect roles of Mg^{2+} in modulating protein synthesis [44] and stabilizing nucleic acid [45].

Also, at the cardiac level, no systematic study has investigated the effect of acute or chronic EtOH consumption on cardiac Mg^{2+} content. At variance with that reported for the skeletal muscle, in which acute and chronic EtOH administration results in muscle protein loss, a conundrum is present in the cardiac field. The general view-point is that acute, moderate EtOH consumption exerts beneficial and protective effects on the heart and the cardiovascular system generally [46]. In contrast, excessive alcohol intake is responsible for toxic alcoholic cardiomyopathy [46] and cardiac enlargement [47]. This discrepancy may be explained to some extent by the absence of alcohol dehydrogenase in the cytoplasm of cardiac cells *versus* the presence of alcohol-inducible cytP450-2E1 within the myocyte sarcoplasmic reticulum [46]. Both these enzymes are responsible for the conversion of ethanol to acetaldehyde, to which several deleterious effects in skeletal muscles [37] or liver [48] have been attributed. However, the metabolic conversion of EtOH via cytP450-2E1 is coupled with the production of free radicals, reactive oxygen species and lipid peroxidation products that react highly with phospholipids and proteins [48]. As the cytP450-2E1 is inducible and becomes more operative at higher doses of EtOH, it is a reasonable explanation to justify the different effects of moderate *vs* chronic EtOH consumption on cardiac function. The production of large quantities of acetaldehyde within the cardiac myocytes, in fact, depresses the cardiac contractile function [49] and results in the release of significant amounts of troponin C into the extracellular space [50]. As already observed for the brain [32], exogenous Mg^{2+} supplementation in the diet ameliorates the myocardial dysfunction induced by acute or chronic ethanol administration, renormalizing heart size, isometric force and isotonic shortening [47]. How exactly Mg^{2+} elicits these effects has not been investigated. However, because Mg^{2+} is considered to act as a natural Ca^{2+}-channel blocker, it is reasonable to hypothesize that the changes in force development and cell shortening depend on the restoration of normal cellular Ca^{2+} levels, which directly impact on the contractile myofilaments. Less clear is whether the renormalization of heart size depends on the restoration of normal cellular Ca^{2+} levels or, alternatively, on direct effects of Mg^{2+} on protein synthesis and mRNA translation.

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Effect of ethanol administration on Mg^{2+} homeostasis in liver cells

The last organ to be discussed in this review is the liver, for which more mechanistic studies have been carried out in our and in other laboratories as well. Liver cells represent the most utilized models for ethanol-related studies for obvious reasons.

Acute ethanol administration

Several lines of evidence indicate that EtOH consumption is associated to a decrease in hepatic Mg^{2+} content [19]. However, for the most part these changes in Mg^{2+} content have been investigated following prolonged EtOH administration rather than acute ethanol consumption. Our laboratory has been almost unique in its attempt to elucidate the effect of both acute and chronic ethanol consumption on Mg^{2+} homeostasis and transport in liver cells.

The administration of an acute dose of EtOH to a perfused liver or a suspension of hepatocyes results in a time- and dose-dependent Mg^{2+} extrusion [51]. In both models, the extrusion is maximal within 8 min from the administration of EtOH, irrespective of the dose of alcohol administered or its persistence in the perfusion or incubation system [51]. Maximal Mg^{2+} extrusion is observed at a dose of 1% EtOH (approximately 150 mM), as larger doses of alcohol are associated to the release of cytoplasmic lactate dehydrogenase as a result of plasma membrane leakage [51]. The Mg^{2+} extrusion occurs via an amiloride-inhibited Na^+-dependent pathway (most likely the Na^+/Mg^{2+} exchanger described previously), and as the result of EtOH metabolism, via the cytosolic alcohol dehydrogenase [51]. In fact, Mg^{2+} extrusion is almost completely prevented by pre-treatment of liver cells with the alcohol dehydrogenase inhibitor 4-methylpyrazole [51]. Biochemical analysis of liver cells and liver tissue indicates that the Mg^{2+} extrusion is the result of a transient decrease in cellular ATP [51, 52], which returns to basal levels within 8 min of alcohol administration via activation of the penthose shunt [53]. Remarkably, the time of Mg^{2+} extrusion coincides precisely with the time of ATP restoration, further underlying the importance of this moiety for the maintenance of Mg^{2+} homeostasis [54]. As a result of this association, it is not surprising that Mg^{2+} content is markedly decreased [51, 52] within cytoplasm and mitochondria, the two main cellular compartments in which Mg^{2+} and ATP are stored [1, 54]. The loss of Mg^{2+}, however, is not confined to these two compartments but also affects the endoplasmic reticulum [52], another major pool of Mg^{2+} within the cell, as well as a site of ethanol metabolism [55]. As the cellular pools become depleted of Mg^{2+}, the administration of subsequent doses of EtOH results in progressively smaller mobilizations of Mg^{2+} that, to some extent, have lost their dose-dependent connotation [52].

The acute administration of EtOH also affects how liver cells respond to catecholamine stimulation. The administration of epinephrine or norepinephrine results in a rapid extrusion of Mg^{2+} via the Na^+/Mg^{2+} exchanger [6, 7]. The depletion of cytoplasm, mitochondria and endoplasmic reticulum Mg^{2+} pools by a previous dose of EtOH markedly inhibits the amount of Mg^{2+} mobilized by catecholamine [52]. The larger the dose of EtOH used, the larger the depletion of the cellular pools and consequently the inhibition of catecholamine-induced Mg^{2+} extrusion. Interestingly, this inhibition persists even in liver cells pre-treated with 4-methylpyrazole [52], implying a possible direct effect of EtOH on the G protein signaling machinery responsible for cAMP generation within the cell. When the sequence of administration of EtOH and catecholamine is reversed, however, the Mg^{2+} extrusion elicited by catecholamine does not impair the subsequent mobilization of Mg^{2+} elicited by EtOH [52]. Biophysical analysis of plasma membrane vesicles purified from livers perfused with EtOH indicates that the operation of the Na^+-dependent and Na^+-independent Mg^{2+} extrusion mechanisms in a system devoid of signaling and buffering components as well as cellular compartments is completely unaffected by the administration of EtOH during the perfusion or directly on plasma membrane suspension [52, 56].

Because of the Mg^{2+} loss induced by the EtOH administration, it could be expected that Mg^{2+} depleted hepatocytes would be prone to rapidly accumulate Mg^{2+} and restore its cellular homeostasis. However, this is not the case. The administration of a dose of ethanol as small as 0.01% (*i.e.* 1.5 mM) is already sufficient to inhibit Mg^{2+} accumulation for up to 60 min (Torres *et al.*, unpublished). This inhibition appears to depend on a defective redistribution of PKCε to the cell membrane following administration of phorbol-myristate acetate derivates, diacylglycerol analogs or vasopressin (Torres *et al.*, unpublished). Consistent with this hypothesis, the restoration of Mg^{2+} accumulation follows closely the time course of PKCε relocation to the cell membrane upon removal of EtOH and the addition of any of the indicated agents (Torres *et al.*, unpublished). As a result of the cellular refilling,

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catecholamine-induced Mg^{2+} extrusion also return to the level observed in EtOH-untreated hepatocytes within the same time frame (Torres *et al.*, unpublished).

Chronic athanol administration

As expected, based upon the effects described in the previous section, the chronic administration of EtOH in the diet for 3 weeks, according to the Lieber-De Carli protocol, results in a loss of Mg^{2+} that accounts for 22% to 25% of total hepatic Mg^{2+} content [57]. This loss is associated to a 17% decrease in the ATP level, and affects all the main cellular Mg^{2+} pools (namely cytoplasm, mitochondria and endoplasmic reticulum) [57]. Due to the massive depletion in cellular Mg^{2+} obtained in this EtOH model, the Mg^{2+} extrusion induced by catecholamine is completely abolished [60]. At variance with observations in the acute model, the Mg^{2+} depletion induced by EtOH in this chronic model is only a partial explanation for the defective Mg^{2+} mobilization observed here. Analysis of liver plasma membrane vesicles isolated from the liver of rats fed ethanol in the diet for 3 weeks indicates that both the Na^{+}-dependent and Na^{+}-independent Mg^{2+} extrusion mechanisms are impaired to a comparable extent (~75%) [56, 57]. The reasons for such an inhibition could be multiple and not mutually exclusive, ranging from an abnormal presence of phosphatidylethanolamine within the cell membrane [58] to the formation of non-functional adducts between protein or phospholipids and 1-hydroxy-ethyl radical [59] or other free radical derivates, including malonyldialdehyde or 4-hydroxynonenal [48, 60].

The same defect in PKCε translocation observed in liver cells acutely treated with EtOH is present following chronic EtOH administration. However, in this model, the time necessary for Mg^{2+} accumulation to return to basal level (i.e. to the level observed in hepatocytes not treated with EtOH) is approximately 10 to 12 days instead of about 1 hour both in intact cells and plasma membrane vesicles (Torres *et al.*, unpublished). As Mg^{2+} accumulation improves and returns to normal levels, catecholamine-induced Mg^{2+} extrusion also returns to the levels observed in hepatocytes from control-diet rats [58]. Because of the similar time necessary for the full restoration of cellular Mg^{2+} content and transport, it is tempting to explain the latter process with the restoration of Mg^{2+} accumulation and the refilling of the cellular pools. Yet, this explanation does not provide a full justification as to why the transport in plasma membrane vesicles remains defective at early time points upon EtOH removal from the diet. Because the assessment of Mg^{2+} transport in plasma membrane vesicles was performed in the presence of 20 mM intravesicular Mg^{2+} (*i.e.* a concentration that resembles the concentration present within the cell) under all the experimental conditions, it can be excluded that the limited Mg^{2+} extrusion depends on the amount of Mg^{2+} present within the vesicles. Hence, defects in phospholipid composition or the formation of adducts between proteins and phospholipids and lipid peroxidation products, as indicated above, must be involved. Alternatively, the time necessary for Mg^{2+} transport, and Mg^{2+} accumulation in particular, to be restored to basal level would be consistent with the time necessary for the expression in the membrane of newly expressed and synthesized Mg^{2+} transporters. Future studies are needed to elucidate this possibility for the various Mg^{2+} entry mechanisms so far identified. At least for the time being, similar studies are precluded for the Na^{+}-dependent and Na^{+}-independent Mg^{2+} extrusion mechanisms, since neither of these transporters has been cloned up to date.

Possible implications of Mg^{2+} loss for liver functions

Despite recent advances in understanding how acute and chronic ethanol administration impair hepatic Mg^{2+} homeostasis and transport, we are far from being able to associate in a cause-effect relationship the observed changes in Mg^{2+} levels with any of the short- or long-term complications observed in ethanol-related liver pathologies. However, some possibilities worth exploring in future studies can be mentioned.

Previous work by the group of Rayssiguier [61] has indicated that loss of hepatic Mg^{2+} by EtOH or CCl4 administration is associated with an increased collagen deposition. This observation would then suggest that a decrease in hepatic Mg^{2+} content may spell dire consequence for the development of hepatic cirrhosis. It is important to note that, despite a relatively large percentage of people being affected by steatohepatitis, the initial and relatively benign level of alcohol-related liver alteration, only 15% to 20% of this population evolves towards alcoholic hepatitis first and then cirrhosis [62]. At the present time, however, no epidemiology study has investigated the possibility that the percentage of people progressing towards cirrhosis is somehow associated with the pre-existing hepatic level of Mg^{2+} or, alternatively, with the PKCε redistribution within liver cells. In fact, this PKC isoform

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has been associated with the modulation of fibrogen deposition within cardiac tissue [63, 64]. Similar studies have yet to be carried out in liver tissue. Unique among the various PKC isoforms, PKCε displays the highest affinity for Mg^{2+}, with a half-maximal activation at 1 mM [65], a concentration that is well within the range of cytoplasm free Mg^{2+} concentration [1, 2]. Hence, a scenario can be envisioned in which changes in PKCε translocation result in a reduced Mg^{2+} accumulation within the hepatocyte which, in turn, further affects PKCε activity and translocation, impacting on its ability to mediate Mg^{2+} accumulation. The decrease in PKCε translocation and activity would also affect other cellular functions, including fibrogen and collagen deposition. On the other hand, a decrease in cellular Mg^{2+} content can affect cation compartmentation and negatively impact on mitochondria bioenergetics, which depend highly on a proper intramitochondrial Mg^{2+} content [66, 67]. Lastly, the possible role of a Mg^{2+} deficit in the inflammatory response induced by EtOH in the liver has to be considered. Under physiological conditions, Mg^{2+} modulates the production and release of anti-inflammatory cytokines positively, while inhibiting the release of pro-inflammatory ones [68]. As inflammation plays a key role in the progression of steatohepatitis towards full blown alcohol liver disease (ALD) [62], it can be easily envisioned how a marked decrease of cellular Mg^{2+} within circulating macrophages and leukocytes, as well as *in situ* Kuppfer cells, can impact on the inflammatory response of these cells, with major consequences for liver function and cyto-architectonics.

Conclusions and future directions

The last twenty years have registered a significant improvement in our understanding of the role of Mg^{2+} in several cellular functions. Although many aspects of Mg^{2+} homeostasis and regulation still need to be elucidated, a picture is emerging whereby Mg^{2+} has become a key element to be taken into account for both physiological and pathological conditions. In the case of alcohol consumption, it is now apparent that ethanol affects whole body Mg^{2+} homeostasis with dire consequences at the level of brain, vasculature, skeletal muscles, heart, and liver. As a more detailed picture of the functions and mechanisms regulated by Mg^{2+} unfolds, new therapeutic approaches and strategies may become available to restore Mg^{2+} homeostasis and reverse or prevent short- and long-term complications of chronic alcohol consumption in humans.

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