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Lipid-Based Therapeutic Strategies for Sarcopenic and Dystrophic Muscular Impairments

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Abstract

The ability to perform muscular work is critical for maintaining health and performing activities of daily living. However, numerous individuals are afflicted by pathologies that limit strength or endurance, such as sarcopenia of aging or muscular dystrophy. These pathologies can be associated with increased mortality risk and shortened lifespan; therefore, improved therapies are needed for treating disorders of skeletal muscle and those that generally affect physical function. Esterified and non-esterified lipids within muscle and other cell types exert metabolic and structural effects that alter biochemistry and physiological functions. Importantly, the composition of dietary fat impacts the variety of fatty acids [FAs] in tissue lipids. In this report, recent discoveries on the effects of dietary FA composition are discussed for pathologies related to skeletal muscle and physical work capacity, with the focus placed upon muscular dystrophy and sarcopenia of aging. Potential effects of n-9 monounsaturated FAs [MUFAs], n-3 polyunsaturated FAs [PUFAs], n-6 PUFAs, and trans-FAs [TFAs] are discussed. Potential benefits of MUFA and discordant findings for n-3 PUFA in muscular dystrophy are discussed, as well as the detriments of TFA and benefits of n-3 PUFA in sarcopenia. Other observations and inferences are considered, and areas for future work are identified.

Keywords: Inflammation; Ageing; Senescence; Disability; Sarcolemma

Abbreviations: ALA: α-Linolenic acid; ARA: Arachidonic Acid; BMD: Becker Muscular Dystrophy; CK: Creatine Kinase; CVD: Cardiovascular Disease; DHA: Docosahexaenoic Acid; DMD: Duchenne Muscular Dystrophy; DPA: Docosapentaenoic Acid; EPA: Eicosapentaenoic Acid; FA: Fatty Acid; FID: Flame Ionization Detection; GC: Gas Chromatography; H&E: Hematoxylin And Eosin; HPLC: High Performance Liquid Chromatography; LA: Linoleic Acid; LGMD: Limb-Girdle Muscular Dystrophy; Mtor: Mammalian Target Of Rapamycin; MUFA: Monounsaturated Fatty Acid; NF-Kb: Nuclear Factor Kappa B; PL: Phospholipid; PLA2: Phospholipase A2; PUFA: Polyunsaturated Fatty Acid; ROS: Reactive Oxygen Species; SAMP8: Senescence-Accelerated Mouse P8; SFA: Saturated Fatty Acid; TFA: Trans-Fatty Acid; TG: Triglyceride; TNF-A: Tumor Necrosis Factor-A; UV-Vis: Ultraviolet/Visible; VO_2peak: Peak Oxygen Consumption.

Background

Introduction

The composition of fatty acids [FAs] in the diet influences physiology in multiple tissues and ultimately the health of an individual. Recent discoveries have shed light on the potential of the dietary FA composition to assist in the management of muscular diseases. This published literature is reviewed here and the discoveries are considered as catalysts for future work in this important area of investigation. In this manuscript, the well-characterized effects as well as the additional possible impacts of the abundance of specific FAs in the diet are discussed in the context of two major types of muscular pathology: muscular dystrophy and the sarcopenia of aging. These two pathologies are discussed in the same report because they are both disorders of muscle weakness and degeneration for which recent discoveries indicate a potentially important role for dietary FA composition. The effects of specific dietary FAs on functional outcomes, intracellular signaling, and cellular integrity are discussed, and future directions for research are identified.

Muscular dystrophy

Duchenne muscular dystrophy [DMD] is a degenerative X-linked muscle disease that is caused by a mutation in the dystrophin gene that leads to loss of dystrophin protein expression [1]. Progressive loss of physical function in DMD generally leads to use of a wheelchair during childhood and then significant loss of respiratory muscle function by early adulthood [1]. Becker Muscular Dystrophy [BMD] is a less severe form of this condition because some expression of a truncated version of dystrophin remains [1]. DMD affects approximately 1 in 3500 boys and BMD is far less common [1]. The mdx mouse is an animal model for DMD [2] and data obtained on this model are reported in this manuscript. Limb Girdle Muscular Dystrophy [LGMD], another type of dystrophic condition, is caused by a mutation in the δ-sarcoglycan gene, and work on a hamster model of this disorder are also discussed in this report. In these conditions that lead to a dystrophic muscle phenotype, the skeletal muscle sarcolemma is destabilized. Corticosteroid treatment is useful, but there is a need for additional treatments and lifestyle recommendations, because corticosteroids are far from being sufficiently effective [3]. Dietary factors could be important as treatments to further blunt the trajectory of disease progression. Little is known regarding appropriate dietary recommendations for patients with DMD and related muscular dystrophies. However, some recent discoveries have been made for the dietary FA composition in animal models, and these findings are discussed here. Skeletal muscle in DMD patients experiences substantial inflammation as evidenced by increased tumor necrosis factor-α [TNF-α] protein expression [4] and enhanced nuclear factor kappa B [NF-κB] activity [5], as well as high sarcolemmal leakiness as indicated by

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serum creatine kinase [CK] activity [1]. Mdx mice recapitulate this phenotype successfully as they exhibit elevated TNF- α expression and NF-κB activity [6-12] in muscle, as well as elevated serum CK activity [9,14,15] compared to wildtype controls. Studies on skeletal muscle from mdx mice suggest that membrane permeability [19,20] and inflammation [8,10] are centrally involved in disease progression, as pharmaceutical-based countermeasures directed at these aspects of cellular function reduced the degree of pathology [8,10,19,20]. It is also known that these processes can be modulated by specific FAs [21,22] therefore, the dietary FA composition likely could also play a role in the design of optimal treatments. Specifically, dietary lipids that could reduce inflammation and/or reduced membrane permeability could be useful. n-3 polyunsaturated FAs [n-3 PUFAs] are generally considered to exert anti-inflammatory properties and are useful as treatments in some inflammatory conditions [21,23,24]. However, use of n-3 PUFAs as anti-inflammatory compounds in dystrophin-deficiency is not a simple solution, because membrane permeability could be exacerbated by enhanced n-3 PUFA content [9,25,26]. Thus, careful consideration and preclinical research is needed to understand the potential for lipid-based therapies in DMD that are aimed at reducing inflammation and improving the integrity of the plasma membrane.

Sarcopenia of aging

Sarcopenia is another pathological condition of skeletal muscle for which dietary lipids could be relevant. In aging, there is a significant loss of motor units [27] though only a modest loss of muscle mass [28]. The changes in muscle and lean body mass are not the sole or most impressive markers of the pathology. That is to say, specific strength [strength per unit of muscle mass] declines far more than the absolute muscle mass [29,30] supporting the interpretation that loss of functional strength could be a better description of sarcopenia than simply the loss of muscle mass. It is not yet clear, but this significant loss of specific strength in sarcopenia may likely be a combination of impaired motor unit recruitment and reduced intrinsic contractility of muscle tissue. The elderly individuals experiencing the highest degree of sarcopenia experience the most risk of disability and loss of independence [31] and if sarcopenia were prevented, a very sizable proportion of the disability experienced by elderly individuals could be eliminated [32]. In addition to strength losses, in sarcopenia an age-related decline in aerobic exercise capacity [VO2peak] is also observed [30,33]. Reductions in strength and aerobic capacity limit quality of life through reduced endurance, decline in mobility, and diminished work capacity, and these changes lead to an increased risk of metabolic disease [34] as well as increased risk for needing hospital-based medical care [32]. Thus, it is critical to identify dietary factors that over a lifetime can alter the trajectory of the age-related decrements in physical function and muscle quality. Since studies of lifelong dietary interventions are not feasible in humans, an animal model is needed for such work. The senescence accelerated SAMP8 mouse [senescence-accelerated prone P8] has been used as a model for sarcopenia [30,35-37] and published findings for effects of dietary lipid in this model are discussed here. Causes of sarcopenia could include inflammatory signaling and related gene expression [38,39] oxidative stress [40] and activation of the molecular program for lipogenesis in muscle [41] and muscle triglyceride [TG] accumulation [42].

Fatty acids

α-Linolenic acid [ALA] is the dietary essential n-3 PUFA, and linoleic acid [LA] is the dietary essential n-6 PUFA. These can be ingested from vegetable oils such as canola oil, which contains both of these FAs to an appreciable extent, flaxseed oil which is highly enriched in ALA, and safflower oil and a variety of other oils which are highly enriched in LA. Additionally, longer chain n-3 PUFAs are enriched in wild-caught fish products. *In vivo* mammals can elongate and desaturate ALA to longer chain n-3 PUFAs such as eicosapentaenoic acid [EPA] and docosahexaenoic acid [DHA], and LA is elongated and further desaturated to arachidonic acid [ARA]. Another FA class discussed in this report is monounsaturated FAs [MUFAs]. It is possible that other positional isomers have distinct biological activities in the context of muscle pathology, but the common n-9 MUFA [oleic acid, 18:1n-9] is the MUFA that is primarily discussed in this report. Oleic acid is highly enriched in olive oil and is found in appreciable amounts in many other oils as well. The vast majority of FAs consumed from natural sources are in the cis-conformation at the double bond positions, but even the relatively small intake of those in the trans-conformation are of clinical significance. During the hydrogenation process to produce partially hydrogenated vegetable oil, cis-unsaturated FAs such as oleic acid are converted to trans-FAs [TFAs] such as elaidic acid, and most TFA in the diet come from partially hydrogenated oils [43]. There is also some evidence that TFAs can be produced *in vivo* from other FAs which is achieved by a free radical mechanism in which the unsaturated FAs of membrane phospholipid [PL] are converted from cis to trans conformation [44]. Within tissues, FAs are primarily stored in esterified forms in TG or PL. The TG pool acts as a fuel depot, while PL is primarily structural in the membranes of organelles and in the plasma membrane [referred to as the sarcolemma for muscle]. Each FA is generally found in both TG and PL within a muscle cell, but some specific FAs are more highly enriched in one or the other [15]. For example, DHA and EPA are an order of magnitude more relatively abundant [% of total acyl chains] in PL than TG of skeletal muscle [15]. In investigations of the role of FAs in skeletal muscle, it is useful to broadly profile the abundances of esterified FAs within the tissue. To measure FAs, either gas chromatography [GC] or high performance liquid chromatography [HPLC] can be used. For each, mass spectrometry can be used as the detection mode or traditional detectors can be used such as a flame ionization detector [FID] for GC or UV-Vis detector for HPLC [9,15,45- 49]. GC of FAs was first described over 60 years ago [50], approximately 2 decades before HPLC instrumentation existed even in its most rudimentary form [51]. Following its development, HPLC use underwent an exponential growth due to its utility for analysis of a wide variety of compounds [51], and while GC is currently the routine procedure for FA analysis, reversed phase HPLC of UV-absorbing or fluorescent derivatives is an approach that can be comparable and potentially better for resolution of certain FA isomers [47]. GC [52-54] and HPLC [9,15,49] have been successfully applied in assessment of the FA composition in skeletal muscle lipids. Of particular challenge is separation of positional isomers [e.g., vaccenic acid vs. oleic acid] as well as geometric isomers [cis vs trans], and careful selection of the analytical method can allow for separation and quantitation of these FAs in the study of skeletal muscle physiology [47].

Esterified Fatty Acids in Tissues and as Potential Therapeutics for Muscular Dystrophy

Tissue fatty acid composition

Compared with their wildtype counterparts on the same diet, the skeletal muscle PL in mdx mice at 12 weeks of age exhibited a nearly 2-fold increase in oleic acid [18:1n-9] and a 15% increase in LA [18:2n-6], as well as a 16% reduction in DHA [22:6n-3] content [15]. Of these changes, the elevation of oleic acid is the most striking in magnitude [2-fold higher than wildtype]. The elevation was specific to 18:1n-9 [oleic acid] rather than 18:1n-7 [cis-vaccenic acid], so an elaborate

chromatographic separation [47] was critical in fully appreciating this adaptation. The relative reduction of DHA content in PL was modest in 12 week old mdx mice compared with wildtype, and DHA levels were still relatively high in both wildtype and mdx muscle PL [because mice generally display very high DHA content in muscle PL such as between 15-20% of acyl chains] [15]. However, this reduction in DHA may be a symptom of cellular stresses, and it is possible that this decrement would be exacerbated under higher levels of stress such as younger ages during active necrosis in mdx skeletal muscle or during exercise training in mdx mice which causes disruption of the sarcolemma. However, it is also possible that reduced DHA content is an appropriate adaptive response to muscular dystrophy. In a different animal model for muscular dystrophy, the dy/dy mouse, the content of DHA was lower than controls in muscle PL [55,56] similar to that which occurs in mdx mice [15]. There may be something about the stress of chronic skeletal muscle pathology that ultimately reduces the content of DHA in PL, as an aspect of a detrimental outcome or as a purposeful cellular remodeling process [15]. Studies of skeletal muscle FA composition in humans with DMD could be considered to be unsatisfactory due to a lack of dietary assessment and dietary control [57-59] as well as issues related to age-matching and tissue sample handling [58]. Muscles of humans with DMD and mdx mice experience increased oxidative stress [15,60-62] which might oxidatively modify DHA in cell mebranes. Phospholipase A2 [PLA2], an enzyme that would remove DHA from membranes, has a preference for peroxidized FAs [63], so it is possible that oxidative damage to DHA in mdx mice leads to its reduced abundance in PL. As inflammation exacerbates muscle damage and degeneration in mdx mice [8,10] and n-3 PUFAs exhibit anti-inflammatory properties [21,23,24] one could speculate that reduction in DHA content may be unfavorable if it were sufficiently severe. A reduction in DHA content theoretically could cause further challenge to the impairment in calcium homeostasis of dystrophic muscle, as treatment of endothelial cells with DHA was reported to reduce oxidative stress-induced calcium influx through effects on ion channels [65]. Elevated calcium influx in dystrophic muscle is expected to cause degradation of membrane-bound proteins through activation of the calpain system [66], activation of cytosolic PLA2, as well as reactive oxygen species [ROS] production which could enhance NF-κB activity [67]. Dietary strategies to reduce calcium influx, through effects on calcium channels as well as through effects on the general permeability of the sarcolemma, could be important. Reductions of DHA content in cellular PL could decrease the cell membrane permeability [25,26] though, which could be advantageous, so it is unclear if the reduced DHA in dystrophic muscle is deleterious or beneficial. Indeed excessive sarcolemmal permeability is generally considered to be the primary detriment of dystrophin deficiency, so if reduced DHA were to dampen this permeability, it would be reasonable to consider the reduction in membrane DHA to be of benefit to dystrophic muscle.

Unlike n-3 PUFAs which are believed to act as anti-inflammatory compounds, n-6 PUFAs are generally believed to exhibit pro-inflammatory properties and thus to be potentially harmful when overly abundant [68]. LA, the dietary essential n-6 PUFA, was more abundant in muscle PL of mdx than wildtype mice at 12 weeks of age when the mice were on identical diets [15]. LA content of muscle PL in mdx mice was correlated with serum CK activity [a marker of membrane leakiness] and was also significantly correlated with protein carbonylation [marker of oxidative stress] [15]. It is possible, but to our knowledge currently untested, that elevations of LA in muscle PL of mdx mice would lead to exacerbated sarcolemmal damage which could be related to oxidative stress. Furthermore, there is some evidence that the n-6 PUFA content in tissues can impact cell integrity. For example, expression of the C. Elegans fat-1 gene, which converts LA to ALA, and thus reduces the amount of LA, leads to improved survival of rat neurons [69]. As well, it was shown in immune cells that LA, as compared with other FAs, strongly stimulates ROS production [70,71]. Thus, there is some evidence that LA supply to muscle causes pathological stress. However, as an example of an actual benefit of LA, in a rat model of heart failure, supplementation of the diet with an oil that is high in LA, such as safflower oil, leads to improved survival, potentially through effects on mitochondria [72]. Hence, it is possible that the dietary supply of LA exerts unfavorable impacts on ROS production which seems to be elevated in muscle of mdx mice [15,62] and humans with DMD [61] and thus on the activation of NF-κB [6-12] but it is also possible that LA is actually protective in some ways. In summary, based upon the FA profile of PL in dystrophic muscle, there was reason to suspect that the dietary supply of n-3 PUFA, n-9 MUFA, and n-6 PUFA could impact the disease severity. Below, recent findings for effects of dietary n-3 PUFAs [ALA, DHA, and EPA] in muscular dystrophy are discussed as well as recent results for n-9 MUFA [oleic acid] intake. The discussion is limited to these FA classes, because we are not aware of any published data yet on the topic of LA intake in a muscular dystrophy model.

Effects of n-3 polyunsaturated fatty acid [PUFA] intake

As discussed above, ALA is an n-3 PUFA, and along with the longer chain n-3 PUFAs such as EPA and DHA, it is expected to increase membrane fluidity and have anti-inflammatory properties [21,23,24]. However, n-3 PUFA in cell PLs also lead to increased membrane permeability [25,26] which would be undesirable in dystrophic muscle. Inflammation appears to play a role in the progression of DMD, but membrane permeability and leakiness are the major cellular defects. Supplementation with n-3 PUFA has been considered by some to be a possible future component of treatment for DMD, because these FAs [ALA, EPA, DHA] generally exert anti-inflammatory effects. Conversely, n-6 PUFAs [LA and ARA] are considered pro-inflammatory [68]. n-3 PUFAs suppress abundance of inflammatory cytokines and reduce NF-κB activity [23,24]. NF-κB exists as homo- and hetero-dimers of the protein subunits, p65 and p50/52, and regulates the transcription of genes involved in inflammatory responses. When NF-κB is quiescent, it is located in the cytoplasm in an inactive form bound to its inhibitor IκB [73] with the p65 subunit in its unphosphorylated form [74]. Phosphorylation of p65 at serine536 enhances translocation to the nucleus and transcriptional activity and so is closely associated with activation of this inflammatory factor [74]. Interestingly, mdx mice exhibit a higher abundance of phospho-p65Ser536 in skeletal muscle compared to wildtype controls [9,74] which suggests that inflammation is a component of the pathology.

Recently, using the mdx mouse model, a high-ALA [n-3 PUFA] diet was compared to a high-oleic acid [n-9 MUFA] diet [9]. In the n-3 PUFA enriched diet, oleic acid level was reduced and replaced with ALA. Breeding mice were assigned to one of the two diets and remained on the diet during pregnancy. Next, from weaning to the conclusion of the study at 8 weeks of age, the mdx mouse offspring were fed the same diet as their mother had consumed. Skeletal muscle histopathology as well as markers of membrane leakiness and inflammation were assessed. The major finding was that the high-ALA diet was associated with approximately 50% higher serum CK activity than the MUFA-based diet. Based on the anti-inflammatory properties of ALA, we had expected to see a reduction in skeletal muscle inflammation. However, apparent NF-κB activity [phospho-p65ser536] was unchanged and membrane leakiness was exacerbated with high ALA intake [ALA

substituted for oleic acid]. There were no significant effects of diet on skeletal muscle histopathology in this study as assessed by hematoxylin and eosin [H&E] staining and no diet effect on grip strength. Thus, these results suggest that membrane properties in mdx mice were worsened with the high dose of n-3 PUFAs without a corresponding change in muscle degeneration. However, it is possible that over the lifespan of the mice, if studied over a longer period, that the high level of membrane leakiness would eventually exert an effect on functional outcomes.

In contrast to the findings discussed above, nutritional supplementation using n-3 PUFA could generally be expected to exert therapeutic effects on a variety of inflammatory disorders. DMD is a disease characterized by high levels of inflammation in skeletal muscle, so one may reasonably expect utility of n-3 PUFA intake for dystrophic muscle on a theoretical basis. One could wonder if the previously reported detrimental effects of a high n-3 PUFA intake [9] would have been because ALA was administered rather than another n-3 PUFA such as EPA. However, in this work, consumption of the ALA-enriched diet resulted in increased EPA content in mdx skeletal muscle PL [presumably because of chain lengthening and desaturation in vivo], and high ALA intake also tended to increases DHA content in muscle PL. Therefore, it appears that the effects of ALA treatment on membrane characteristics exerted a challenge to muscle cell homeostasis that outweighed effects on inflammatory signaling. Furthermore, as discussed below, it is possible that oleic acid [an n-9 MUFA] may be more beneficial than n-3 PUFAs in dystrophic skeletal muscle, and thus the differences in MUFA content between the diets could have explained the results.

In other studies, n-3 PUFA supplementation with pure EPA or with fish oil decreased muscle degeneration and inflammation in mdx mice [75,76]. This result is dissimilar to the findings with ALA administration discussed above. It may be that, even despite effects of ALA ingestion on tissue EPA levels [9] the effects of ALA intake [plant-based n-3 PUFA] on muscle could be unique from direct consumption of EPA and DHA [fish/marine-based n-3 PUFAs]. Yet, other explanations for the disparity in results between these previous studies need to be considered. The age of the mice when studied may have contributed, as very young mice were investigated in the studies of EPA/DHA [75,76] compared with that of ALA [8], as the dietary lipid requirements might be different at such a young age during rapid growth and during the early severe cycles of degeneration/regeneration in mdx mice. It is also possible that fetal programming, as a result of maternal diet, would explain discrepancies between these studies, as in the work on ALA in mdx mice, the pregnant mice were exposed to the respective experimental diets before and during pregnancy [9]. Additionally, the duration of treatments were different, as mice were supplemented with pure EPA [76] or fish oil [75] for a sixteen-day period beginning at fourteen days of age. Those mice were still very young [approximately four weeks old] at the time of tissue collection, and it is possible that this age was too early to show the prolonged effects of n-3 PUFA intake which might eventually become detrimental. In contrast, mice on ALA-enriched diets were fed the diets until 8 weeks of age [8]. If benefits of n-3 PUFAs are transient, then the time course of study would be critical, and future work is needed on this issue. Mice in the work on ALA were treated for 8 weeks [9] and those taking marine-type n-3 PUFAs for 2 weeks [75,76] so there were numerous factors that could explain the disparity in the results, related to the type of FA, dose, age, and treatment duration. Nonetheless, the disparity leads to concern over use of any n-3 PUFA supplementation in humans with DMD and related muscle disorders. Future work should examine a more detailed and extended time course for effects of ALA and the other n-3 PUFAs such as pure EPA, DHA, perhaps docosapentaenoic acid [DPA], and fish oil. Additionally, periodized/intermittent treatment regimens could be considered if benefits of supplementation are transient.

Contrary to findings from work on ALA intake in a mouse model of DMD [9], using a hamster model of LGMD investigators concluded that consumption of an ALA-enriched diet from weaning to 150 days of age improved skeletal muscle histological appearance [64]. However, there are significant concerns over the dietary design in the study on hamsters. The animals on the control diet were fed a standard pelleted rodent chow, but the animals on the ALA-enriched diet consumed flaxseeds, apples, and carrots. While the investigators observed an association between the n-3 PUFA [ALA] enriched diet and muscle health in dystrophic hamsters, there would have also been extensive nutritional differences between the diets that go well beyond the FA profile that could have impacted muscle pathology. In contrast, in the work on ALA intake in mdx mice, as should be generally expected in preclinical work, the diets were matched nutritionally with respect to micronutrients, total fat, carbohydrate, and protein [9]. It is also possible that the disparity between our conclusions on dystrophic mice [9] and that of others on dystrophic hamsters [64] are related to differences in the disease model [mice vs hamster, dystrophin vs δ-sarcoglycan deficiency], but it appears also likely that the degree of dietary control was an issue. Indeed, other work in the δ-sarcoglycan deficient hamster model [77] was generally in agreement with our work on mdx mice [9]. This previous work on the hamster model of LGMD demonstrated a superiority of saturated fat over n-3 PUFA for longevity of the animals [77]. So lesser degrees of dietary FA desaturation may be preferable in dystrophic pathology, as discussed in detail below in the context of MUFA intake.

Effects of monounsaturated fatty acid [MUFA] intake

Though PUFA, namely n-3 PUFA, seem to attract the most attention as potential lipid-based therapeutic compounds, a high intake of other FA classes such as MUFA could possibly be beneficial for muscle. Intake of high MUFA levels is associated with reduced risk for cardiovascular disease [CVD] in humans [78,79] and perhaps this class of FA also directly impacts skeletal muscle. Oleic acid [an n-9 MUFA] is the most abundant MUFA in the diet, and this specific FA may be important for muscle health. Mdx mice displayed a 2-fold higher oleic acid content in skeletal muscle PL than wildtypes [15], and it is possible that this increase in oleic acid content is adaptive to cellular stresses experienced by dystrophic muscle. If so, a high-MUFA diet, providing ample oleic acid, might assist dystrophic muscle in coping with the pathology. Currently the mechanism for this possible relationship is not clear, but this could be addressed in future work if our hypothesis is supported by subsequent studies on oleic acid intake and muscle health. Similarly, it has been reported that exercise training in healthy humans increases the abundance of oleic acid in skeletal muscle PL [52], so it is apparent that content of oleic acid in muscle PL can be altered by adaptation to stressors. Above we discussed work in mdx mice comparing a high PUFA diet [high in ALA] to a MUFA-based diet [high in oleic acid]. We interpreted this work above in the context of n-3 PUFA supplementation, but it is also useful for inferring possible health effects of a high MUFA diet [compared with high PUFA]. High oleic acid intake was associated with reduced serum CK [a marker of sarcolemma leakiness] compared with the high-PUFA diet in mdx mice [9]. Therefore, it appears that a higher degree of saturation of FA in membrane PL may reduce the severity of pathology in dystrophic muscle. Along with the higher oleic acid content in PL, mdx mice also exhibit reduced content of DHA which is a highly unsaturated FA,

and this shift toward more highly saturated FAs in membranes may be important for tolerance of dystrophin-deficiency [15]. This finding has also been supported by *in vitro* preparations of rodent skeletal muscle. A standard laboratory rat strain was fed either fish oil [high in n-3 PUFAs] or corn oil [as a control, very low in n-3 PUFA content] as their primary dietary lipid from gestation until approximately nine weeks of age, and then skeletal muscle was exposed to a chemical insult ex vivo [calcium ionophore to raise intracellular calcium to harmful levels] [82]. This could be considered to be a model that mimics dystrophic pathology to a reasonable extent as intracellular calcium is elevated in mdx mice [15-17,83]. In response to this intracellular calcium elevation, the CK release [membrane leakiness] was significantly higher in the rats that consumed fish oil rather than corn oil [82]. Thus, again it appears that higher degrees of FA desaturation [more double bonds in the FAs] can make the sarcolemma prone to lesions under certain stresses. It has been demonstrated that pharmacological interventions to seal or reduce severity of lesions to mdx muscle are useful as treatments [18,19] and the work described above indicates that diet may play a role as well.

MUFA may be a more desirable dietary constituent than PUFA because they could allow for a more dense packing of PL molecules in the muscle membranes, reducing the leakiness. This supposition is based upon the well-accepted understanding that double bonds with cis conformation create a kink in FA structure, essentially widening the footprint of the FA within the membrane. Based upon FA geometry, it is even conceivable [but currently untested] that saturated FA could be even more beneficial than MUFA for reducing dystrophic muscle membrane permeability. Much more work is needed in the preclinical and clinical setting before strongly advocating for specific dietary prescription approaches in DMD, but currently it appears that it may be advisable to focus on oils with high MUFA content and reasonably low PUFA content [e.g., olive oil]. And again, it should be noted that it would be worth testing in the future if SFA is better than MUFA [or theoretically even TFAs could be of benefit for membrane properties because of the altered FA geometry when converting from cis to trans conformation at the double bond positions]. However, these FAs with favorable geometry for formation of a less-leaky membrane [SFA and TFA], if consumed at high enough levels, could theoretically exacerbate inflammation and cardiovascular disease risk, so this trade-off should be investigated in the future.

Future directions for research in dystrophic muscle

While significant gains in knowledge of the effects of dietary n-3 PUFAs and n-9 MUFA have been made (Figure 1), there is a need to test the effects of the dietary LA intake on muscle health in these models. Specific oils are highly enriched in LA [e.g., safflower and soybean oils] while others are not [e.g., olive and palm oils], so the choice of oils in the diet of dystrophic individuals could drastically impact the supply of LA to skeletal muscle. Work on SFA is also needed to be compared with apparent benefits of MUFA intake. Furthermore, additional work on dose-responses is needed for n-3 PUFA and n-9 MUFA as well as work comparing different n-3 PUFAs to one another. Then ultimately, as caution is needed in making inferences about human nutrition from data on rodent models, findings will need to be tested and potentially confirmed in trials on human study participants.

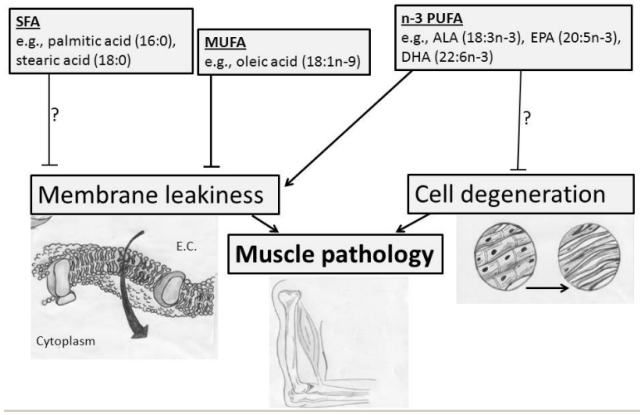

Figure 1: Muscular dystrophy.

Esterified Fatty Acids in Tissues and as Potential Therapeutics for Sarcopenia

Tissue fatty acid composition

Young rats consuming no TFA still have low but detectable amounts of TFA in various tissues, and injecting a pro-oxidant compound in rats results in accumulation of TFA in multiple tissue types [81,82]. Moreover, there was a significant age-related increase of TFA content in PL of rat tissues even when no TFA were consumed, presumably due to age-related oxidative stress [81,82]. It is possible that the oxidative stress per se leads to weakness during the aging process [sarcopenia], but it is also possible that the TFA per se cause neuromuscular dysfunction. As discussed below, we tested this hypotheses by studying the effects of TFA intake in a sarcopenic mouse model and discovered that TFA intake exacerbates the severity of age-related sarcopenia [30]. In addition to potential increases in TFA exposure in muscle with age, there are other changes in the FA composition of the membrane PL as well. Specifically, an age-related decrease in the relative abundance of DHA was reported for skeletal muscle of rats as well as an increase in LA [83] which is remarkably similar to the changes in dystrophic muscle [15].

Effects of n-3 polyunsaturated fatty acid [PUFA] intake

Even when normalized to muscle mass or fat-free mass, strength is reduced with aging [29,30]. In addition to reduced skeletal muscle tissue quality, some of this strength reduction could be a result of neural mechanisms such as altered motor unit recruitment patterns, reduced ability to recruit high threshold motor units, or reduced total number of motor units [27]. Protein turnover in skeletal muscle is important for maintenance, repair, and remodeling. The reductions in rates of protein synthesis with aging in the basal state [84-86] and in response to nutritional stimuli [87-89] likely contributes to susceptibility for sarcopenia and the development of a frail phenotype. Thus, it is of interest to develop strategies to prevent age-related declines in skeletal muscle protein synthesis. Muscle protein synthesis has been used as a marker of efficacy in preclinical studies, such in the testing of n-3 PUFA supplementation as discussed below. Humans 65 years and older were studied before and after 8 weeks of n-3 PUFA supplementation in a double-blinded, placebo-controlled fashion [90]. Lovaza [a mixture of EPA and DHA ethyl esters] was administered at a dose of 4 grams per day and compared to corn oil. Subjects were studied in the postabsorptive state and in response to a hyperaminoacidemic hyperinsulinemic clamp [intravenous infusion of insulin, glucose, and amino acids]. Using stable isotope tracer methodology, the authors discovered that n-3 PUFA treatment led to an enhanced response of muscle protein synthesis to the nutrient and hormone infusion. mTOR signaling, which leads to initiation of translation [protein synthesis] was also enhanced by the supplementation [90]. These results indicated that n-3 PUFA supplementation may be a useful treatment for the sarcopenia of aging.

Physical function has been assessed in other studies of n-3 PUFA supplementation in the elderly. There was no significant effect of 30 g per day of flaxseed oil [approximately half of the FAs in the supplement would be ALA] [91]. In this study, humans older than 60 years were treated during a 12 week resistance training program with either the flaxseed oil or placebo [corn oil], and there was no clear effect on strength gains [91]. However, in individuals of an approximately similar age, daily fish oil supplementation [2 g/d] during chronic resistance training led to enhanced gains of strength over the 3 month training period [92]. Thus, it appears that fish oil may lead to chronic improvements in neuromuscular function [presumably a result of the DHA or EPA], and these results are consistent with previous work on muscle protein turnover [90]. However, it should be noted that the work on strength gains during fish oil supplementation was not placebo-controlled [92], so in the future it would be informative to see results from a double blinded study on the topic.

Effects of trans-fatty acid [TFA] intake

In a study of SAMP8 mice, a senescence-accelerated mouse model of sarcopenia, the effects of dietary TFA were investigated. Two percent of dietary energy was from TFA, using partially hydrogenated oils as the TFA source, which is a typical TFA intake for Americans [93]. This diet was compared to a control diet with very little TFA and no hydrogenated oils [30]. The diets were fed from weaning until 60 weeks of age. Major functional changes were observed from adulthood [25 weeks] to old age [60 weeks] in SAMP8 mice along with biochemical changes. Even on the control diet, grip strength declined with age, and there was a further exacerbation of this age-related sarcopenia when the mice were assigned to the TFA-containing diet [30]. The diet containing partially hydrogenated oils exacerbated the age-related decline in functional strength by approximately 2-fold. Aerobic capacity [VO2peak normalized to fat-free mass] also declined with age and the decrements occurred at a younger age if SAMP8 mice consumed TFA [30].

TFA intake is known to unfavorably alter serum lipid profiles in humans [93-96] and common laboratory mouse [97,98] and rat strains [99,101]. Furthermore, along with the exacerbation of the sarcopenia of aging, serum lipid changes were observed in SAMP8 mice with TFA intake [30]. Blood TG was increased by TFA intake at both young and old age, and serum total cholesterol rose to levels seen in old mice by a younger age when on the TFA diet. Dyslipidemia was present even at young age in SAMP8 mice that consumed partially hydrogenated oils at a similar level to that in Western Societies that have not yet banned this oil from the food supply [93], and the cumulative exposure of the vasculature to a dyslipidemic environment could have been a factor in the development of sarcopenia with age. For example, work on the apolipoprotein E [apoE] knockout mouse has shown that dyslipidemia impairs exercise capacity and vasodilatory function [105]. Additionally, vasodilatory function in vessels feeding skeletal muscle is blunted with age in humans, and this is believed to be a factor in the supply of nourishment to muscle [102].

Possible mechanisms for sarcopenia could generally be expected to be related to inflammation [38,39] oxidative stress [40] and vascular dysfunction [102]. TFA intake could theoretically worsen these factors. In SAMP8 mice, we did not observe any age-related or TFA-related changes in protein carbonylation [a marker of oxidative stress] or serum TNF-α [marker of systemic inflammation] [30] therefore, age- and diet-related reductions in strength and aerobic capacity may have occurred independently of these types of cellular stresses, though it is possible that assessment of other markers of oxidative stress and inflammation would have led to different conclusions. A deterioration in vascular function, as noted above, could provide an alternative explanation for susceptibility to sarcopenia with age. The vascular dysfunction that accompanies dysregulated muscle protein metabolism with age in humans is related to elevated endothelin-1 in circulation and a blunted vasodilation response to insulin [102]. Elevated TFA intake in humans also reduces flow-mediated dilation, another measure of vasodilatory function, so it seems possible that TFA intake could accelerate this vascular component of sarcopenia. Other possible explanations for sarcopenia could include altered protein structure and stability, as well as changes in muscle architecture or changes in the central or peripheral nervous system. It is also possible that the detriments of TFA on sarcopenia in our work were related to neural effects [30] as the SAMP8 mouse model is commonly used as a model for age-related dementia clearly the nervous system in this model is vulnerable to age-related deterioration. Thus, we cannot exclude the possibility that effects of TFA on volitional strength [grip strength] were related to changes in the central nervous system. Nonetheless, though mechanisms are not yet entirely clear, it appears that TFA avoidance could be a useful strategy for reducing the incidence or severity of sarcopenia [30].

Future directions for research in sarcopenic muscle

The results for n-3 PUFA supplementation on muscle protein synthesis in the elderly are promising, and double-blinded placebo-controlled clinical trials testing the long-term functional benefits are needed. Furthermore, additional development work to identify the optimal doses will be useful. Additionally, work in a mouse model of sarcopenia was promising regarding the potential of TFA avoidance to reduce the trajectory of age-related sarcopenia. In future observational studies, as more countries move toward banning TFA from the food supply, it could be determined if the reductions in TFA intake are associated with reduced sarcopenia incidence over time. Finally, to expand knowledge beyond what is currently known (Figure 2), additional preclinical work is needed on FA types beyond n-3 PUFA and TFA, such as those discussed in the context of muscular dystrophy above. For example, work on n-9 MUFA intake in aging is needed. Reduced spontaneous physical activity is a feature of the sarcopenia of aging [100], and a high oleic acid diet led to increased physical activity compared with a high SFA diet in young individuals [104]. If a shift toward higher n-9 MUFA intake in elderly people caused a similar accentuation of physical activity as seen in young individuals, this could potentially treat an important aspect of sarcopenia. MUFA in addition to other FA classes clearly deserve attention in the future, such that we may develop a strong understanding of ideal diet prescription for sarcopenia.

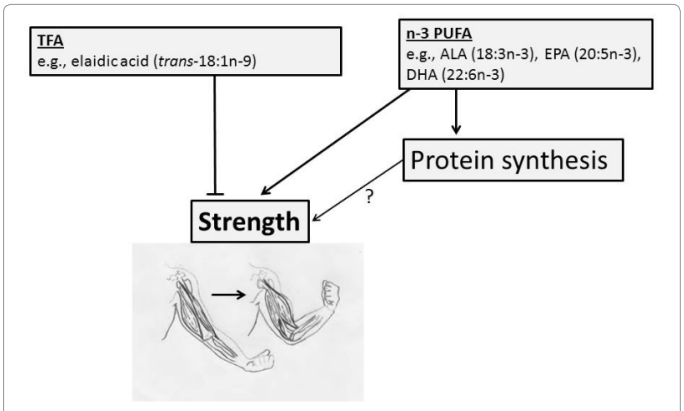

Figure 2: Sarcopenia of aging.

Summary and Final Conclusions

As discussed, the supply of specific FAs to skeletal muscle can alter intracellular signaling in numerous ways, including through mTOR anabolic signaling, NF-κB inflammatory signaling, and through structural changes such as membrane permeability that alter the intracellular milieu. A variety of FA classes appear to have unique impacts on these cellular factors in muscle, such as n-9 MUFA, n-3 PUFA, n-6 PUFA, and TFA. Recent evidence has shown that a higher ratio of dietary MUFA vs n-3 PUFA reduces membrane permeability in mdx mice. On the contrary, there is other evidence showing that n-3 PUFA supplementation in elderly humans is of benefit to skeletal muscle, so it is possible that the effects of n-3 PUFA in muscular dystrophy and in sarcopenia are not similar to one another. It is currently unknown if the intake of MUFA is important in sarcopenia, while MUFA appear to be potentially beneficial for dystrophic muscle. It was recently shown that TFA worsen the severity of sarcopenia, but it is unknown how TFA intake impacts muscular dystrophy. Though one can reasonably speculate, it is currently unknown how the supply of n-6 PUFA to muscle affects disease severity in dystrophic and sarcopenic tissues. Much of the work in this area of investigation has been on animal models, as is appropriate in the early stages of preclinical research. However, to confirm relevance to humans, when appropriate, it will be important to conduct work on human subjects to test if effects are similar to those discovered on animal models. Overall, recent work has provided exciting new data on ways that n-9 MUFA, n-3 PUFA, and TFA may alter biochemistry, physiology, and health, but extensive investigative efforts are needed in this area of lipid-based therapeutic strategies, including use of these dietary manipulations in concert with primary drug-based treatments of sarcopenic and dystrophic muscular impairments.

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