Lipids in Health and Disease

REVIEW

Open Access

Targeting intramyocellular lipids to improve aging muscle function

David W. Russ[1,*], Ravikumar Manickam[2] and Srinivas M. Tipparaju[2]

Abstract

Decline of skeletal muscle function in old age is a significant contributor to reduced quality of life, risk of injury, comorbidity and disability and even mortality. While this loss of muscle function has traditionally been attributed to sarcopenia (loss of muscle mass), it is now generally appreciated that factors other than mass play a significant role in age-related muscle weakness. One such factor gaining increased attention is the ectopic accumulation of lipids in skeletal muscle, in particular, intramyocellular lipids (IMCLs). It has been appreciated for some time that metabolic flexibility of several tissues/organs declines with age and may be related to accumulation of IMCLs in a "vicious cycle" whereby blunted metabolic flexibility promotes accumulation of IMCLs, which leases to lipotoxicity, which can then further impair metabolic flexibility. The standard interventions for addressing lipid accumulation and muscle weakness remain diet (caloric restriction) and exercise. However, long-term compliance with both interventions in older adults is low, and in the case of caloric restriction, may be inappropriate for many older adults. Accordingly, it is important, from a public health standpoint, to pursue potential pharmacological strategies for improving muscle function. Because of the success of incretin-analog drugs in addressing obesity, these medications may potentially reduce IMCLs in aging muscles and thus improve metabolic flexibility and improve muscle health. A contrasting potential pharmacological strategy for addressing these issues might be to enhance energy provision to stimulate metabolism by increasing NAD + availability, which is known to decline with age and has been linked to reduced metabolic flexibility. In this narrative review, we present information related to IMCL accumulation and metabolic flexibility in old age and how the two major lifestyle interventions, caloric restriction and exercise, can affect these factors. Finally, we discuss the potential benefits and risks of select pharmacologic interventions in older adults.

Keywords Sarcopenia, Aging, Lipotoxicity

*Correspondence:
David W. Russ
Druss2@usf.edu
[1]School of Physical Therapy and Rehabilitation Sciences, Morsani College of Medicine, University of South Florida, 12901 Bruce B. Downs Blvd., MDC77, Tampa, FL 33612-4799, USA
[2]Department of Pharmaceutical Sciences, Taneja College of Pharmacy, University of South Florida, Tampa, FL, USA

Introduction

Muscle weakness contributes to >50% of all chronic conditions in people over 50 [1], with one estimate suggesting that it may account for 1.5% (i.e., >$40 billion) of total U.S. healthcare expenditures [2]. These costs are largely attributed to the losses of independence [3], increased risk of frailty and injury from falls [4–7]. At the extreme end of negative consequences is an independent association between weakness and increased mortality [8]. These costs and consequences are predicted to increase in a number of countries, including the U.S., due to the increasing age of their populations [9]. The emergence of the term sarcopenia to describe the age-related loss of muscle mass in the late 1980s [10] brought an increased interest in and focus on addressing muscle size deficits in older adults [11–17].

However, even at the outset of the increased study of sarcopenia, data emerged suggesting that loss of muscle size could not explain most, or even a major portion, of the impaired muscle performance (force, power, etc.) and physical function observed in old age [4, 18–20], findings also observed in animal studies [21–25]. Several terms have been used to express this age-related impairment in muscle performance that exceeds loss of muscle size (i.e., force per unit muscle tissue), including, but not limited to, specific force/tension, muscle quality, and dynapenia [26–30]. Although reductions in habitual physical activity with aging can no doubt contribute to the observed losses in muscle performance, the fact that aging tends to be associated with a fast to slow twitch myosin heavy chain (MHC) phenotype shift [13, 31, 32], while the opposite shift is more typical for disuse [33–36], suggests that reduced activity/exercise does not drive all of the changes seen in old age. Regardless of the term used, the preponderance of evidence from years indicates that loss of muscle performance has a greater negative impact on older adults than loss of mass [18, 20–25, 37–39]. These findings, coupled with observations that the hypertrophic response of aging skeletal muscle may be blunted [40–43], may explain why human and animals studies of interventions targeting muscle mass have often produced disappointing results with regard to performance gains interventions targeting mass often give limited, disappointing results with regard to strength [44–51]. Given these observations, identifying and targeting factors contributing to the age-related impairment of muscle function is an important clinical priority. Several factors including, but not limited to, central neural drive, neuromuscular transmission, excitation-contraction coupling and myosin heavy chain profile have been explored, for reviews, see [30, 52, 53]. For this review, we will focus on ectopic lipid deposition (aka intramyocellular lipids) as a potential target for improving muscle performance in older adults, in part because of the recent increase in the use of the use of incretin-agonist drugs and the potential for these drugs to target ectopic muscle lipids. In addition, we discuss some alternative classes of drugs with potential to address age-related accumulation of ectopic lipids in skeletal muscle.

Age-related metabolic changes and muscular ectopic lipid deposition

One putative factor in the age-related decline of skeletal muscle function that has received increased attention over the past decade or more is accumulation of intramyocellular lipids (IMCLs), sometimes referred to as myosteatosis - though this term may also include subcutaneous and inter-muscular adipose tissue [54]. This ectopic deposition of lipids in muscle is a hallmark of pathology in several conditions and is associated with inflammation, oxidative injury, insulin resistance and disruption of several cell functions, a condition referred to as lipotoxicity [55–57]. Skeletal muscle is no exception, and excess IMCLs, largely in the form of lipid droplets (LDs), are associated with impaired muscle function in conditions such as Type 2 diabetes and obesity, which have been described as accelerating aging, leading to increased adipose tissue combined with decreased muscle and bone content that can impair quality of life [58, 59]. However, aging and disuse, even in the absence of insulin resistance are associated with increased IMCLs [57, 60–63]. These excess ectopic lipids are believed to induce lipotoxicity, where dysfunctional lipid metabolism promotes inflammation, oxidative injury and disruption of several cell functions, including maintenance of the neuromuscular junction (NMJ) [55–57, 64], all of which could impair neuromuscular function. Accordingly, we suggest a hypothesis whereby aging muscles lack the metabolic flexibility to process the IMCLs in the way that younger muscles do [62, 65, 66], leading to lipotoxicity and its associated impairments. A set of interesting experiments in transgenic mice (carnitine palmitoyltransferase 1B knockout) have suggested that IMCL accumulation may not directly induce insulin-resistance, but even these animals' exhibited markers of muscle injury, disrupted metabolism and functional impairment, which were partially ameliorated by a low-fat diet [67–69]. Compared to healthy older adults, studies report greater IMCLs in frail, mobility-impaired and high fall-risk older adults with reduced muscle performance, who are at increased risk of death and disability ([63, 70, 71]. Longitudinal studies indicate that increased IMCLs are predictive of losses of physical function (e.g., gait speed) that are associated with increased rates of morbidity and mortality (Beavers, 2013) [54]. Preclinical studies even report an association between lifespan and increased IMCLs (Schmeisser, 2019). Of note, increased IMCLs are observed in concert with reduced muscle

function in human and rodent models of disuse regardless of age, though the deficit is exacerbated by the combination of old age and disuse [60, 72].

Interestingly, ectopic muscle lipid accumulation is not necessarily pathogenic, as illustrated by a phenomenon known as the *Athlete's Paradox*. First reported, to our knowledge, in 2001 [73], this term has been used to describe the observation that fit, well-trained aerobic athletes exhibit high levels of IMCLs/LDs with no lipotoxicity or loss of muscle function [73–75]. While apparently contradictory, this observation likely highlights the metabolic differences between exercise adaptation and the aging process with regard to metabolic flexibility. First described in the context of a shift between aerobic and anaerobic metabolism in parasitic worms [76], the term was adopted to describe the capacity to adjust metabolic rate and substrate utilization to changing environmental demands and substrate availability [77, 78]. Thus, the well described shift from lipid to carbohydrate (CHO) oxidation with increasing exercise intensity [79–81] and back to lipid oxidation during rest and recovery is one example of metabolic flexibility related to metabolic demand. Indeed, IMCLs are reduced less following exercise in older vs. young adults, though later in the recovery period, the IMCL levels are similar [66]. This is interesting, because during the fasting and resting phase, metabolic flexibility drives a shift toward lipid vs. CHO metabolism. Older adults exhibit an elevated respiratory exchange ratio (reflecting greater CHO vs. lipid metabolism) during assessment of resting metabolic rate, and respiratory exchange ratio is positively associated with weight (fat) gain in older adults (JC Seidell, 1992). With imposed muscle disuse (e.g., bedrest, immobilization), normal resting lipid oxidation is disrupted and these conditions are also associated with increased IMCL [62, 72, 82]. Together, these data suggest that loss of metabolic flexibility with aging leads to incomplete lipid oxidation that could contribute to IMCL accumulation. On the supply-side, changes in metabolic flexibility are commonly assessed by manipulating diet. For example, muscle β-oxidation increases in response to a high-fat diet (HFD) [83–86], although excessive lipid content or duration of such diets can exceed this capacity, leading to ectopic lipid accumulation and impaired lipid metabolism [87–91]. Impaired metabolic flexibility with regard to both supply and demand perturbations has been reported in old age [65, 80, 89, 92] and ectopic lipid accumulation with aging, diabetes and obesity is associated with a blunted adaptation to exercise, all conditions that are associated with mitochondrial impairments. As normal mitochondrial function is essential to metabolic flexibility [93], the cause and effect sequence of IMCL accumulation and mitochondrial function remains equivocal and the two factors are likely linked in a "vicious cycle [94]. " However, in old age, a metabolic shift resulting in reduced lipid turnover has been reported to precede the onset of sarcopenia [95]. Thus, impaired metabolic flexibility with aging leads to accumulation and reduced utilization of ectopic lipids that persist in skeletal muscle and may lead to increased serum free-fatty acids from non-muscle sources [74, 96]. In contrast, healthy, trained individuals exhibit functional metabolic flexibility, and the training-induced increases in the storage and utilization of carbohydrate (glycogen) and lipid [97–99] result in consistent, appropriate utilization of IMCLs. This prevents the lipotoxic effects on contractile and metabolic performance of skeletal muscle seen in aging [100], and explains the athlete's paradox.

Lifestyle interventions for age-associated ectopic lipid accumulation

To date, the main interventions available to combat sarcopenia and age-related muscle weakness are nutrition and exercise (Fig. 1). The effectiveness of these approaches is in line with the preceding discussion of metabolic flexibility, as they affect the supply and demand aspects of metabolism, respectively, and have been shown to influence IMCL levels in both aging and obesity [59, 97, 99, 101, 102].

Exercise

Aging is generally associated with a reduction in overall physical activity [103]. As noted above, muscle disuse leads to increased muscle lipid deposition and loss of muscle performance (weakness, fatigue, insulin resistance, etc.). It is no surprise then that both life-long exercise and exercise initiated at older ages can positively affect both muscle performance and muscle lipids in both human and animal models. Although there are effects of exercise on appetite that may increase caloric intake in older adults [104], the main effect is a regular increase in metabolic demand that can reduce IMCL accumulation. Indeed aerobic exercise in healthy, young adults has been shown to acutely reduce IMCL (Bucher, 2014), which may account for the Athlete's Paradox described earlier. Thus, adoption of a regular exercise program may or may not reduce resting IMCLs, but promotion of appropriate metabolic utilization and turnover of IMCL to prevent the negative impact of lipotoxicity [105] might be more important than actual IMCL content. For example, high running wheel activity in a rat model of hyperphagic obesity exhibited reduced muscle lipid peroxidation vs. sedentary controls, despite similarly high levels of total muscle lipids [106]. Additionally, aerobic exercise may alter the muscle lipid profile and reducing lipotoxic intermediates (Mendham 2021). Exercise has also been found to enhance metabolic flexibility of muscle [107], promoting the complete oxidation of palmitate, the incomplete

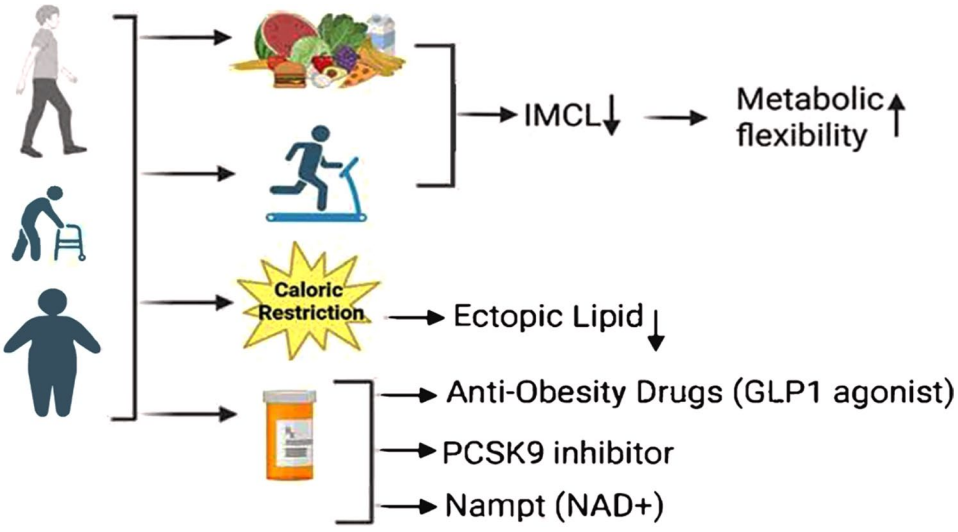

Fig. 1 Lifestyle and pharmacologic interventions for reducing IMCLs. Young, physically active adults generally maintain normal IMCLs with a varied, isocaloric diet. Older adults can reduce IMCLs with regular exercise. Individuals with obesity can reduce IMCLs with caloric restriction or anti-obesity medications. The use of such medications is being considered for older adults. Alternative pharmacologic approaches might also improve muscle lipid status, but current data are largely from preclinical studies. Created with https://BioRender.com

oxidation of which is associated with lipotoxic intermediates in muscle cells [108, 109]. Whether these effects of exercise are the same in aged individuals, as opposed to adults with obesity remains equivocal [110–112] and is likely influenced by long-term diet and physical activity patterns. A further concern for older adults is that aging increases risks for the frequency and duration of episodes of injury and illness resulting in muscle disuse relative to younger adults [113]. Evidence suggests that muscle disuse leads to a shift away from lipid toward carbohydrate oxidation has been shown with muscle disuse [62, 72, 82] similar to that seen with old age, potentially compounding deficits in metabolic flexibility and leading to further IMCL accumulation. Furthermore, evidence suggests that the return to normal metabolism following disuse may also be blunted in old age (impaired "metabolic elasticity" [81]), leading to a progressive increase in IMCL and decline in muscle performance with every episode of disuse.

Caloric restriction

Given the association between excess caloric intake, obesity and IMCL, it is reasonable that a calorie restricted diet (CR) might reduce ectopic lipid accumulation in aging muscle. Indeed, a long-term calorie-restricted diet has been recognized for many years as a way to extend lifespan and reduce age-related physiological impairments in a variety of organisms [114–116]. Classic CR can involve a reduction of 20% to as much as 50% of average caloric intake and tend to induce greater weight loss than exercise interventions alone [115, 117, 118]. Because of the long lifespan of humans, trials investigating truly long-term CR in humans are problematic, but lifespan, health span and muscular benefits of shorter-term CR have been reported in both human and animal models [119–121]. With regard to overall body weight and fat loss, evidence suggests that CR is more effective than exercise alone at reducing body weight and fat mass [115, 122]. With regard to IMCLs greater loss of overall fat mass has been associated with greater loss of IMCL [114, 123, 124], but the effects of CR have been shown to be mixed, with studies reporting increases, decreases and no change [124–127]. In addition, concerns with initiating CR in aged individuals have been raised for many years [128–130]. The main thrust of these reservations is centered around the fact that even overweight older adults can exhibit reduced muscle mass (sarcopenic obesity) and loss of muscle mass during CR could put older adults at high risk for clinically meaningful (i.e., disabling) weakness. Several reports have raised valid methodological issues with common measures and terminology related to fat-free mass, lean mass and muscle mass such that the oft-touted "25% rule" (~ 25% of weight lost with a typical lifestyle intervention regimen is muscle/lean mass [115]) is likely much more variable with regard to its impact on muscle [131, 132]. Nevertheless, any substantial impact on muscle performance with CR could pose problems for older adults by the accelerated development or progression of sarcopenia, which could further impair muscle performance and functional mobility. A potential further complication of the use of CR in an older population is common observation, in humans and animals, that weight regain following CR interventions appears to preferentially favor increased fat mass vs. lean/muscle mass [123, 133], potentially due to altered lipid oxidation [134]. Indeed, the greater loss of weight and lean mass in CR vs.

exercise may put individuals at greater risk of weight and fat mass regain following CR [133, 135]. In older adults, who may exhibit reduced metabolic flexibility, this could promote further impair proper metabolic oxidation of lipids and increase ectopic lipid composition in addition to a relative loss of muscle: body mass.

Pharmacologic interventions to address ectopic muscle lipids

While the lifestyle interventions of exercise and nutrition described in previous sections are mainstays of preserving aging muscle performance and combatting sarcopenia, they have their limitations. Unfortunately, exercise participation among older adults is low and in a number of older adults may be precluded or limited due to age-related co-morbidities, as well as social and environmental barriers [136–140]. Adherence to prolonged dietary restriction in humans is also poor [141] and so, while exercise and dietary strategies for older adults should be promoted and supported, pharmacological interventions should also be pursued.

Anti-obesity drugs: In recent years, the use of pharmacological analogs for the incretins Glucagon-Like Peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (aka gastric inhibitory peptide; GiP) [142, 143] has greatly increased. Initially developed as anti-diabetic medications targeting reduced blood glucose and glycosylated hemoglobin, the findings that use of these drugs could lead to weight loss has led to their use in treating obesity. Indeed, they have been found to significantly improve the efficacy and effectiveness of pharmacological approaches to weight loss. A number of physiological aspects of aging are also found in obesity and diabetes (Fig. 2) including accumulation of ectopic lipids and lipotoxicity in metabolic organs such as liver, skeletal muscle and heart, as well as decreased mitochondrial number and activity [144–147]. These changes are associated with increased long and medium chain acylcarnitines and incomplete β-oxidation, which is further exacerbated in Type 2 diabetes [148–151] in skeletal muscle. Because of the shared metabolic impairments in aging, obesity and diabetes, the idea of potentially using these new drugs to address sarcopenia and IMCL accumulation in aging muscle is now being considered. While some newer, FDA-approved anti-diabetic drug classes are currently being studied for their possible effects on obesity (e.g., dipeptidyl peptidase-4 (DPP-4) inhibitors; sodium-glucose cotransporter-2 (SGLT2) inhibitors), fewer data on weight loss and body composition are available compared to the incretin analog medications [143].

Although a number of direct metabolic effects of these anti-obesity drugs have been evaluated in different tissues, the majority of their effects are attributed to appetite control and satiety [142]. Thus, they function largely through CR with minimal effects on energy expenditure, consistent with reports that muscles do not express GLP-1 or GIP receptors [152]. Along with the positive effects on blood glucose, the popularity of these drugs centers around their greater effectiveness with regard to weight loss (and thus associated fat loss) when compared with CR through lifestyle intervention alone [115, 122]. Since fat mass and IMCL tend to be positively associated, these drugs may have potential to reduce IMCL in aging muscle. There are few studies of anti-obesity drug treatment that directly evaluate IMCLs in either humans or animals [153–156], which report either decreases or no change in IMCL. However, none of these studies were conducted in aged humans or animals, and all involved models of obesity and/or Type 2 diabetes. Thus, the potential to improve muscle function in sarcopenia via IMCL metabolism remains unexplored. Moreover, this possible application comes with concerns similar to those for CR regarding effects on lean/muscle mass balanced against the potential ability to control lipid accumulation

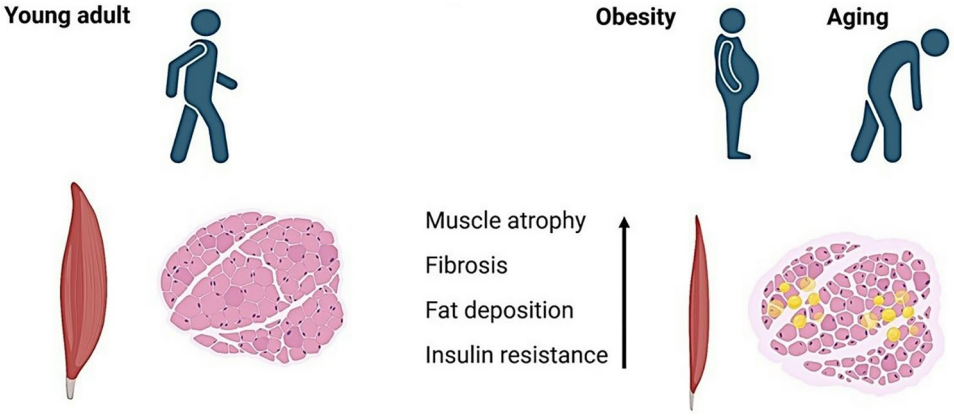

Fig. 2 Common mechanisms of reduced muscle mass and increased lipid accumulation in aging and obesity as compared to healthy, young adults. Created with https://BioRender.com

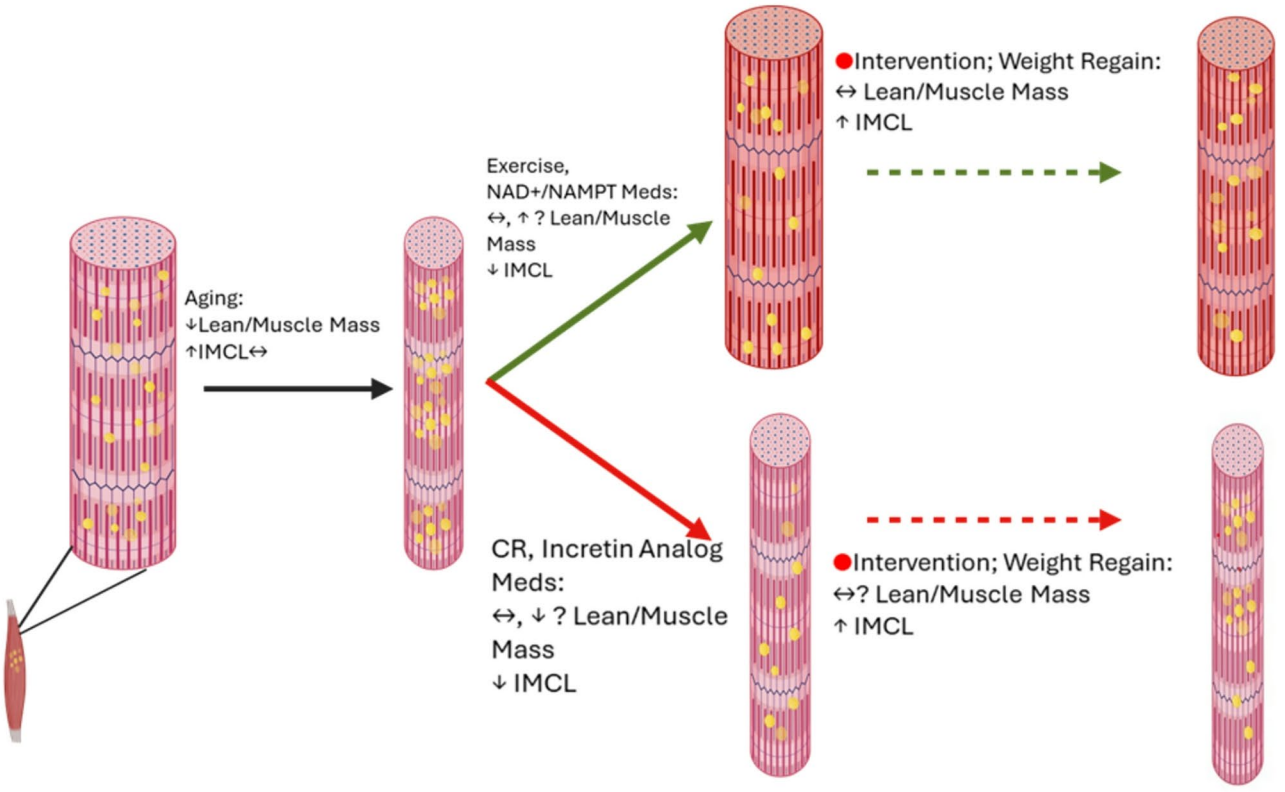

Fig. 3 Potential divergent effects of "stimulatory" (green arrows) vs. "inhibitory" interventions (red arrows) on muscle size and IMCLs. While both interventions can reduce IMCLs, the stimulatory approaches may carry greater benefits related to promotion of muscle mass, as well as maintenance during periods where the intervention is removed (red circles: ●). Created with https://BioRender.com

in older adults, as these drugs also can reduce lean mass [142, 143, 157].

In addition, since the bulk of the weight- and fat-loss effects of these drugs is due to CR, driven by reduced appetite, weight regain tends to recur at a fairly rapid rate unless the drug regimen is retained. Long term compliance with medications can be quite variable, and truly long-term GLP there is a similar concern that, during weight regain following treatment, accrual of fat mass and IMCLs could outstrip return of lean/muscle mass (Fig. 3). Indeed, human and animal studies report a greater proportion of fat as a fraction regained weight following GLP-1 receptor agonist treatment has been shown to exceed that seen when it is combined with exercise or as compared to exercise alone [123, 135]. Interestingly, a recent study using a rodent model of obesity found that GLP-1 receptor agonist (exenatide) treatment at a dose that improved serum glucose and insulin sensitivity, but not body or fat mass, reduced IMCL [154]. Though these animals were not aged, this finding does support further investigation of the potential for GLP-1 agonist drugs as a method for improving aged muscle quality, particularly in the absence of obesity. Finally, it should be noted that the bulk of the studies of these anti-obesity drugs have been understandably conducted in models of obesity and or diabetes, these conditions are not necessarily affecting older adults, though incidence of both increases with age. Further, despite increased interest in sarcopenic obesity, few studies (human or animal) have evaluated these drugs in older individuals. For example, a recent review paper [142] found only 4 of 24 studies involved participants with a mean age of ≥60 years and the study with the oldest sample (68 years) consisted of only 9 participants. Given the plethora of metabolic and neuromuscular changes occurring in old age, application of the new and future generations of anti-obesity drugs to older adults should probably best proceed on the assumption that results will differ from those seen on younger adults.

PCSK9 Inhibition

The proprotein convertase subtilisin/kexin type 9 (PCSK9) is a protease that degrades low-density lipoprotein receptors [158]. The pharmacological mechanism of PCSK9 inhibitors is it allows the availability of LDL receptor, which thereby results in increased binding of LDL to its receptor and therefore removal of LDL from blood stream, overall this process is beneficial in disease conditions such as hypercholesterolemia, obesity and Type 2 diabetes [159, 160]. Inhibiting PCSK9 receptor binding and signaling significantly lowers the low-density

lipoprotein levels in the blood similar to HMG-CoA reductase inhibitors (statins) and are aimed at treating obesity and diabetes associated cardiovascular diseases by lowering blood lipids. Lowering blood lipids is associated with reduced risk of such diseases, and might also mitigate the accumulation of IMCLs [161]. However, there are some concerns that PCSK9 inhibition might promote intrahepatic lipid accumulation [162]. New interventions such as MEDI4166, a PCSK9 antibody and glucagon-like peptide-1 (GLP-1) fusion molecule require further testing in models of aging and Type 2 diabetic patients [163].

NAD+-directed Medications

In contrast to the mechanistic strategy of the incretin-analogue drugs, i.e., reducing the stimulus for excessive energy (food) intake, an alternative pharmacologic approach could be to address the metabolic demand side of metabolic flexibility to target age-related accumulation of ectopic lipids. As noted above, a key manifestation of metabolic flexibility is appropriate shifting between lipid and CHO metabolism and data indicate that sarcopenic older adults manifest reduced lipid oxidation (though not lipolysis) during post-prandial resting metabolism and aerobic exercise [80] and has been found to precede onset of sarcopenia [95]. If an appropriate target for restoring metabolic flexibility in aged muscle could be identified and targeted, aging muscle might be (at least partially) maintained or restored. We propose that NAD+ (nicotinamide adenine dinucleotide), an essential cofactor in the TCA cycle where lipid and CHO metabolism intersect is a potential target. It is a key factor in metabolism of all major biomolecules (lipids, carbohydrates, amino acids) as well as ATP synthesis by the electron transport chain [164, 165], and reduced NAD + accompanies a loss of metabolic flexibility [166]. Reduced muscle NAD + is observed with aging, attributed to both increased consumption for specific biochemical processes and reduced synthesis. Increased consumption has been linked to excessive activation of poly-ADP ribose polymerase to address DNA damage, which is known to increase with aging [167, 168]. Reduced synthesis is associated with reduced abundance and/or activity of nicotinamide phosphoribosyltransferase (NAMPT), the rate-limiting enzyme of intramuscular NAD + synthesis [169–171]. Some of the benefits of exercise in aged muscle may be a result of the fact that aerobic exercise training can increase muscle NAMPT activity and NAD+ [170, 172, 173]. Thus, a drug that could produce a similar effect could have significant benefits for addressing sarcopenia and age-related muscle weakness. Pharmacological increases in NAD + has been shown to restore mitochondrial function in various preclinical models of aging [174]. We have found that administration of 3,6-dibromo-α-[(phenylamino)methyl]-9 H-carbazol-9-ethanol (P7C3), a NAMPT activator, increases NAD+, enhances voluntary strength and muscle contractility [145] (in response to direct stimulation), and realigns pro-and anti-inflammatory lipids in a murine model of type 2 diabetes to better approximate those of healthy control mice, suggesting that metabolic function in muscle was reset to more complete lipid oxidation. A similar association between increased NAD + and reduced hepatic ectopic lipids has been reported in a mouse model of diet-induced obesity [175]. As diabetes is associated with impaired metabolic flexibility [176], we suggest that P7C3 might have similar benefits for aging muscle. We hypothesize that increasing NAD + availability by NAMPT activation will normalize aging metabolic flexibility and restore muscle β-oxidation, which will reduce the IMCL accumulation and lipotoxicity that inhibit recovery in aged muscle. Improved cellular energy stores might also result from NAMPT activation, which could enhance protein synthesis needed to rebuild muscle, remodel the NMJ [177], and promote repair following the mechanical injury [178], all of which might improve muscle performance. To date, data from NAMPT activating drugs has been limited to preclinical studies. A few human studies have attempted to address NAD + deficiency in aging and obesity though the use of exogenous NAD + precursors (e.g., nicotinamide riboside). However, these approaches have not shown improved muscle function [179], nor have they shown increased muscle NAD+ [180, 181]. However, other studies suggest that aging muscle is already not using endogenous precursors, as indicated by increased urinary 1-methylnicotinamide [182] (a marker of breakdown of unused NAD + precursors [183]). We suggest substrate provision is insufficient if NAMPT activity remains low, making development of pharmacological interventions to increase NAD + via NAMPT activation to be a more promising strategy.

Summary

Maintaining sufficient muscle function to limit the risk of disability in older adults is a critical goal for geriatric care. Accumulating findings indicate that accumulation of ectopic lipids in muscle may be an important contributor to age-related muscle weakness, due to a loss of metabolic flexiblity. Although the long-established interventions of exercise and diet can improve muscle function and lipid status, they require long-term behavior changes that older adult may not be able, or willing, to adopt, for a variety of reasons. One potential alternative strategy for addressing the contribution of ectopic lipids to age-related muscle impairments is the application of the incretin-analog drugs that are increasingly used to treat obesity. Notably, the application of these drugs in human and animal models of aging is limited, with a lack

of human clinical trials, raising potential concerns for their use in treating muscle weakness. The incretin analog medications do reduce fat mass in obesity and DM, but there are concerns that they may reduce lean/muscle mass as well. In older adults, where loss of muscle mass is already an established problem, such an effet might have negative consequences that offset, or exceed the benefits accrued from a reduction in fat mass. Further study in aging models is needed to address these concerns. One aspect of such medication regimens that has been explored even less is related to the discontinuation of treatment. During periods when older adults might suspend medication use, that fat mass will be regained to a greater extent than lean mass and potentially exacerbate the problem. A potential alternative pharmacological avenue for targeting ectopic lipids in aging muscle is increasing the supply of NAD + to promote metabolic flexibility. As metabolic flexibility is directly tied to mitochondrial function, the cause and effect relationship between IMCLs and mitochondrial dysfunction remains equivocal, but a "vicious cycle," where each factor reinforces the other is likely present in aging. Though studies to date have been mostly limited to preclinical work, data suggest that increasing NAD + can stimulate metabolic flexibility, reduce ectopic lipids and improve muscle function in animal models of diabetes. Given that aging and diabetes share several metabolic and muscular impairments, it is not unreasonable to think NAD+ - promoting medications could have similar benefits for aging muscle, with less risk for loss of muscle mass than incretin- analog medications might pose. We suggest that further investigation of this possibility is warranted.

Abbreviations

CHO	Carbohydrate
DPP-4	Dipeptidyl peptidase 4
FO	Fish oil
GiP	Gastric inhibitory peptide
GLP-1	Glucagon-like peptide 1
HFD	High-fat diet
HMG CoA	3-hydroxy-3-methylglutaryl coenzyme A
IMCL	Intramyocellular lipids
LD	Lipid droplet
MHC	Myosin Heavy Chain
NAD +	Nicotinamide adenine dinucleotide
NAMPT	Nicotinamide phosphoribosyltransferase
NMJ	Neuromuscular junction
P7C3	3,6-dibromo-α-[(phenylamino)methyl]-9 H-carbazol-9-ethanol
PCSK9	Proprotein convertase subtilisin/kexin type 9
SGLT2	Sodium-glucose transporter 2

Acknowledgements
Not applicable.

Author contributions
All authors (DWR, RM, SMT) contributed to the conception, writing, and revision of the paper, and all of them read and approved the final manuscript.

Funding
Saunders Endowment for Geriatric Pharmacotherapy and NIH DK119066 (to SMT).

Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
Not applicable; Review Article.

Consent for publication
Not applicable; Review Article.

Competing interests
The authors declare no competing interests.

Received: 11 March 2025 / Accepted: 22 May 2025
Published online: 31 May 2025

References

1. Jacobs JJ, Andersson GBJ, Bell J-E, Weinstein SL, Dormans JP, Gnatz SM, et al. The burden of musculoskeletal diseases in the united States. Rosemont, IL: American Academy of Orthopedic Surgeons; 2008.
2. Janssen I, Shepard DS, Katzmarzyk PT, Roubenoff R. The healthcare costs of sarcopenia in the united States. J Am Geriatr Soc. 2004;52(1):80–5.
3. Bean JF, Kiely DK, Herman S, Leveille SG, Mizer K, Frontera WR, et al. The relationship between leg power and physical performance in mobility-limited older people. J Am Geriatr Soc. 2002;50(3):461–7.
4. Brown M, Sinacore DR, Host HH. The relationship of strength to function in the older adult. J Gerontol Biol Sci Med Sci. 1995;50(Spec No):55–9.
5. Schwendner KI, Mikesky AE, Holt WS Jr., Peacock M, Burr DB. Differences in muscle endurance and recovery between fallers and nonfallers, and between young and older women. J Gerontol Biol Sci Med Sci. 1997;52(3):M155–60.
6. Fried LP, Tangen CM, Walston J, Newman AB, Hirsch C, Gottdiener J, et al. Frailty in older adults: evidence for a phenotype. J Gerontol Biol Sci Med Sci. 2001;56(3):M146–56.
7. Xue QL, Bandeen-Roche K, Varadhan R, Zhou J, Fried LP. Initial manifestations of frailty criteria and the development of frailty phenotype in the women's health and aging study II. J Gerontol Biol Sci Med Sci. 2008;63(9):984–90.
8. Metter EJ, Talbot LA, Schrager M, Conwit R. Skeletal muscle strength as a predictor of all-cause mortality in healthy men. J Gerontol Biol Sci Med Sci. 2002;57(10):B359–65.
9. Nations U. Department of economic and social affairs. Popul Div (2017) World Popul Prospects. 2017;2.
10. Rosenberg IH. Sarcopenia: origins and clinical relevance. J Nutr. 1997;127(5 Suppl):S990–1.
11. Greenlund LJ, Nair KS. Sarcopenia–consequences, mechanisms, and potential therapies. Mech Ageing Dev. 2003;124(3):287–99.
12. Grinspoon S, Corcoran C, Rosenthal D, Stanley T, Parlman K, Costello M, et al. Quantitative assessment of cross-sectional muscle area, functional status, and muscle strength in men with the acquired immunodeficiency syndrome wasting syndrome. J Clin Endocrinol Metab. 1999;84(1):201–6.
13. Lexell J, Taylor CC, Sjostrom M. What is the cause of the ageing atrophy? Total number, size and proportion of different fiber types studied in whole Vastus lateralis muscle from 15- to 83-year-old men. J Neurol Sci. 1988;84(2–3):275–94.
14. Kallman DA, Plato CC, Tobin JD. The role of muscle loss in the age-related decline of grip strength: cross-sectional and longitudinal perspectives. J Gerontol. 1990;45(3):M82–8.
15. Baumgartner RN, Stauber PM, McHugh D, Koehler KM, Garry PJ. Cross-sectional age differences in body composition in persons 60 + years of age. J Gerontol Biol Sci Med Sci. 1995;50(6):M307–16.
16. Evans WJ, What is sarcopenia?. J Gerontol Biol Sci Med Sci. 1995;50(Spec No):5–8.
17. Baumgartner RN, Koehler KM, Gallagher D, Romero L, Heymsfield SB, Ross RR, et al. Epidemiology of sarcopenia among the elderly in new Mexico. Am J Epidemiol. 1998;147(8):755–63.
18. Visser M, Newman AB, Nevitt MC, Kritchevsky SB, Stamm EB, Goodpaster BH, et al. Reexamining the sarcopenia hypothesis. Muscle mass versus muscle strength. Health, aging, and body composition study research group. Ann N Y Acad Sci. 2000;904:456–61.

19. Goodpaster BH, Carlson CL, Visser M, Kelley DE, Scherzinger A, Harris TB, et al. Attenuation of skeletal muscle and strength in the elderly: the health ABC study. J Appl Physiol. 2001;90(6):2157–65.
20. Visser M, Goodpaster BH, Kritchevsky SB, Newman AB, Nevitt M, Rubin SM, et al. Muscle mass, muscle strength, and muscle fat infiltration as predictors of incident mobility limitations in well-functioning older persons. J Gerontol Biol Sci Med Sci. 2005;60(3):324–33.
21. Gonzalez E, Messi ML, Delbono O. The specific force of single intact extensor digitorum longus and soleus mouse muscle fibers declines with aging. J Membr Biol. 2000;178(3):175–83.
22. Horner AM, Russ DW, Biknevicius AR. Effects of early-stage aging on locomotor dynamics and hindlimb muscle force production in the rat. J Exp Biol. 2011;214(Pt 21):3588–95.
23. Moran AL, Warren GL, Lowe DA. Soleus and EDL muscle contractility across the lifespan of female C57BL/6 mice. Exp Gerontol. 2005;40(12):966–75.
24. Payne AM, Dodd SL, Leeuwenburgh C. Life-long calorie restriction in Fischer 344 rats attenuates age-related loss in skeletal muscle-specific force and reduces extracellular space. J Appl Physiol. 2003;95(6):2554–62.
25. Russ DW, Grandy JS, Toma K, Ward CW. Ageing, but not yet senescent, rats exhibit reduced muscle quality and sarcoplasmic reticulum function. Acta Physiol (Oxf). 2011;201(3):391–403.
26. Clark BC, Manini TM. Sarcopenia =/= dynapenia. J Gerontol Biol Sci Med Sci. 2008;63(8):829–34.
27. Delbono O. Molecular mechanisms and therapeutics of the deficit in specific force in ageing skeletal muscle. Biogerontology. 2002;3(5):265–70.
28. Deschenes MR. Effects of aging on muscle fibre type and size. Sports Med. 2004;34(12):809–24.
29. Reeves ND, Narici MV, Maganaris CN. Effect of resistance training on skeletal muscle-specific force in elderly humans. J Appl Physiol. 2004;96(3):885–92.
30. Russ DW, Gregg-Cornell K, Conaway MJ, Clark BC. Evolving concepts on the age-related changes in muscle quality. J Cachexia Sarcopenia Muscle. 2012;3(2):95–109.
31. Klitgaard H, Zhou M, Schiaffino S, Betto R, Salviati G, Saltin B. Ageing alters the myosin heavy chain composition of single fibres from human skeletal muscle. Acta Physiol Scand. 1990;140(1):55–62.
32. Sullivan VK, Powers SK, Criswell DS, Tumer N, Larochelle JS, Lowenthal D. Myosin heavy chain composition in young and old rat skeletal muscle: effects of endurance exercise. J Appl Physiol. 1995;78(6):2115–20.
33. Blottner D, Hastermann M, Weber R, Lenz R, Gambara G, Limper U, et al. Reactive jumps preserve skeletal muscle structure, phenotype, and myofiber oxidative capacity in bed rest. Front Physiol. 2019;10:1527.
34. Brooks MJ, Hajira A, Mohamed JS, Alway SE. Voluntary wheel running increases satellite cell abundance and improves recovery from disuse in gastrocnemius muscles from mice. J Appl Physiol. 2018;124(6):1616–28.
35. Ciciliot S, Rossi AC, Dyar KA, Blaauw B, Schiaffino S. Muscle type and fiber type specificity in muscle wasting. Int J Biochem Cell Biol. 2013;45(10):2191–9.
36. Sergeeva XV, Lvova ID, Sharlo KA. Disuse-Induced muscle fatigue: facts and assumptions. Int J Mol Sci. 2024;25(9).
37. Cawthon PM, Fox KM, Gandra SR, Delmonico MJ, Chiou CF, Anthony MS, et al. Do muscle mass, muscle density, strength, and physical function similarly influence risk of hospitalization in older adults? J Am Geriatr Soc. 2009;57(8):1411–9.
38. Manini TM, Clark BC. Dynapenia and aging: an update. J Gerontol Biol Sci Med Sci. 2011.
39. Newman AB, Kupelian V, Visser M, Simonsick EM, Goodpaster BH, Kritchevsky SB, et al. Strength, but not muscle mass, is associated with mortality in the health, aging and body composition study cohort. J Gerontol Biol Sci Med Sci. 2006;61(1):72–7.
40. Degens H, Alway SE. Skeletal muscle function and hypertrophy are diminished in old age. Muscle Nerve. 2003;27(3):339–47.
41. Raue U, Slivka D, Minchev K, Trappe S. Improvements in whole muscle and myocellular function are limited with high-intensity resistance training in octogenarian women. J Appl Physiol. 2009;106(5):1611–7.
42. Hikida RS, Staron RS, Hagerman FC, Walsh S, Kaiser E, Shell S, et al. Effects of high-intensity resistance training on untrained older men. II. Muscle fiber characteristics and nucleo-cytoplasmic relationships. J Gerontol Biol Sci Med Sci. 2000;55(7):B347–54.
43. Hwee DT, Bodine SC. Age-related deficit in load-induced skeletal muscle growth. J Gerontol Biol Sci Med Sci. 2009;64(6):618–28.
44. Personius KE, Jayaram A, Krull D, Brown R, Xu T, Han B, et al. Grip force, EDL contractile properties, and voluntary wheel running after postdevelopmental myostatin depletion in mice. J Appl Physiol. 2010;109(3):886–94.
45. Matsakas A, Macharia R, Otto A, Elashry MI, Mouisel E, Romanello V, et al. Exercise training attenuates the hypermuscular phenotype and restores skeletal muscle function in the myostatin null mouse. Exp Physiol. 2012;97(1):125–40.
46. Schirwis E, Agbulut O, Vadrot N, Mouisel E, Hourde C, Bonnieu A, et al. The beneficial effect of myostatin deficiency on maximal muscle force and power is attenuated with age. Exp Gerontol. 2013;48(2):183–90.
47. Amthor H, Macharia R, Navarrete R, Schuelke M, Brown SC, Otto A, et al. Lack of myostatin results in excessive muscle growth but impaired force generation. Proc Natl Acad Sci U S A. 2007;104(6):1835–40.
48. Chikani V, Ho KK. Action of GH on skeletal muscle function: molecular and metabolic mechanisms. J Mol Endocrinol. 2014;52(1):R107–23.
49. Isidori AM, Giannetta E, Greco EA, Gianfrilli D, Bonifacio V, Isidori A, et al. Effects of testosterone on body composition, bone metabolism and serum lipid profile in middle-aged men: a meta-analysis. Clin Endocrinol (Oxf). 2005;63(3):280–93.
50. Meinhardt U, Nelson AE, Hansen JL, Birzniece V, Clifford D, Leung KC, et al. The effects of growth hormone on body composition and physical performance in recreational athletes: a randomized trial. Ann Intern Med. 2010;152(9):568–77.
51. Thomas DK, Quinn MA, Saunders DH, Greig CA. Protein supplementation does not significantly augment the effects of resistance exercise training in older adults: A systematic review. J Am Med Dir Assoc. 2016;17(10):e9591–9.
52. de Lucena Alves CP, de Almeida SB, Lima DP, Neto PB, Miranda AL, Manini T, et al. Muscle quality in older adults: A scoping review. J Am Med Dir Assoc. 2023;24(4):462–e712.
53. Tieland M, Trouwborst I, Clark BC. Skeletal muscle performance and ageing. J Cachexia Sarcopenia Muscle. 2018;9(1):3–19.
54. Correa-de-Araujo R, Addison O, Miljkovic I, Goodpaster BH, Bergman BC, Clark RV, et al. Myosteatosis in the context of skeletal muscle function deficit: an interdisciplinary workshop at the National Institute on aging. Front Physiol. 2020;11:963.
55. Engin AB. What is lipotoxicity?? Adv Exp Med Biol. 2017;960:197–220.
56. Brookheart RT, Michel CI, Schaffer JE. As a matter of fat. Cell Metab. 2009;10(1):9–12.
57. Carter CS, Justice JN, Thompson L. Lipotoxicity, aging, and muscle contractility: does fiber type matter? Geroscience. 2019;41(3):297–308.
58. Ostler JE, Maurya SK, Dials J, Roof SR, Devor ST, Ziolo MT, et al. Effects of insulin resistance on skeletal muscle growth and exercise capacity in type 2 diabetic mouse models. Am J Physiol Endocrinol Metab. 2014;306(6):E592–605.
59. Handy RM, Holloway GP. Insights into the development of insulin resistance: unraveling the interaction of physical inactivity, lipid metabolism and mitochondrial biology. Front Physiol. 2023;14:1151389.
60. Vigelso A, Gram M, Wiuff C, Hansen CN, Prats C, Dela F, et al. Effects of immobilization and aerobic training on proteins related to intramuscular substrate storage and metabolism in young and older men. Eur J Appl Physiol. 2016;116(3):481–94.
61. Wall BT, Dirks ML, Snijders T, Stephens FB, Senden JM, Verscheijden ML, et al. Short-term muscle disuse atrophy is not associated with increased intramuscular lipid deposition or a decline in the maximal activity of key mitochondrial enzymes in young and older males. Exp Gerontol. 2015;61:76–83.
62. Vigelso A, Gram M, Dybboe R, Kuhlman AB, Prats C, Greenhaff PL, et al. The effect of age and unilateral leg immobilization for 2 weeks on substrate utilization during moderate-intensity exercise in human skeletal muscle. J Physiol. 2016;594(8):2339–58.
63. Gueugneau M, Coudy-Gandilhon C, Theron L, Meunier B, Barboiron C, Combaret L, et al. Skeletal muscle lipid content and oxidative activity in relation to muscle fiber type in aging and metabolic syndrome. J Gerontol Biol Sci Med Sci. 2015;70(5):566–76.
64. Miao Y, Xie L, Song J, Cai X, Yang J, Ma X, et al. Unraveling the causes of sarcopenia: roles of neuromuscular junction impairment and mitochondrial dysfunction. Physiological Rep. 2024;12(1):e15917.
65. Zhang X, Trevino MB, Wang M, Gardell SJ, Ayala JE, Han X, et al. Impaired mitochondrial energetics characterize poor early recovery of muscle mass following Hind limb unloading in old mice. J Gerontol Biol Sci Med Sci. 2018;73(10):1313–22.
66. Tsintzas K, Stephens FB, Snijders T, Wall BT, Cooper S, Mallinson J, et al. Intramyocellular lipid content and lipogenic gene expression responses following a single bout of resistance type exercise differ between young and older men. Exp Gerontol. 2017;93:36–45.
67. Warfel JD, Bermudez EM, Mendoza TM, Ghosh S, Zhang J, Elks CM, et al. Mitochondrial fat oxidation is essential for lipid-induced inflammation in skeletal muscle in mice. Sci Rep. 2016;6:37941.

68. Warfel JD, Vandanmagsar B, Wicks SE, Zhang J, Noland RC, Mynatt RL. A low fat diet ameliorates pathology but retains beneficial effects associated with CPT1b knockout in skeletal muscle. PLoS ONE. 2017;12(12):e0188850.
69. Wicks SE, Vandanmagsar B, Haynie KR, Fuller SE, Warfel JD, Stephens JM, et al. Impaired mitochondrial fat oxidation induces adaptive remodeling of muscle metabolism. Proc Natl Acad Sci U S A. 2015;112(25):E3300–9.
70. St-Jean-Pelletier F, Pion CH, Leduc-Gaudet JP, Sgarioto N, Zovile I, Barbat-Artigas S, et al. The impact of ageing, physical activity, and pre-frailty on skeletal muscle phenotype, mitochondrial content, and intramyocellular lipids in men. J Cachexia Sarcopenia Muscle. 2017;8(2):213–28.
71. Al Saedi A, Debruin DA, Hayes A, Hamrick M. Lipid metabolism in sarcopenia. Bone. 2022;164:116539.
72. Black MN, Wilkinson JA, Webb EK, Kamal M, Bahniwal R, McGlory C, et al. Two weeks of single-leg immobilization alters intramyocellular lipid storage characteristics in healthy, young women. J Appl Physiol. 2021;130(4):1247–58.
73. Goodpaster BH, He J, Watkins S, Kelley DE. Skeletal muscle lipid content and insulin resistance: evidence for a paradox in endurance-trained athletes. J Clin Endocrinol Metab. 2001;86(12):5755–61.
74. Dube JJ, Amati F, Stefanovic-Racic M, Toledo FG, Sauers SE, Goodpaster BH. Exercise-induced alterations in intramyocellular lipids and insulin resistance: the athlete's paradox revisited. Am J Physiol Endocrinol Metab. 2008;294(5):E882–8.
75. van Loon LJ, Koopman R, Manders R, van der Weegen W, van Kranenburg GP, Keizer HA. Intramyocellular lipid content in type 2 diabetes patients compared with overweight sedentary men and highly trained endurance athletes. Am J Physiol Endocrinol Metab. 2004;287(3):E558–65.
76. Kohler P. The strategies of energy conservation in helminths. Mol Biochem Parasitol. 1985;17(1):1–18.
77. Smith RL, Soeters MR, Wust RCI, Houtkooper RH. Metabolic flexibility as an adaptation to energy resources and requirements in health and disease. Endocr Rev. 2018;39(4):489–517.
78. Kelley DE, Mandarino LJ. Fuel selection in human skeletal muscle in insulin resistance: a reexamination. Diabetes. 2000;49(5):677–83.
79. Brooks GA, Mercier J. Balance of carbohydrate and lipid utilization during exercise: the crossover concept. J Appl Physiol. 1994;76(6):2253–61.
80. Shoemaker ME, Pereira SL, Mustad VA, Gillen ZM, McKay BD, Lopez-Pedrosa JM, et al. Differences in muscle energy metabolism and metabolic flexibility between sarcopenic and nonsarcopenic older adults. J Cachexia Sarcopenia Muscle. 2022;13(2):1224–37.
81. Zhou Q, Yu L, Cook JR, Qiang L, Sun L. Deciphering the decline of metabolic elasticity in aging and obesity. Cell Metab. 2023;35(9):1661–71. e6.
82. Blanc S, Normand S, Pachiaudi C, Fortrat JO, Laville M, Gharib C. Fuel homeostasis during physical inactivity induced by bed rest. J Clin Endocrinol Metab. 2000;85(6):2223–33.
83. Dasari S, Newsom SA, Ehrlicher SE, Stierwalt HD, Robinson MM. Remodeling of skeletal muscle mitochondrial proteome with high-fat diet involves greater changes to beta-oxidation than electron transfer proteins in mice. Am J Physiol Endocrinol Metab. 2018;315(4):E425–34.
84. Garcia-Roves P, Huss JM, Han DH, Hancock CR, Iglesias-Gutierrez E, Chen M, et al. Raising plasma fatty acid concentration induces increased biogenesis of mitochondria in skeletal muscle. Proc Natl Acad Sci U S A. 2007;104(25):10709–13.
85. Li M, Chen F, Wang H, Wu W, Zhang X, Tian C, et al. Non-invasive assessment of phosphate metabolism and oxidative capacity in working skeletal muscle in healthy young Chinese volunteers using (31)P magnetic resonance spectroscopy. PeerJ. 2016;4:e2259.
86. Miller WC, Bryce GR, Conlee RK. Adaptations to a high-fat diet that increase exercise endurance in male rats. J Appl Physiology: Respiratory Environ Exerc Physiol. 1984;56(1):78–83.
87. Eshima H, Tamura Y, Kakehi S, Kurebayashi N, Murayama T, Nakamura K et al. Long-term, but not short-term high-fat diet induces fiber composition changes and impaired contractile force in mouse fast-twitch skeletal muscle. Physiological Rep. 2017;5(7).
88. Koves TR, Ussher JR, Noland RC, Slentz D, Mosedale M, Ilkayeva O, et al. Mitochondrial overload and incomplete fatty acid oxidation contribute to skeletal muscle insulin resistance. Cell Metab. 2008;7(1):45–56.
89. Messa GAM, Piasecki M, Hurst J, Hill C, Tallis J, Degens H. The impact of a high-fat diet in mice is dependent on duration and age, and differs between muscles. J Exp Biol. 2020;223(Pt 6).
90. Shortreed KE, Krause MP, Huang JH, Dhanani D, Moradi J, Ceddia RB, et al. Muscle-specific adaptations, impaired oxidative capacity and maintenance of contractile function characterize diet-induced obese mouse skeletal muscle. PLoS ONE. 2009;4(10):e7293.
91. Hancock CR, Han DH, Chen M, Terada S, Yasuda T, Wright DC, et al. High-fat diets cause insulin resistance despite an increase in muscle mitochondria. Proc Natl Acad Sci U S A. 2008;105(22):7815–20.
92. Brooks SP, Lampi BJ. Enzymes of carbohydrate metabolism in young and adult rats fed diets differing in fat and carbohydrate. Mol Cell Biochem. 1996;159(1):55–63.
93. Muoio DM. Metabolic inflexibility: when mitochondrial indecision leads to metabolic gridlock. Cell. 2014;159(6):1253–62.
94. Devries MC, Samjoo IA, Hamadeh MJ, McCready C, Raha S, Watt MJ, et al. Endurance training modulates intramyocellular lipid compartmentalization and morphology in skeletal muscle of lean and obese women. J Clin Endocrinol Metab. 2013;98(12):4852–62.
95. Pugh TD, Conklin MW, Evans TD, Polewski MA, Barbian HJ, Pass R, et al. A shift in energy metabolism anticipates the onset of sarcopenia in rhesus monkeys. Aging Cell. 2013;12(4):672–81.
96. Shaw CS, Swinton C, Morales-Scholz MG, McRae N, Erftemeyer T, Aldous A, et al. Impact of exercise training status on the fiber type-specific abundance of proteins regulating intramuscular lipid metabolism. J Appl Physiol. 2020;128(2):379–89.
97. Fritzen AM, Lundsgaard AM, Kiens B. Tuning fatty acid oxidation in skeletal muscle with dietary fat and exercise. Nat Rev Endocrinol. 2020;16(12):683–96.
98. Richter EA, Hargreaves M, Exercise. GLUT4, and skeletal muscle glucose uptake. Physiol Rev. 2013;93(3):993–1017.
99. Bosma M. Lipid homeostasis in exercise. Drug Discov Today. 2014;19(7):1019–23.
100. Kwon OS, Nelson DS, Barrows KM, O'Connell RM, Drummond MJ. Intramyocellular ceramides and skeletal muscle mitochondrial respiration are partially regulated by Toll-like receptor 4 during hindlimb unloading. Am J Physiol Regul Integr Comp Physiol. 2016;311(5):R879–87.
101. Kiens B, Alsted TJ, Jeppesen J. Factors regulating fat oxidation in human skeletal muscle. Obes Reviews: Official J Int Association Study Obes. 2011;12(10):852–8.
102. Corpeleijn E, Saris WH, Blaak EE. Metabolic flexibility in the development of insulin resistance and type 2 diabetes: effects of lifestyle. Obes Reviews: Official J Int Association Study Obes. 2009;10(2):178–93.
103. Manini TM. Energy expenditure and aging. Ageing Res Rev. 2010;9(1):1–11.
104. Jadczak AD, Visvanathan R. Anorexia of Aging - An updated short review. J Nutr Health Aging. 2019;23(3):306–9.
105. Zacharewicz E, Hesselink MKC, Schrauwen P. Exercise counteracts lipotoxicity by improving lipid turnover and lipid droplet quality. J Intern Med. 2018;284(5):505–18.
106. Morris RT, Laye MJ, Lees SJ, Rector RS, Thyfault JP, Booth FW. Exercise-induced Attenuation of obesity, hyperinsulinemia, and skeletal muscle lipid peroxidation in the OLETF rat. J Appl Physiol. 2008;104(3):708–15.
107. Laye MJ, Rector RS, Borengasser SJ, Naples SP, Uptergrove GM, Ibdah JA, et al. Cessation of daily wheel running differentially alters fat oxidation capacity in liver, muscle, and adipose tissue. J Appl Physiol. 2009;106(1):161–8.
108. Capel F, Cheraiti N, Acquaviva C, Henique C, Bertrand-Michel J, Vianey-Saban C, et al. Oleate dose-dependently regulates palmitate metabolism and insulin signaling in C2C12 myotubes. Biochim Biophys Acta. 2016;1861(12 Pt A):2000–10.
109. Pinel A, Rigaudiere JP, Laillet B, Pouyet C, Malpuech-Brugere C, Prip-Buus C, et al. N-3PUFA differentially modulate palmitate-induced lipotoxicity through alterations of its metabolism in C2C12 muscle cells. Biochim Biophys Acta. 2016;1861(1):12–20.
110. Cartee GD, Farrar RP. Muscle respiratory capacity and VO2 max in identically trained young and old rats. J Appl Physiol. 1987;63(1):257–61.
111. Chee C, Shannon CE, Burns A, Selby AL, Wilkinson D, Smith K, et al. Relative contribution of intramyocellular lipid to Whole-Body fat oxidation is reduced with age but subsarcolemmal lipid accumulation and insulin resistance are only associated with overweight individuals. Diabetes. 2016;65(4):840–50.
112. Latimer LE, Constantin-Teodosiu D, Popat B, Constantin D, Houchen-Wolloff L, Bolton CE et al. Whole-body and muscle responses to aerobic exercise training and withdrawal in ageing and COPD. Eur Respir J. 2022;59(5).
113. Wall BT, Dirks ML, van Loon LJ. Skeletal muscle atrophy during short-term disuse: implications for age-related sarcopenia. Ageing Res Rev. 2013;12(4):898–906.
114. Most J, Gilmore LA, Smith SR, Han H, Ravussin E, Redman LM. Significant improvement in cardiometabolic health in healthy Nonobese individuals

during caloric restriction-induced weight loss and weight loss maintenance. Am J Physiol Endocrinol Metab. 2018;314(4):E396–405.
115. Weinheimer EM, Sands LP, Campbell WW. A systematic review of the separate and combined effects of energy restriction and exercise on fat-free mass in middle-aged and older adults: implications for sarcopenic obesity. Nutr Rev. 2010;68(7):375–88.
116. Xie WQ, Xiao WF, Tang K, Wu YX, Hu PW, Li YS, et al. Caloric restriction: implications for sarcopenia and potential mechanisms. Aging. 2020;12(23):24441–52.
117. Abe T, Song JS, Bell ZW, Wong V, Spitz RW, Yamada Y, et al. Comparisons of calorie restriction and structured exercise on reductions in visceral and abdominal subcutaneous adipose tissue: a systematic review. Eur J Clin Nutr. 2022;76(2):184–95.
118. Verheggen RJ, Maessen MF, Green DJ, Hermus AR, Hopman MT, Thijssen DH. A systematic review and meta-analysis on the effects of exercise training versus hypocaloric diet: distinct effects on body weight and visceral adipose tissue. Obes Reviews: Official J Int Association Study Obes. 2016;17(8):664–90.
119. Dorling JL, van Vliet S, Huffman KM, Kraus WE, Bhapkar M, Pieper CF, et al. Effects of caloric restriction on human physiological, psychological, and behavioral outcomes: highlights from CALERIE phase 2. Nutr Rev. 2021;79(1):98–113.
120. Lopez-Lluch G, Navas P. Calorie restriction as an intervention in ageing. J Physiol. 2016;594(8):2043–60.
121. Rizza W, Veronese N, Fontana L. What are the roles of calorie restriction and diet quality in promoting healthy longevity? Ageing Res Rev. 2014;13:38–45.
122. Ard J, Fitch A, Fruh S, Herman L. Weight loss and maintenance related to the mechanism of action of Glucagon-Like peptide 1 receptor agonists. Adv Therapy. 2021;38(6):2821–39.
123. Chmelo EA, Beavers DP, Lyles MF, Marsh AP, Nicklas BJ, Beavers KM. Legacy effects of short-term intentional weight loss on total body and thigh composition in overweight and obese older adults. Nutr Diabetes. 2016;6(4):e203.
124. Kristensen MD, Petersen SM, Moller KE, Lund MT, Hansen M, Hansen CN, et al. Obesity leads to impairments in the morphology and organization of human skeletal muscle lipid droplets and mitochondrial networks, which are resolved with gastric bypass surgery-induced improvements in insulin sensitivity. Acta Physiol (Oxf). 2018;224(4):e13100.
125. Coker RH, Robinette L, Kern PA. Minimal alteration in muscle lipid genes following stabilized weight loss. Appl Physiol Nutr Metab. 2017;42(12):1277–82.
126. Snel M, Gastaldelli A, Ouwens DM, Hesselink MK, Schaart G, Buzzigoli E, et al. Effects of adding exercise to a 16-week very low-calorie diet in obese, insulin-dependent type 2 diabetes mellitus patients. J Clin Endocrinol Metab. 2012;97(7):2512–20.
127. Nadeau KJ, Ehlers LB, Aguirre LE, Moore RL, Jew KN, Ortmeyer HK, et al. Exercise training and calorie restriction increase SREBP-1 expression and intramuscular triglyceride in skeletal muscle. Am J Physiol Endocrinol Metab. 2006;291(1):E90–8.
128. Darmon P. Intentional weight loss in older adults: useful or wasting disease generating strategy? Curr Opin Clin Nutr Metab Care. 2013;16(3):284–9.
129. Locher JL, Goldsby TU, Goss AM, Kilgore ML, Gower B, Ard JD. Calorie restriction in overweight older adults: do benefits exceed potential risks? Exp Gerontol. 2016;86:4–13.
130. Papageorgiou M, Kerschan-Schindl K, Sathyapalan T, Pietschmann P. Is weight loss harmful for skeletal health in obese older. Adults? Gerontol. 2020;66(1):2–14.
131. Heymsfield SB, Gonzalez MC, Shen W, Redman L, Thomas D. Weight loss composition is one-fourth fat-free mass: a critical review and critique of this widely cited rule. Obes Reviews: Official J Int Association Study Obes. 2014;15(4):310–21.
132. Tinsley GM, Heymsfield SB. Fundamental body composition principles provide context for Fat-Free and skeletal muscle loss with GLP-1 RA treatments. J Endocr Soc. 2024;8(11):bvae164.
133. Calonne J, Arsenijevic D, Scerri I, Miles-Chan JL, Montani JP, Dulloo AG. Low 24-hour core body temperature as a thrifty metabolic trait driving catch-up fat during weight regain after caloric restriction. Am J Physiol Endocrinol Metab. 2019;317(4):E699–709.
134. Steig AJ, Jackman MR, Giles ED, Higgins JA, Johnson GC, Mahan C, et al. Exercise reduces appetite and traffics excess nutrients away from energetically efficient pathways of lipid deposition during the early stages of weight regain. Am J Physiol Regul Integr Comp Physiol. 2011;301(3):R656–67.
135. Jensen SBK, Blond MB, Sandsdal RM, Olsen LM, Juhl CR, Lundgren JR, et al. Healthy weight loss maintenance with exercise, GLP-1 receptor agonist, or both combined followed by one year without treatment: a post-treatment analysis of a randomised placebo-controlled trial. EClinicalMedicine. 2024;69:102475.
136. (US) NCfHS, Health US. 2014: With Special Feature on Adults Aged 55–64. Health, Hyattsville (MD): United States; 2015.
137. Beck KL, Weeks LE, Montelpare WJ, MacDonald DJ. Identifying important factors for older adults' physical activity participation across individual/group, structured/unstructured contexts. Eur J Ageing. 2016;13(3):209–18.
138. Harvey JA, Chastin SF, Skelton DA. Prevalence of sedentary behavior in older adults: a systematic review. Int J Environ Res Public Health. 2013;10(12):6645–61.
139. Jancey J, Lee A, Howat P, Clarke A, Wang K, Shilton T. Reducing attrition in physical activity programs for older adults. J Aging Phys Act. 2007;15(2):152–65.
140. Hui EK, Rubenstein LZ. Promoting physical activity and exercise in older adults. J Am Med Dir Assoc. 2006;7(5):310–4.
141. Coker RH, Wolfe RR. Weight loss strategies in the elderly: A clinical conundrum. Obesity. 2018;26(1):22–8.
142. Dubin RL, Heymsfield SB, Ravussin E, Greenway FL. Glucagon-like peptide-1 receptor agonist-based agents and weight loss composition: filling the gaps. Diabetes Obes Metab. 2024;26(12):5503–18.
143. Sargeant JA, Henson J, King JA, Yates T, Khunti K, Davies MJ. A review of the effects of Glucagon-Like Peptide-1 receptor agonists and Sodium-Glucose cotransporter 2 inhibitors on lean body mass in humans. Endocrinol Metab (Seoul). 2019;34(3):247–62.
144. Kelley DE, He J, Menshikova EV, Ritov VB. Dysfunction of mitochondria in human skeletal muscle in type 2 diabetes. Diabetes. 2002;51(10):2944–50.
145. Manickam R, Tur J, Badole SL, Chapalamadugu KC, Sinha P, Wang Z, et al. Nampt activator P7C3 ameliorates diabetes and improves skeletal muscle function modulating cell metabolism and lipid mediators. J Cachexia Sarcopenia Muscle. 2022;13(2):1177–96.
146. Morino K, Petersen KF, Dufour S, Befroy D, Frattini J, Shatzkes N, et al. Reduced mitochondrial density and increased IRS-1 Serine phosphorylation in muscle of insulin-resistant offspring of type 2 diabetic parents. J Clin Invest. 2005;115(12):3587–93.
147. Ritov VB, Menshikova EV, He J, Ferrell RE, Goodpaster BH, Kelley DE. Deficiency of subsarcolemmal mitochondria in obesity and type 2 diabetes. Diabetes. 2005;54(1):8–14.
148. Kim JY, Hickner RC, Cortright RL, Dohm GL, Houmard JA. Lipid oxidation is reduced in obese human skeletal muscle. Am J Physiol Endocrinol Metab. 2000;279(5):E1039–44.
149. Mihalik SJ, Goodpaster BH, Kelley DE, Chace DH, Vockley J, Toledo FG, et al. Increased levels of plasma acylcarnitines in obesity and type 2 diabetes and identification of a marker of glucolipotoxicity. Obesity. 2010;18(9):1695–700.
150. Mogensen M, Sahlin K, Fernstrom M, Glintborg D, Vind BF, Beck-Nielsen H, et al. Mitochondrial respiration is decreased in skeletal muscle of patients with type 2 diabetes. Diabetes. 2007;56(6):1592–9.
151. Ritov VB, Menshikova EV, Azuma K, Wood R, Toledo FG, Goodpaster BH, et al. Deficiency of electron transport chain in human skeletal muscle mitochondria in type 2 diabetes mellitus and obesity. Am J Physiol Endocrinol Metab. 2010;298(1):E49–58.
152. Hammoud R, Drucker DJ. Beyond the pancreas: contrasting cardiometabolic actions of GIP and GLP1. Nat Rev Endocrinol. 2023;19(4):201–16.
153. Watanabe T, Tamura Y, Kakehi S, Funayama T, Gastaldelli A, Takeno K, et al. Effects of sitagliptin on ectopic fat contents and glucose metabolism in type 2 diabetic patients with fatty liver: A pilot study. J Diabetes Investig. 2015;6(2):164–72.
154. Xu F, Cao H, Chen Z, Gu H, Guo W, Lin B, et al. Short-term GLP-1 receptor agonist exenatide ameliorates intramyocellular lipid deposition without weight loss in Ob/ob mice. Int J Obes. 2020;44(4):937–47.
155. Kakegawa T, Sugimoto K, Saito K, Yunaiyama D, Araki Y, Wada T, et al. Favorable liver and skeletal muscle changes in patients with MASLD and T2DM receiving glucagon-like peptide-1 receptor agonist: A prospective cohort study. Med (Baltim). 2024;103(23):e38444.
156. Xiang J, Qin L, Zhong J, Xia N, Liang Y. GLP-1RA liraglutide and semaglutide improves Obesity-Induced muscle atrophy via SIRT1 pathway. Diabetes Metab Syndr Obes. 2023;16:2433–46.
157. Neeland IJ, Linge J, Birkenfeld AL. Changes in lean body mass with glucagon-like peptide-1-based therapies and mitigation strategies. Diabetes Obes Metab. 2024;26(Suppl 4):16–27.
158. Liu C, Chen J, Chen H, Zhang T, He D, Luo Q, et al. PCSK9 Inhibition: from current advances to evolving future. Cells. 2022;11:19.

159. Ibarretxe D, Girona J, Plana N, Cabre A, Ferre R, Amigo N, et al. Circulating PCSK9 in patients with type 2 diabetes and related metabolic disorders. Clin Investig Arterioscler. 2016;28(2):71–8.
160. Levenson AE, Shah AS, Khoury PR, Kimball TR, Urbina EM, de Ferranti SD, et al. Obesity and type 2 diabetes are associated with elevated PCSK9 levels in young women. Pediatr Diabetes. 2017;18(8):755–60.
161. Schrauwen-Hinderling VB, Hesselink MK, Schrauwen P, Kooi ME. Intramyocellular lipid content in human skeletal muscle. Obesity. 2006;14(3):357–67.
162. Ioannou GN, Lee SP, Linsley PS, Gersuk V, Yeh MM, Chen YY, et al. Pcsk9 deletion promotes murine nonalcoholic steatohepatitis and hepatic carcinogenesis: role of cholesterol. Hepatol Commun. 2022;6(4):780–94.
163. Jain M, Carlson G, Cook W, Morrow L, Petrone M, White NE, et al. Randomised, phase 1, dose-finding study of MEDI4166, a PCSK9 antibody and GLP-1 analogue fusion molecule, in overweight or obese patients with type 2 diabetes mellitus. Diabetologia. 2019;62(3):373–86.
164. Arnold PK, Finley LWS. Regulation and function of the mammalian Tricarboxylic acid cycle. J Biol Chem. 2023;299(2):102838.
165. Egan B, Zierath JR. Exercise metabolism and the molecular regulation of skeletal muscle adaptation. Cell Metab. 2013;17(2):162–84.
166. Franczyk MP, Qi N, Stromsdorfer KL, Li C, Yamaguchi S, Itoh H et al. Importance of adipose tissue NAD + Biology in regulating metabolic flexibility. Endocrinology. 2021;162(3).
167. Imai SI. NAD world 3.0: the importance of the NMN transporter and eNAMPT in mammalian aging and longevity control. NPJ Aging. 2025;11(1):4.
168. Xu Y, Xiao W. NAD+: an old but promising therapeutic agent for skeletal muscle ageing. Ageing Res Rev. 2023:102106.
169. Frederick DW, Loro E, Liu L, Davila A Jr., Chellappa K, Silverman IM, et al. Loss of NAD homeostasis leads to progressive and reversible degeneration of skeletal muscle. Cell Metab. 2016;24(2):269–82.
170. Koltai E, Szabo Z, Atalay M, Boldogh I, Naito H, Goto S, et al. Exercise alters SIRT1, SIRT6, NAD and NAMPT levels in skeletal muscle of aged rats. Mech Ageing Dev. 2010;131(1):21–8.
171. Xie N, Zhang L, Gao W, Huang C, Huber PE, Zhou X, et al. NAD(+) metabolism: pathophysiologic mechanisms and therapeutic potential. Signal Transduct Target Ther. 2020;5(1):227.
172. Sun X, Su L, Bu T, Zhang Y. Exercise training upregulates intracellular nicotinamide phosphoribosyltransferase expression in humans: a systematic review with meta-analysis. Front Public Health. 2023;11:1287421.
173. de Guia RM, Agerholm M, Nielsen TS, Consitt LA, Sogaard D, Helge JW, et al. Aerobic and resistance exercise training reverses age-dependent decline in NAD(+) salvage capacity in human skeletal muscle. Physiological Rep. 2019;7(12):e14139.
174. Romani M, Sorrentino V, Oh CM, Li H, de Lima TI, Zhang H, et al. NAD(+) boosting reduces age-associated amyloidosis and restores mitochondrial homeostasis in muscle. Cell Rep. 2021;34(3):108660.
175. Di Francesco A, Choi Y, Bernier M, Zhang Y, Diaz-Ruiz A, Aon MA, et al. NQO1 protects obese mice through improvements in glucose and lipid metabolism. NPJ Aging Mech Disease. 2020;6(1):13.
176. Goodpaster BH, Sparks LM. Metabolic flexibility in health and disease. Cell Metab. 2017;25(5):1027–36.
177. Lundt S, Zhang N, Polo-Parada L, Wang X, Ding S. Dietary NMN supplementation enhances motor and NMJ function in ALS. Exp Neurol. 2024;374:114698.
178. Kanazawa Y, Ikegami K, Sujino M, Koinuma S, Nagano M, Oi Y, et al. Effects of aging on basement membrane of the soleus muscle during recovery following disuse atrophy in rats. Exp Gerontol. 2017;98:153–61.
179. Connell NJ, Grevendonk L, Fealy CE, Moonen-Kornips E, Bruls YMH, Schrauwen-Hinderling VB, et al. NAD+-Precursor supplementation with L-Tryptophan, nicotinic acid, and nicotinamide does not affect mitochondrial function or skeletal muscle function in physically compromised older adults. J Nutr. 2021;151(10):2917–31.
180. Dollerup OL, Chubanava S, Agerholm M, Sondergard SD, Altintas A, Moller AB, et al. Nicotinamide riboside does not alter mitochondrial respiration, content or morphology in skeletal muscle from obese and insulin-resistant men. J Physiol. 2020;598(4):731–54.
181. Jensen JB, Dollerup OL, Moller AB, Billeskov TB, Dalbram E, Chubanava S, et al. A randomized placebo-controlled trial of nicotinamide riboside and pterostilbene supplementation in experimental muscle injury in elderly individuals. JCI Insight. 2022;7:19.
182. Calvani R, Brasili E, Pratico G, Capuani G, Tomassini A, Marini F, et al. Fecal and urinary NMR-based metabolomics unveil an aging signature in mice. Exp Gerontol. 2014;49:5–11.
183. Henderson JD, Quigley SNZ, Chachra SS, Conlon N, Ford D. The use of a systems approach to increase NAD(+) in human participants. NPJ Aging. 2024;10(1):7.

Publisher's note