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Reviews: Current Topics

Alcoholic muscle disease and biomembrane perturbations (Review)

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Abstract

Excessive alcohol ingestion is damaging and gives rise to a number of pathologies that influence nutritional status. Most organs of the body are affected such as the liver and gastrointestinal tract. However, skeletal muscle appears to be particularly susceptible, giving rise to the disease entity *alcoholic myopathy*. Alcoholic myopathy is far more common than overt liver disease such as cirrhosis or gastrointestinal tract pathologies.

Alcoholic myopathy is characterised by selective atrophy of Type II (anaerobic, white glycolytic) muscle fibres: Type I (aerobic, red oxidative) muscle fibres are relatively protected. Affected patients have marked reductions in muscle mass and impaired muscle strength with subjective symptoms of cramps, myalgia and difficulty in gait. This affects 40–60% of chronic alcoholics (in contrast to cirrhosis, which only affects 15–20% of chronic alcohol misuers).

Many, if not all, of these features of alcoholic myopathy can be reproduced in experimental animals, which are used to elucidate the pathological mechanisms responsible for the disease. However, membrane changes within these muscles are difficult to discern even under the normal light and electron microscope. Instead attention has focused on biochemical and other functional studies.

In this review, we provide evidence from these models to show that alcohol-induced defects in the membrane occur, including the formation of acetaldehyde protein adducts and increases in sarcoplasmic-endoplasmic reticulum Ca^{2+}-ATPase (protein and enzyme activity). Concomitant increases in cholesterol hydroperoxides and oxysterol also arise, possibly reflecting free radical-mediated damage to the membrane. Overall, changes within muscle membranes may reflect, contribute to, or initiate the disturbances in muscle function or reductions in muscle mass seen in alcoholic myopathy. Present evidence suggest that the changes in alcoholic muscle disease are not due to dietary deficiencies but rather the direct effect of ethanol or its ensuing metabolites.

Keywords: Oxidative stress; Rat; Muscle; Myopathy; Cholesterol hydroperoxides; Adducts

1. Introduction

In the following review we first describe some of the pathological aspects of alcohol misuse and then go on to discuss how this may affect the tissue membrane of skeletal muscle. We draw largely upon animal studies since this form of modelling allows the experimenter to account for nutritional influences on alcohol-induced pathologies. However, it is important to point out that the pathology we describe is expressed differently in the various muscle fibre types. Nevertheless, in this review we present data derived mainly from the analysis of Type II (anaerobic, white glycolytic) fibre-predominant plantaris and gastrocnemius muscles. Where relevant, the response of the Type I (aerobic, red oxidative) fibre-predominant muscles represented by the soleus are mentioned. The governing factors that determine differential fibre sensitivity are however beyond the scope of this review: instead the reader is directed to more detailed articles (see [1–4]).

It is also important to point out that other studies have suggested that alcohol impairs skeletal muscle and its associated membrane. This includes increased activity of Na^+-K^+-ATPase (which may possibly occur via upregulation of [^{3}H]-ouabain binding sites), impairment of calcium trans-

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port and storage (i.e., ascribed as being "*leakier* and *less ordered*") and dilatation of the sarcoplasmic reticulum [5–9]. However, we believe that the laboratory animal studies originating from our group represents the most comprehensive series of investigations into alcoholic myopathy which has employed a wide range of analytical methods, ranging from gene expression to immuno-histochemistry. These studies are described below.

2. Effects of alcohol on the body

By virtue of its extreme reactivity, alcohol has the potential to affect virtually every organ or biochemical pathway in the mammalian body including the liver, heart, reproductive organs, central nervous system, gastrointestinal tract, bone and skin to name but a few examples [10–14]. The prevalence of some forms of cancer may also be caused or exacerbated by chronic alcohol ingestion [15]. These adverse changes arise because of the extreme biochemical or chemical reactivity of ethanol itself or its reactive metabolite, acetaldehyde. Alternatively, damage to cells or organs may arise as a result of the ensuing secondary changes within the whole body, such as free radical generation [16,17] or endocrine disruption [18]. To this list, we must also add membrane changes and to a certain extent these have been reviewed previously though not with respect to skeletal muscle [19–23]. Paradoxically, most attention in the area of alcohol-related membrane pathology seems to revolve around the liver, though cirrhosis is less common than alcoholic myopathy [24]. This supposition is supported by a landmark study investigating the comparative prevalence of various pathologies in 250 chronic alcoholics: cirrhosis was diagnosed in only 20 subjects contrasting with the diagnosis of alcoholic skeletal myopathy in 117 subjects [25]. Peripheral neuropathy was observed in 41 cases and cardiomyopathy in 20 [25]. In other words, the emphasis on the liver as a target organ of ethanol toxicity is misplaced. On the other hand there is some evidence to suggest that in moderate amounts, alcohol imparts a cardioprotective effect [26,27] although this has been challenged [28]. In the following section we describe the deleterious effects of alcohol that arises as a consequence of chronic and sustained alcohol misuse and focus skeletal muscle.

3. Nutritional implications of excessive alcohol abuse and disease

Consideration needs to be given to the fact that alcohol misuse has profound implications for impaired or compromised nutritional status. This may arise via a number of processes. Firstly, organs such as the liver, which are directly involved in secondary processing or storage of nutritional components or metabolites, may be damaged [29]. For example, the liver is a major site of metabolism for the calciferols. Cholecalciferol is converted to 25-hydroxycholecaliferol in the liver and osteoporosis is a common feature in alcoholics. Impaired vitamin D status may contribute to the osteoporosis in alcoholics, and this is responsive to supplementation [30]. Secondly, there may be reduced dietary intakes in alcoholics in general. Such a statement needs to be treated with caution. Although as a rule of thumb, 50% of alcoholics will have deficiencies in one or more macro- or micronutrients, some sub-populations (middle and upper class versus lower and working class; European regions with diets high in anti-oxidants versus those with lower dietary antioxidants) may have adequate nutrition. Financial displacement of nutrient-rich food items for alcoholic-beverages may also contribute to malnutrition in alcohol-misuse. Finally, there may be malabsorption and/or maldigestion. Virtually every single region of the gastrointestinal tract is affected in alcoholism (though not in every individual) [31]. (For selective reviews on alcohol and nutrition see [32–38].

4. Effects of alcohol on skeletal muscle

Excessive and prolonged alcohol intake causes a defined myopathic lesion characterised by selective atrophy of Type II (i.e., white or anaerobic, glycolytic fast twitch) fibres [24]. The Type I (i.e., red or aerobic, oxidative slow twitch) fibres are relatively protected unless there is severe alcohol exposure in which case Type I fibres may also atrophy. In the initial stages of the disease there is some evidence of a Type I fibre hypertrophy, though the significance of this is unclear [24]. These changes are accompanied by reductions in muscle mass (by an average of 22%) and body mass index (15%) [39]. Functional impairments include cramps with frequent falls and myalgic symptoms [24]. Muscle strength is also impaired by alcohol which is related to life-time cumulative intake [40–42].

Alcoholic cirrhosis *per se* reduces muscle strength [43,44]. However, alcoholic myopathy is not related to overt liver disease [24,41]. Indeed, the notion that alcoholic myopathy may be mediated directly by excessive alcohol ingestion has been addressed by a number of studies and there is convincing evidence to show it occurs independently of either neuropathy [45], malnutrition [39] or endocrine abnormalities such as glucocorticoid excess [46]. Nevertheless, a modulating, rather than a causative role for some of these factors have been described (for example, for nutritional influences, see [25,47]).

Concomitant changes within the muscle include a reduction in muscle protein content [48,49] which implicates defects in protein metabolism. This is supported by the observation that muscle protein synthesis is reduced in alcoholic patients with myopathy [50] and protein degradation may be either unchanged or reduced [51]. Similar results have been obtained in animal models of alcoholic myopathy (reviewed in [1–4]).

5. Animal model of alcoholic myopathy

We have comprehensively investigated the pathogenic processes involved in alcoholic myopathy with suitable animal models. To investigate the putative responses of the Type I fibres, the soleus has been examined whereas the plantaris and gastrocnemius are taken to represent the Type II fibres [52,53].

In acute studies, rats are treated with a bolus of ethanol at a standard dose of 75 mmol/kg body weight, intraperitoneally. This ensures a rapid and sustained circulating level of ethanol. Thus, plasma levels of ethanol are approximately 450, 375, 290 185 and 0 mg/100 ml, at 0.33, 1, 2.5, 6 and 24 hours respectively [54]. In our studies, animals are sacrificed at various time internals up to 24 hours, though most studies entail a 2.5 hour dosing period [55]. Reductions in protein muscle protein synthesis occur after 1 hour and the effects persist for 24 hours when there is no measurable blood alcohol [54]. Reductions in total RNA (i.e., largely ribosomal) and muscle protein contents are observed only after 24 hours [54]. Molecular changes within muscle exposed acutely to alcohol include increased in the mRNA levels encoding c-myc [56]. Skeletal muscle protease activity also decreases acutely [54]. Other changes have been reviewed previously [1–4]). The intraperitoneal route is used in acute dosing studies as this ensures complete bioavailability of the test substance [55]. For example, giving rats the same dose of ethanol by gavage (75 mmol/kg body weight) produces significantly lower levels of blood alcohol at 2.5 hour probably because of first-pass metabolism [57]. However, even with this acute dosing by gavage, a significant reduction in skeletal muscle protein synthesis still occurs, though the magnitude of the decrease is less than the effect seen in rats treated identically by the intraperitoneal route (Marway, Preedy and Peters, unpublished).

In chronic studies, a different protocol is used to overcome the fact that in a free-choice system, experimental animals have an aversion for alcohol [58,59]. To resolve this rats are fed a nutritionally complete liquid diet, containing ethanol as 35% of total dietary energy [55,58,59]. Controls are fed identical volumes of the same diet in which ethanol is replaced by isocaloric glucose [55,58,59]. Thus, the ensuing effects can be ascribed to ethanol *per se* rather than nutritional limitations. However, this does not overcome the possibility that alcohol may impair either the absorption or metabolism of nutrients. In this model, mean plasma levels of ethanol are approx. 292–377 mg/100 ml, between 1 and 6 weeks [60]. There is a myopathic lesion, as defined by decreases in muscle fibre diameter and reduced protein contents as early as two weeks, particularly in the Type II fibre plantaris [61]. Myofibrillary proteins in general and myosin contents are reduced (Fig. 1) [62]. This may contribute to the muscle weakness seen in rats muscle exposed to alcohol [63] which is similar to the impaired muscle strength of alcoholic patients [40,41]. Concomitant metabolic changes within rat muscle exposed chronically to alcohol include increased RNase activities, reduced protein synthesis and loss of ribosomal RNA (reviewed in [1–4]). There appears to be some tolerance, as some variables seen in acute ethanol dosed rats (such as increased c-myc mRNA) do not appear to occur in chronic ethanol dosed rats [64].

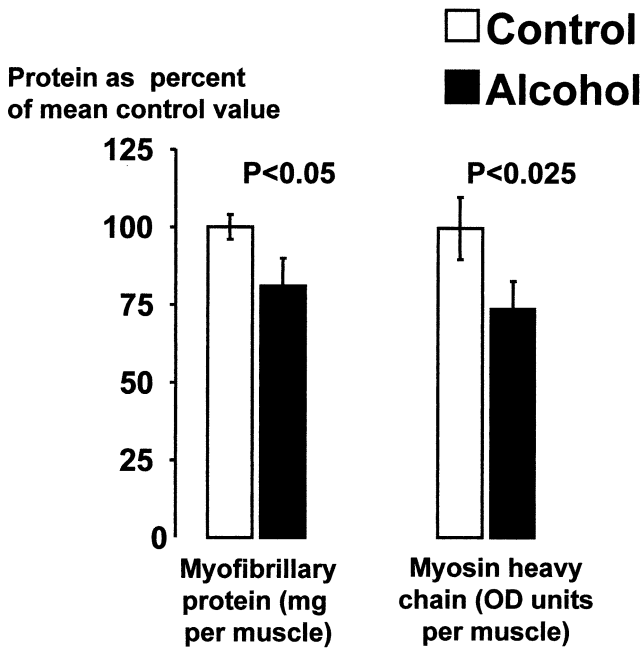

Fig. 1. Myofibrillary and myosin heavy chain protein in rats fed ethanol for 6 weeks. Total myofibrillary protein contents in plantaris muscle from 6-week glucose- and ethanol-fed male Wistar rats (n = 5-6), pair-fed equivalent amounts of iso-caloric, iso-volumetric, liquid diet. Data are means ± SEM. P values are displayed over the relevant histograms. Adapted from data contained in [62].

However, pathological changes within these muscles are difficult to discern even under the normal light and electron microscope [65]. For example, in skeletal muscle of rats fed alcohol for 6 weeks, necrotic fibres undergoing phagocytosis occur only infrequently and there are no observable differences in subcellular organelle structure under the electron microscope [65]. Instead, attention has focused on biochemical and other functional studies. Below we describe possible membrane changes as determined by analysis of adducts, calcium regulatory protein and cholesterol hydroperoxides and oxysterols.

6. Protein-adduct formation

Protein adducts are formed when there is covalent linkage of a peptide with another reactive compound such as acetaldehyde, malondialdehyde or free radicals such as the hydroxyethyl radical [66]. They are extensively formed in the liver [66–68]. Hybrid adducts may also be formed, such as the malondialdehyde-acetaldehyde-protein adduct [66]. The importance of adduct formation pertains to the possi-

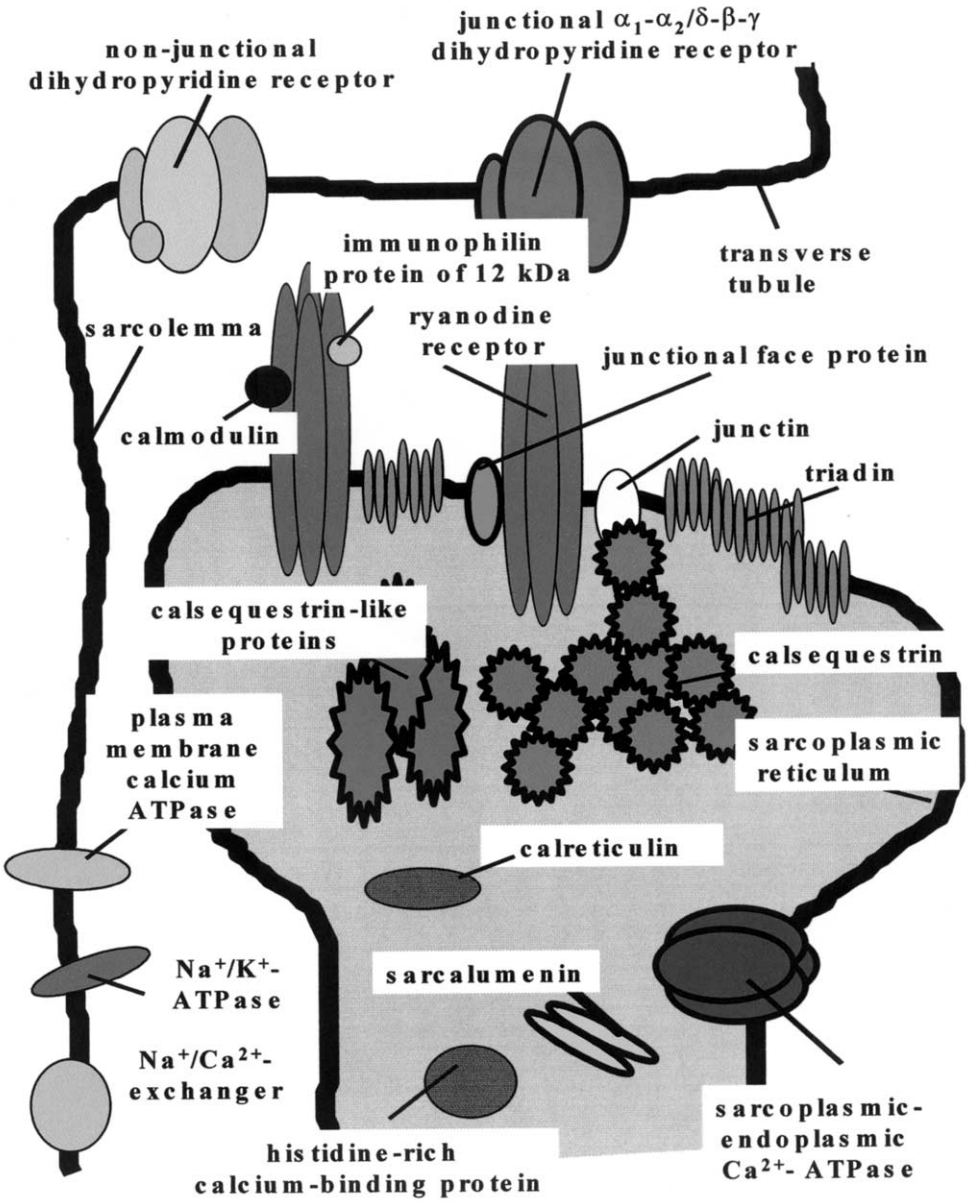

Fig. 2. Skeletal muscle proteins involved in the excitation-contraction-relaxation cycle. Various proteins are involved in the excitation-contraction-relaxation cycle of skeletal muscle, anyone of which has the potential to be targeted in alcoholic myopathy. Proteins in colour were analysed using immunoblotting techniques. (Adapted from [74] and with permission pending).

bility that adducts will (I) render the target protein inoperative by virtue of its conformational change or inactivation of functional sites; (II) initiate an immunogenic reaction or (III) be subjected to altered protein degradation by virtue of abnormal mass, charge or structure [69]. Previous *in vitro* work showed that rabbit skeletal muscle actin (particular G-actin compared to F-actin) forms stable covalent adducts with acetaldehyde in both reducing and non-reducing conditions, possibly by reaction with lysyl residues [70]. Before our involvement in this area, there were no reports of such adducts being formed in skeletal muscle using well validated methods of alcohol dosing *in vivo*. However, when we fed rats alcohol for 6 weeks using a pair-feeding protocol to produce a defined myopathy, we showed increased amounts of unreduced acetaldehyde-protein adducts in plantaris muscle using both ELISA and immuno-histochemical staining techniques [71]. However, we could not detect reduced-acetaldehyde, malondialdehyde, malondialdehyde-acetaldehyde and alpha-hydroxyethyl protein-adducts in muscle from rats fed ethanol for 6 weeks, though all these adduct species increased in liver [71]. The histochemical analysis showed unreduced-acetaldehyde protein adducts were located within the sarcolemmal (i.e., muscle membrane) and sub-sarcolemmal regions though adduct were also located in the intermyofibrillary region [71]. We do not know the nature of these adducts, nor the mechanism of their forma-

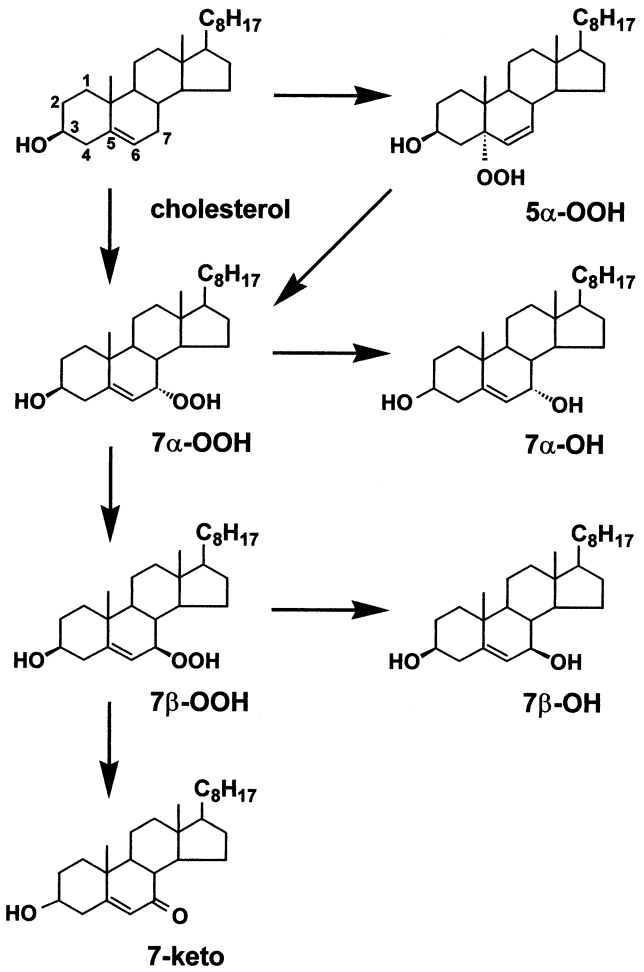

Fig. 3. Schematic representation of pathways for cholesterol hydroperoxide and oxysterol production. Schematic pathway of hydroperoxide and oxysterol production from cholesterol: 7alpha-OOH, 7alpha-hydroperoxycholesterol; 7beta-OOH, 7beta-hydroperoxycholesterol; 7-keto, 7-ketocholesterol; 5alpha-OOH, 5alpha-hydroperoxycholest-6-en-3beta-ol; 7alpha-OH, 7alpha-hydroxycholesterol; 7beta-OH, 7beta-hydroxycholesterol.

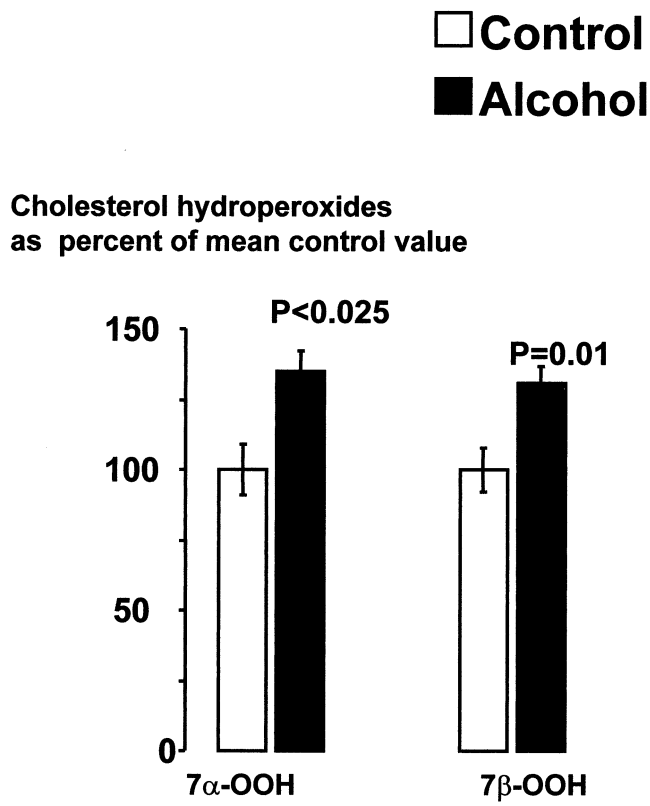

Fig. 4. Cholesterol hydroperoxides in skeletal muscle of rats treated acutely with ethanol (24 hour after administration). Rats were treated with an acute dose of ethanol (75 mmol/kg body weight) and killed 24 hours later. Controls were given an equivalent volume of saline. Cholesterol hydroperoxides were measured in plantaris muscle 7alpha-OOH, 7alpha-hydroperoxycholesterol; 7beta-OOH, 7beta-hydroperoxycholesterol. Data are mean ± SEM. From [80].

tion. Nevertheless, it is worth noting that cytochrome P450 is located in the sarcoplasmic reticulum of skeletal muscle [72]. One can speculate acetaldehyde binds to proteins within its immediate site of production. Alternatively, circulating acetaldehyde in the blood may bind with the first proteins it comes into contact with producing a gradient effects across the sarcolemmal-myofibrillary interface.

Regardless of the explanation, it is important to note that the sarcolemmal and sub-sarcolemmal regions are particularly principal sites of intracellular signalling and receptor-post-receptor cascades [22]. Thus, adducted proteins within these regions have the potential to alter biochemical or mechanical performance of skeletal muscle. It is possible that the receptors themselves will be targeted by adduct formation. We recently tested this assumption by examining regulatory and receptor proteins involved in calcium homeostasis [73].

7. Calcium regulatory proteins

The coupling of excitation-contraction and relaxation in skeletal muscle is regulated by cystosolic Ca^{2+}-levels (reviewed in [74]). There are numerous regulatory and structural proteins involved in this process, and defects in one or more of these may contribute towards the features of alcoholic myopathy. To test this we analysed muscle from rats fed alcohol for 6 weeks and quantified by immunoblotting and enhanced chemiluminescence the alpha1 and alpha2 dihydropyridine receptors, calsequestrin, sarcalumenin, and the 90kDa junctional face protein (Fig. 2; proteins analysed are depicted in different colours) [73,74]. However, the relative abundance of these microsomal proteins was relatively maintained [73]. In contrast, there were significant increases in the relative amounts of sarcoplasmic-endoplasmic reticulum Ca^{2+}-ATPase protein and Ca^{2+}-ATPase activity [73]. The upregulation of SERCA1 protein and Ca^{2+}-ATPase activity may be an adaptive mechanism in alcohol-induced muscle disease. It substantiates the aforementioned work on protein-adducts suggesting the sarcolemmal and/or sub-sarcolemmal regions are targeted by alcohol.

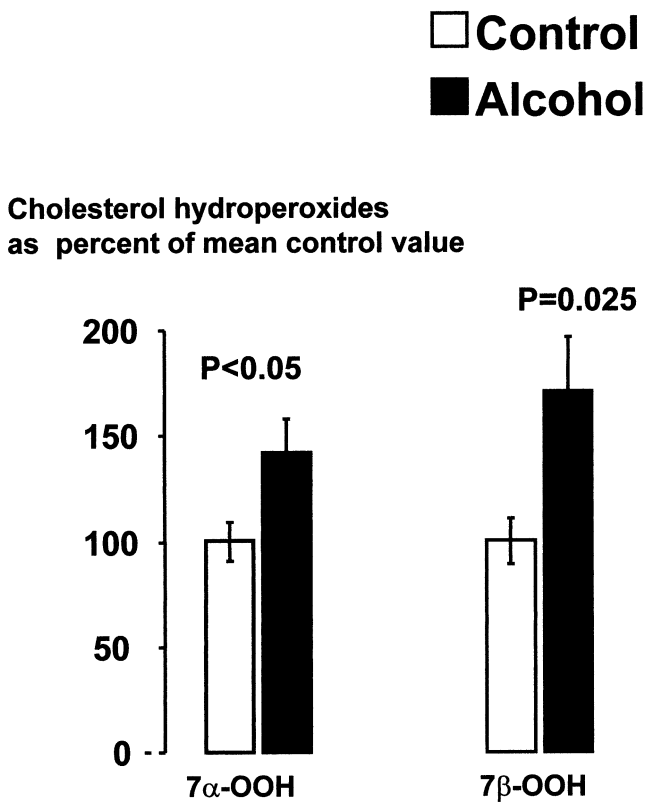

Fig. 5. Cholesterol hydroperoxides in skeletal muscle of rats treated chronically with ethanol (6 weeks continuous administration). Rats were treated with ethanol (35% of total dietary energy) and killed 6 weeks later. Controls were fed isocaloric glucose. Cholesterol hydroperoxides were measured in plantaris muscle 7alpha-OOH, 7alpha-hydroperoxycholesterol; 7beta-OOH, 7beta-hydroperoxycholesterol. Data are mean ± SEM. From [82].

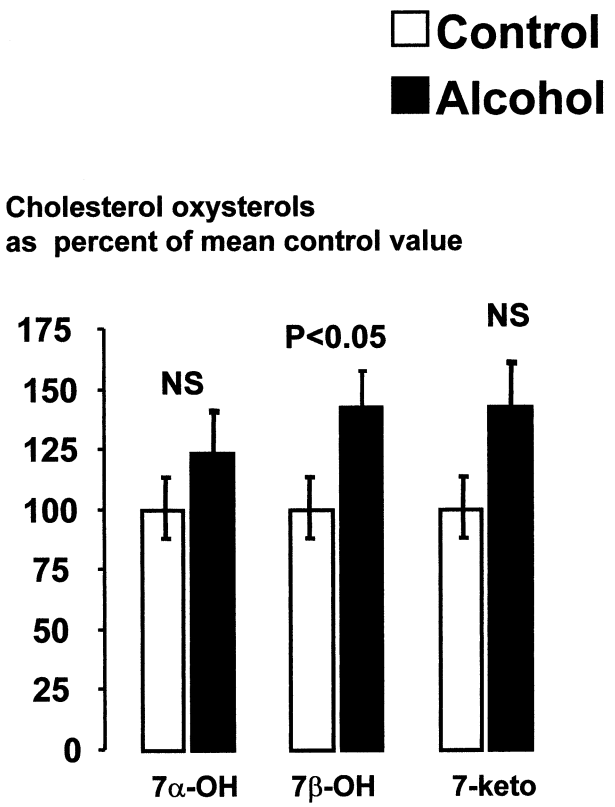

Fig. 6. Cholesterol oxysterols in skeletal muscle of rats treated chronically with ethanol (6 weeks continuous administration). Rats were treated as described in the legend to Fig. 5. Oxysterols were measured in plantaris muscle 7-keto, 7-ketocholesterol; 7alpha-OH, 7alpha-hydroxycholesterol; 7beta-OH, 7beta-hydroxycholesterol. Data are mean ± SEM. From [82].

8. Oxidative stress, and cholesterol hydroperoxides and oxysterols

Alcohol increases oxidative stress in a variety of tissues exposed to ethanol such as the liver [75], CNS [76] and heart [77] though hitherto the response of skeletal muscle has been elusive. Carbonyl concentration is a marker of oxidative stress albeit reflecting a non-enzymatic modification to proteins rather than lipids [78,79]. However, protein carbonyl does not alter in muscles of either acutely or chronically ethanol dosed rats and there is even some evidence that a small reduction occurs [80,81]. We have, nevertheless, identified and confirmed the presence of two cholesterol-derived hydroperoxides, 7α-hydroperoxycholest-5-en-3β-ol (7α-OOH) and 7β-hydroperoxycholest-5-en-3β-ol (7β-OOH) in skeletal muscle (Fig. 3) [82]. These compounds arise directly because of reactive oxygen species per se rather than other routes of metabolism [83]. Muscle concentrations of 7α- and 7β-OOH are elevated equally in soleus and plantaris muscle 24 hours after acutely dosing rats with ethanol (Fig. 4) [80]. Studies at earlier time points, i.e., 2.5 hours, demonstrate significant increases in 7α-OOH and 7β-OOH in plantaris, but the soleus is less sensitive confirming a Type II fibre specificity [84]. Chronic ethanol feeding for 6 weeks also increases 7α-OOH and 7β-OOH in both plantaris (Fig. 5) and soleus muscle (data for soleus not shown) [82].

The oxysterols 7α- and 7β-hydroxycholesterol (7α-OH and 7β-OH), and 3β-hydroxycholest-5-en-7-one (also termed 7-ketocholesterol; 7-keto) have also been measured in rats fed ethanol for 6 weeks [82]. Previously, 7-keto, 7α-OH and 7β-OH have been identified in pig and mouse skeletal muscle [85] but not in rat muscle especially with a myopathic lesion. In response to chronic alcohol feeding, increases in the oxysterols 7α-OH, 7β-OH and 7-keto occur in soleus muscle whereas significant increases in the plantaris only relate to 7β-OH [82] (Fig. 6). There are no studies at present on the acute effects of alcohol on the 7α-OH, 7β-OH and 7-keto oxysterols in muscle.

The importance of these results from the analysis of hydroperoxides and oxysterol is three fold. Firstly, oxysterols may have putative cytotoxic effects [86–88]. For example, both 7β-OH and 7-keto impair cell adhesion and increase cell permeability [89]. Oxysterols also increase apoptosis in rat and human smooth muscle cells [89,90]. It is unclear at present however, how this cytotoxicity relates to the myopathic lesions seem in alcoholism or whether the

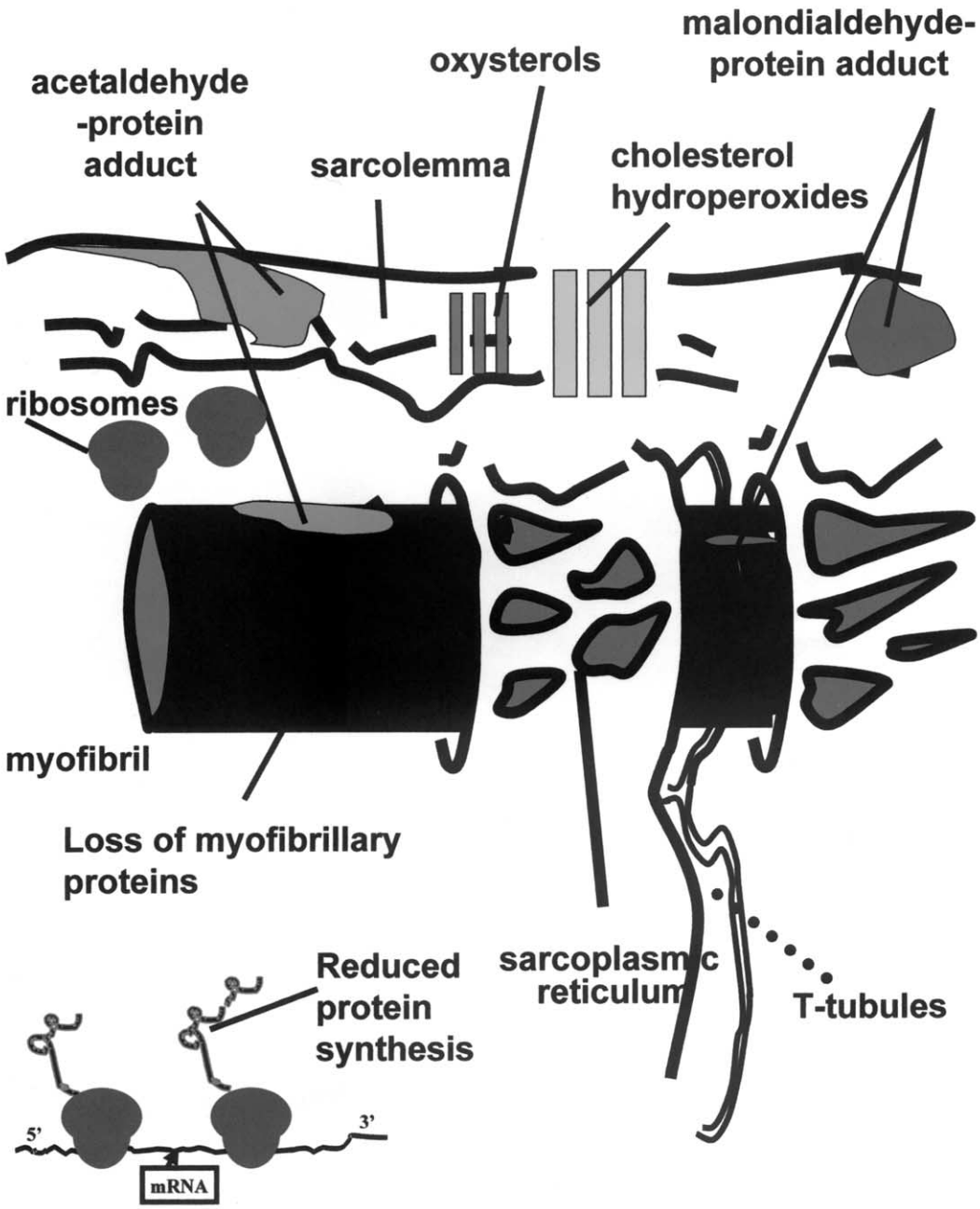

Fig. 7. Schematic summary of changes in alcohol expose muscle. Summary figure of the changes in muscle. Location of oxysterols and cholesterol hydroperoxides are putative and will occur as a result of oxidative stress.

increased oxysterols occurs bound (i.e., conjugated) or mobile within the membrane domain. Secondly, oxysterols are indicative of lipid peroxidation: for example their levels can be reduced with vitamin E in smooth muscle cells *in vitro* [91]. Finally, most of the cholesterol in skeletal muscle is associated with the membrane fraction and thus increased hydroperoxides and oxysterol represent disruption of this subcellular region [92–94].

The fatty acid composition of muscle phospholipids is altered in response to ethanol, including an increase in 18:2 and a decrease in the relative non-essential/essential fatty acid ratio [80]. This also has implications for membranes fluidity which is determined by fatty acid composition [95–98]. Overall, these changes suggest significant perturbations in the membrane lipid domain of skeletal muscle in response to ethanol.

9. Concluding comments

Alcohol is an important constituent in most diets of the Western and developed world. However, excessive exposure to alcohol induces defects in muscle including the

formation of aldehyde protein adducts, loss of ribosomes, reduced proteins synthesis, loss of myofibrillary proteins, and increases in sarcoplasmic-endoplasmic reticulum Ca^{2+}-ATPase (Fig. 7). Concomitant increases in cholesterol hydroperoxides and oxysterol also occur reflecting a variety of pathogenic processes putatively within the membrane domain. Ultimately, excessive alcohol ingestion leads to the disease entity alcoholic myopathy. However, it is uncertain whether these muscle changes may reflect, contribute to, or initiate, the disturbances in muscle function or reductions in muscle mass in this disease.

Alcoholic subjects with myopathy may lose up to 30% of their entire musculature [39]. This should be considered in the context that skeletal muscle contributes to 40% of body weight and approximately one-fifth to one quarter of whole-body protein turnover. For example, whole-body nitrogen excretion is enhanced in alcohol-consuming subjects [99-101]. Thus, the associated chronic alcoholic myopathy has profound implications for the physiology of the whole body and protein kinetics [50].

Acknowledgments

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