Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Food Science

Translating novel insights from age-related loss of skeletal muscle mass and phenotypic flexibility into diet and lifestyle recommendations for the elderly

Joost JGC van den Borne, Urszula Kudla and Jan MW Geurts

Aging is associated with a decline in muscle mass and strength (sarcopenia), impacting not only mobility but also metabolic health. Phenotypic flexibility, as a proxy for health, impairs during aging and varies considerably between subjects, as identified from challenge studies with multiple biomarkers. Recent studies have linked transcriptomics and metabolomics in serum and muscle tissue to functional outcomes, such as muscle strength. Age-related changes in skeletal muscle gene expression were partially reversed by resistance exercise, indicating the plasticity of muscle. Supplementation of high-quality dietary proteins enhances the beneficial effects of exercise on sarcopenia. Signaling pathways and molecules associated with atrophy in aged skeletal muscle are increasingly revealed, providing novel opportunities for optimizing and personalizing nutritional concepts for the elderly population.

Address
FrieslandCampina, Stationsplein 4, 3818 LE Amersfoort, The Netherlands

Corresponding author: Geurts, Jan MW (jan.geurts@frieslandcampina.com)

Current Opinion in Food Science 2016, **10**:60–67

This review comes from a themed issue on **Foodomics technologies**

Edited by **Stanley Brul**

http://dx.doi.org/10.1016/j.cofs.2016.08.006

Consequences of age-related skeletal muscle atrophy for health

From 1900 to 2015, median life expectancy has increased by approximately 20 years [1]. Aging is typically associated with an increased prevalence of diseases, such as cardiovascular disease, cancer, arthritis, cataracts, osteoporosis, type 2 diabetes, hypertension and Alzheimer's disease. More than half of the elderly population (>65 y old) suffer from two or more of those chronic conditions [2]. The underlying biology of aging is complex [1], but aging is commonly associated with loss of skeletal muscle mass [3] and strength [4,5], and often with accumulation of body fat. The combined loss of muscle mass and function (e.g. gait speed or hand grip strength) [6] is defined as sarcopenia, and strongly contributes to frailty. Epidemiological studies in humans have indicated that muscle strength is the best predictor of mortality and of the risk of developing age-related diseases, highlighting a correlation between muscle dysfunction and derangement of homeostasis [7•]. The most obvious consequence of reduced muscle mass is probably compromised mobility due to reduced muscle strength and impaired physical performance [8–10], but also metabolic health can deteriorate due to a relatively smaller contribution of muscle tissue to body weight [11,12]. Skeletal muscles play a crucial role in regulating glucose utilization, dysfunction of which leads to hyperglycemia, one of the five components of metabolic syndrome [13]. Age-related skeletal muscle atrophy has been associated with insulin resistance in clinical observational studies [14].

A healthy lifestyle, including sufficient physical activity and optimized dietary patterns, can decrease the age-related decline in muscle mass and strength [15,16], which may substantially improve physical performance as well as metabolic health in the elderly population. Contemporary technologies, such as transcriptomics, proteomics and metabolomics, are increasingly used to assess early changes in health (viz. phenotypic flexibility) and muscle function. Detailed understanding of the mechanisms involved in the regulation of muscle metabolism during aging can help to develop effective exercise protocols and tailored dietary concepts for the elderly population. The aim of this paper is twofold; 1/to present advances in omics-driven research in phenotypic flexibility (as a proxy of health), sarcopenia biomarker development and skeletal muscle metabolism in the elderly; and 2/to suggest potential successful dietary and lifestyle strategies to reduce the decline in muscle mass and function during aging.

Variation in phenotypic flexibility

Health is the ability to physically, cognitively, and socially adapt to continuing changes in the environment [17], a concept that was subsequently extended to phenotypic flexibility [18]. Phenotypic flexibility links health to the body's capacity to deal with stressors that challenge its homeostasis, with restoration of homeostasis as the outcome of various physiological systems. Nutrition impacts health every day by inducing subtle and pleiotropic physiological effects, which can manifest after weeks,

years or even after decades. Targeted challenges can increase sensitivity for detecting the often subtle effects of interventions and could therefore permit detection of effects on health at earlier stages of life [19•,20]. Skeletal muscles play a vital role in postprandial energy metabolism and therefore contribute heavily to responses to dietary challenges. Such challenge studies often include metabolomics to quantify responses of multiple biomarkers simultaneously and results can be plotted in a multivariate 'health space' [21]. Recent studies have shown that phenotypic flexibility differs substantially between individual subjects and that this flexibility can adapt to long-term dietary interventions. In 100 persons, Wopereis *et al.* [22] measured a large panel of metabolites related to glucose metabolism, lipid metabolism, protein metabolism, and low grade chronic inflammation in response to a high-fat challenge meal (60 g palm olein, 75 g glucose and 20 g milk protein). Persons of young age with low to normal fat percentage responded different to the challenge when compared to persons of old age with normal to high fat percentage, but there was also much inter-individual variation within each group. In another study, four weeks of a hypercaloric diet significantly altered the response curves of almost all endocrine, metabolic, and inflammatory biomarkers after a high-fat challenge meal, whereas fasting biomarker concentrations remained unchanged [23]. Also dairy consumption may impact phenotypic flexibility. A recent study showed that 51 metabolites responded differently to the challenge after 3 weeks of high dairy (i.e. 750 ml semi-skimmed milk and 60 g of cheese per day) compared to a dairy-free diet, indicating increased whole-body fat oxidation due to alterations in glycolysis, fatty acid ß oxidation, and TCA cycle activity (unpublished). This 3-week crossover study included 10 healthy male participants (30–40 y) and multivariate biomarker profiles were measured after a high-fat challenge meal. Challenges for assessing phenotypic flexibility are not limited to meals. For example, Duivenvoorde *et al.* [24] conducted a study in which young (10-week old) and old (72-week old) mice were subjected to oxygen restriction (14.5% O_2) as a challenge test. Old mice did not maintain reduced oxygen consumption during oxygen restriction, whereas young mice did. Biochemical and gene expression analyses showed that oxygen restriction affected glucose and lactate homeostasis in liver and white adipose tissue of young mice, supporting the observed differences in whole body oxygen consumption [24]. These studies show that aging and age-related changes in body composition may result in a loss of phenotypic flexibility, which is partly reflected in reduced ability to adapt fuel utilization to fuel availability, and that dietary or exercise strategies can be used to enhance phenotypic flexibility.

Similarly, aging is associated with the development of anabolic resistance, implying that higher doses of dietary protein are required for older subjects to generate equivalent anabolic responses (i.e. muscle protein synthesis) to younger subjects, also when the protein dose is expressed relative to body weight [25,26••]. This reduced sensitivity to respond to the anabolic properties of dietary protein bears a striking resemblance to the concept of phenotypic (in)flexibility indicating the development of insulin resistance and chronic metabolic disease [27]. Whole body insulin sensitivity is closely related to muscle mass and therefore often expressed per kg fat-free mass [28]. Insulin sensitivity, measured as glucose infusion rate in μmol/kg fat-free mass per min, typically reduces during aging. This is however primarily driven by age-related changes in the content and distribution of adipose tissue and independent of muscle mitochondrial function or chronological age [29]. More detailed understanding of the relationship between phenotypic flexibility, anabolic resistance and skeletal muscle mass will likely allow the development of more effective nutritional, exercise and/or pharmacological strategies to support healthy aging. Such research will benefit from appropriate biomarkers for sarcopenia and mechanistic understanding of skeletal muscle adaptation to aging and interventions.

Biomarkers for sarcopenia

Skeletal muscle mass decreases substantially (1–2%/y) from the age of 50 onwards [30], and prevalence of sarcopenia has been estimated at 1–29% in community-dwelling populations, 14–33% in long-term care populations and 10% in the only acute hospital-care population, based on 18 prevalence studies and including regional and age-related variation [31]. Several reviews describe recent efforts to identify biomarkers for sarcopenia that can be used in longitudinal studies [32–37]. Many single biomarkers have been proposed, including anabolic hormones [e.g., testosterone, growth hormone (GH), insulin-like growth factor-1 (IGF-1)], inflammatory biomarkers (e.g., C-reactive protein, interleukin-6, tumor necrosis factor-α), and products of oxidative damage (e.g., advanced glycation end-products, protein carbonyls, oxidized low-density lipoproteins) [38]. Such biomarkers are often identified by mass spectrometry or nuclear magnetic resonance spectroscopy in blood samples [39,40], and validated against measures of muscle mass (estimated from e.g. magnetic resonance imaging, computed tomography, and dual energy X-ray absorptiometry) and muscle strength (e.g. hand grip strength or lower extremity muscle power) [32]. For example, butyryl-cholinesterase [41], C1q [42], branched chain amino acid-related metabolites [43], myostatin [44], and the activin A-to-follistatin ratio [45] have recently been added to the longlist. Although the use of single biomarkers (i.e. reductionist approach) may have been successful, a large proportion of the phenotypic variance remains unknown. Combinations of biomarkers have therefore been applied to explain more of the observed variation. For example, a urine proteomics panel for estimating muscle protein breakdown allowed simultaneous assessment of multiple

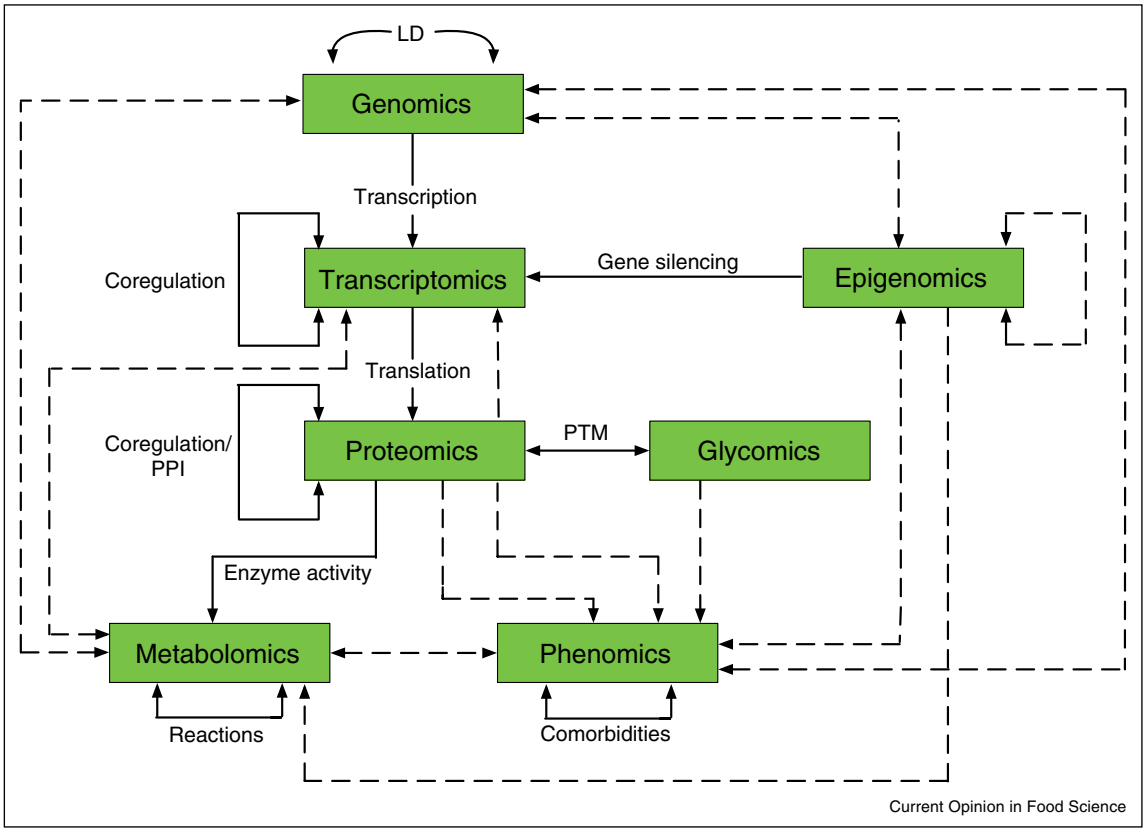
biomarkers [33]. Next to the bivariate association of sarcopenia (or related outcomes) with one type of omics data, there are strong interdependencies within and between the different omics data (see Figure 1). Correlations can be observed practically between all levels of biological organization. Systems biology approaches have therefore been implemented to integrate datasets from different platforms (e.g. transcriptomics, proteomics, metabolomics) by adopting multidimensional and multivariate analyses [32,46•]. Especially proteomic studies are a complementary tool and relevant to support transcriptome studies at a functional level and to provide insight in the role of secondary protein modifications, which increasingly emerges as an important level of regulation [47]. Also, complementary biomarkers (likely belonging to multiple classes: imaging, biological markers, and functional tests) have been suggested to be most effective [33]. Integration comprises network analysis and annotation of the pathways involved, as well as interactions with key environmental factors, such as nutrition and other lifestyle factors [48].

Interestingly, Peters *et al.* [49] recently performed a whole-blood gene expression meta-analysis in 14 983 individuals of European ancestry, and identified 1497 genes that were differentially expressed with chronological age (although not specifically related to muscle tissue). Based on expression profiles in blood, they proposed 'transcriptomic age' as a surrogate biomarker. This large study showed that differences between transcriptomic age and chronological age are associated with biological features linked to aging, such as blood pressure, cholesterol levels, fasting glucose, and body mass index [49], i.e. features that can be modulated by nutrition and lifestyle. Hence, integrating data from different platforms and using complementary biomarkers may explain more variation in the age-related reduction of skeletal muscle mass and whole-body phenotypic flexibility.

Metabolic signature of skeletal muscle during aging

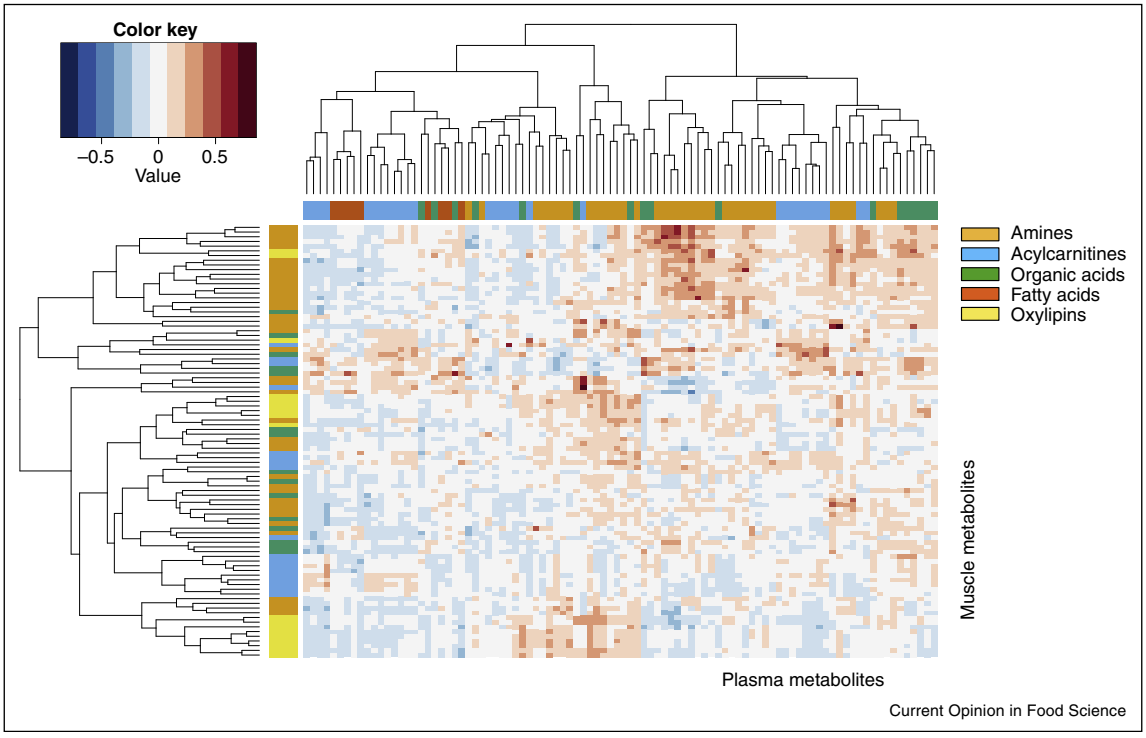
It can be questioned whether surrogate biomarkers such as transcriptomic age can predict age-associated decline in specific tissues, like skeletal muscle. Nonetheless, a recent meta-analysis of whole blood gene expression (overall 17 534 unique genes measured by microarray) and hand-grip strength in four independent cohorts (n = 7781, ages: 20–104 y) identified robust associations between the expression of 221 genes and muscle strength

Figure 1



Interdependencies of omics data. Solid lines indicate biological processes which cause dependencies, while dashed lines represent observer associations. LD: linkage disequilibrium; PPI: protein–protein interaction; PTM: post-translational modifications. Reproduced with permission from [46•].

Figure 2



Pearson correlation heatmap of muscle-to-plasma metabolites. Red and blue indicate positive and negative correlations, respectively. Thick lines: correlation ~0.5; thin lines: 0.3 < correlation < 0.5. Pink nodes: muscle tissue metabolites; yellow nodes: plasma metabolites. Reproduced with permission from [51•].

in adults [50••]. Although blood is generally easily accessible, plasma levels of biomarkers reflect an overall status and the contribution of individual body tissues is not known [45,49]. Skeletal muscle is the largest organ in the human body, comprising approximately 40% of body weight [26••], but only few studies have measured changes in metabolites in human skeletal muscle tissue. Fazelzadeh *et al.* [51•] measured the baseline metabolome in plasma and muscle biopsies (*m. vastus lateralis*) from 30 young, 66 healthy older subjects, and 43 frail older subjects. Follow-up samples from 38 healthy older and 24 frail older subjects were collected and analyzed after 6 months of prolonged resistance-type exercise training. Surprisingly, they observed only modest correlations between muscle and plasma metabolite levels (Figure 2), which pleads against the use of plasma metabolites as a direct read-out of muscle metabolism and stresses the need for direct assessment of metabolites in muscle tissue biopsies [51•].

In addition, Hangelbroek *et al.* [52•] examined the skeletal muscle transcriptome between healthy young and older subjects and (pre-)frail older adults. After 24 weeks of resistance exercise training, all subjects increased muscle strength (leg extension 1RM) and several age-related changes in skeletal muscle gene expression were partially reversed [52•]. This is in accordance with the study by Su *et al.* [53••], who performed a complete re-annotation of public microarray data from human skeletal muscle biopsies and constructed a muscle expression compendium consisting of nearly 3000 samples ((http://www.ebi.ac.uk/arrayexpress/; accession number E-MTAB-1788). They also found that high physical activity counteracts age-related changes in muscle gene expression [53••]. Interestingly, the study by Hangelbroek *et al.* revealed that especially the protocadherin gamma gene cluster may be related to muscle denervation and re-innervation in aging muscle [52•]. These studies illustrate the plasticity of muscle tissue during lifespan and have identified specific pathways involved. Long-term, longitudinal studies in the aging population, and combining omics technologies in muscle biopsies with functional outcomes, could further increase our insight in underlying mechanisms. Such studies can be used to quantify the interactions between lifestyle and diet with the age-related adaptation of skeletal muscle.

Nutritional propositions for the elderly

Based on our current knowledge, diets for the elderly population can already be optimized to some extent.

Caloric needs are commonly reduced in elderly subjects due to lower physical activity, and aging is often accompanied by a loss of appetite and changes in taste and smell. This can reduce food intake and limit the food choices, hence maintaining a nutrient-dense diet is important to meet nutrient requirements. Affordable and tasty nutrient-rich foods, such as dairy products (important sources of protein, calcium, iodine, vitamins B_2 and B_{12}, magnesium, zinc, phosphorus and retinol) and fruits and vegetables (most important source of vitamin C), are therefore helpful in the diet of elderly people [54,55]. For the elderly, the most prominent nutritional concerns are related to vitamin D, vitamin B_{12} and protein malnutrition [56]. For countering age-related loss of muscle mass and strength [26••], resistance exercise currently remains the most effective therapeutic strategy. In addition, dietary protein supplementation, including branched chain amino acids, has been well established to enhance the beneficial effects of exercise on maintenance of muscle mass during aging [5,57–60]. Recent evidence suggests that the ingestion of the plant-based proteins soy and wheat results in a lower muscle protein synthetic response when compared to milk proteins [61]. The lower anabolic properties of plant-based protein sources may be attributed to a lower digestibility of protein from these sources, in addition to greater splanchnic extraction and subsequent urea synthesis of plant protein-derived amino acids when compared with the ingestion of animal-based proteins [61]. Dairy products are appealing for the elderly population as they are rich in nutrients (incl. high-quality proteins and micronutrients), easy to chew, swallow and digest, and available in multiple product formats (e.g. milk, yoghurt, cheese). An observational study in 1456 older women aged 70–85 years, for example, showed that muscle mass and functional capacity increased with increasing intakes of milk, cheese and yogurt [62].

In addition to protein, vitamin D may be protective for muscle loss, but more studies are required. There is no consensus about the minimum serum levels of 25OHD required for reducing the risk of sarcopenia. According to randomized controlled studies, vitamin D supplementation to ensure mean serum 25OHD levels of 66–84 nmol/L (26.4–33.6 ng/mL) is required to improve muscle performance [63]. For the elderly population, future dose–response studies may be required as the effective supplemental dose ranges widely from 600 to 2000 IU of vitamin D per day for reducing the risk of falls [63]. Molecular insights suggest interactions between protein and vitamin D. For example, addition of vitamin D, as $1,25(OH)_2D$, increased insulin receptor and vitamin D receptor mRNA expression, whereas the Akt/mTOR-dependent pathway was activated by insulin and leucine and further enhanced by $1,25(OH)_2D$ [64,65]. A 13-week intervention study in 380 sarcopenic primarily independent-living older adults with a nutritional supplement containing vitamin D and leucine-enriched whey protein resulted in improvements in muscle mass and lower-extremity function [66].

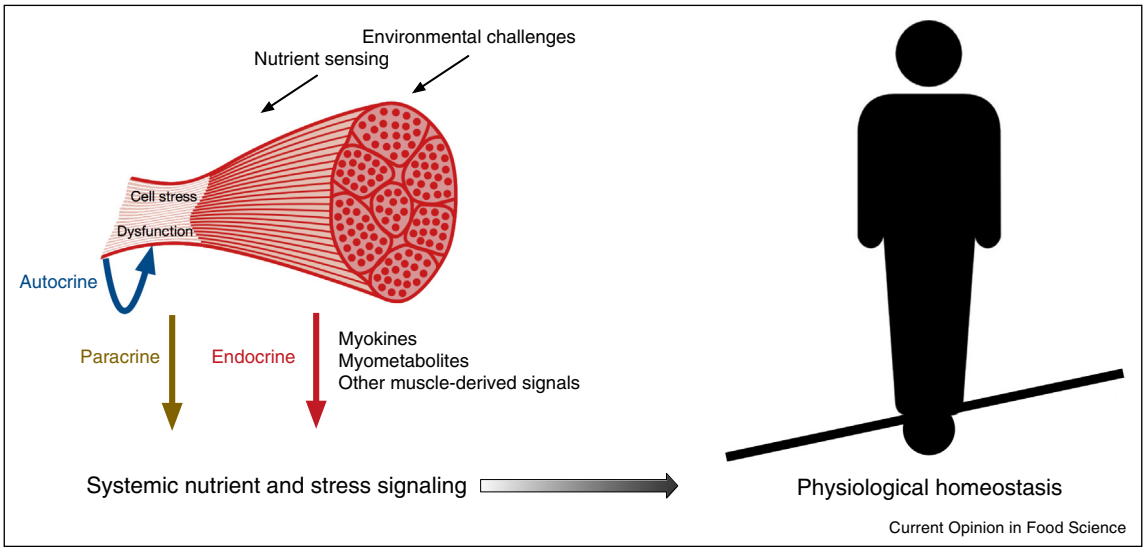
Other nutritional factors, such as a more alkalinogenic diet and diets higher in the anti-oxidant nutrients vitamin C and vitamin E, are potentially involved in preventing muscle loss during aging, but this needs further study [5]. Magnesium, which is a primary regulator of mTOR dynamics and muscle cell proliferation [67], may also help to prevent sarcopenia, but this also needs to be further established. In general, omics techniques are instrumental to identify crucial pathways and link these to dietary components and signaling pathways in order to develop nutritional propositions for the elderly population. For example, the transcription factor ATF4 is involved in age-related muscle weakness and atrophy, and the small molecules ursolic acid and tomatidine reduce ATF4 activity, weakness, and atrophy in aged skeletal muscle [68,69•]. Ursolic acid is present in peels of fruits and herbs, such as apples, cranberries and rosemary, and tomatidine can be found in (green) tomatoes.

Emerging research areas which can be expected to impact, at a longer term, elderly nutrition are nutrient sensing and signaling and host–microbe interactions. The role of skeletal muscle in regulating physiological homeostasis and disease progression in other tissues has been extensively reviewed by Rai and Demontis [7]. Fundamental insights in signaling pathways via myokines (cytokines from muscle) and myometabolites (metabolites from muscle) to other tissues further illustrate the importance of skeletal muscle tissue in whole body homeostasis and health (Figure 3). Targets for nutritional and pharmacological interventions may be identified based on such insights. Host–microbe interactions are increasingly recognized and integrated omics approaches are used [70,71]. A recent study in mice showed that the histone deacetylase inhibitor butyrate improves metabolism and reduces muscle atrophy during aging [72]. This suggests that histone deacetylases contribute to age-related muscle atrophy and may be effective targets for intervention in sarcopenia and age-related metabolic disease.

Integrated omics technologies will help to further tune nutrition and lifestyle to individual needs as recently demonstrated by Zeevi *et al.* [73••]. Technological developments in smart phones and wearable devices may further accelerate this development. Online monitoring of specific non-invasive biomarkers may generate health-related data in the context of our day-to-day life. Such devices can be linked to reference databases and provide us with individual, tailored advices regarding diet and lifestyle.

Recent advances in omics technologies for assessing individual phenotypic flexibility, developing biomarkers

Figure 3



Skeletal muscle responds to environmental and dietary challenges and to organelle and metabolic dysfunction by secreting myokines and myometabolites that regulate systemic nutrient and stress signaling. Systemic signaling by muscle regulates an organism's metabolic and physiological homeostasis and influences aging and the onset and progression of many diseases in several target organs.

for sarcopenia and understanding the plasticity of skeletal muscle during aging are very promising. There are significant leads that age-related loss of muscle mass can be reduced by increasing physical activity and optimizing diets, thereby improving phenotypic flexibility and physical performance. In our opinion, further research should elucidate the most effective nutritional and lifestyle strategies to limit loss of muscle mass during aging and to conserve phenotypic flexibility, preferably at an individual level. From these studies, valuable leads can be obtained for development of specific dietary and lifestyle advices for the elderly and stimulate new product development by food industry.

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Aunan JR, Watson MM, Hagland HR, Søreide K: **Molecular and biological hallmarks of ageing**. *Br J Surg* 2016, **103**:29-46.
2. Marengoni A, Angleman S, Melis R, Mangialasche F, Karp A, Garmen A, Meinow B, Fratiglioni L: **Aging with multimorbidity: a systematic review of the literature**. *Ageing Res Rev* 2011, **10**:430-439.
3. Chang KV, Hung CY, Li CM, Lin YH, Wang TG, Tsai KS, Han DS: **Reduced flexibility associated with metabolic syndrome in community-dwelling elders**. *PLoS One* 2015, **10**:1-11.
4. Mitchell WK, Williams J, Atherton P, Larvin M, Lund J, Narici M: **Sarcopenia, dynapenia, and the impact of advancing age on human skeletal muscle size and strength; a quantitative review**. *Front Physiol* 2012, **3**:1-18.
5. Welch AA: **Nutritional influences on age-related skeletal muscle loss**. *Proc Nutr Soc* 2014, **73**:16-33.
6. Cederholm T, Morley JE: **Sarcopenia**. *Curr Opin Clin Nutr Metab Care* 2015, **18**:1-4.
7. • Rai M, Demontis F: **Systemic nutrient and stress signaling via myokines and myometabolites**. *Annu Rev Physiol* 2016, **78**:85-107.

Review describing how skeletal muscle tissue affects other tissues as well as whole body metabolism via autocrine, paracrine and endocrine mechanisms.

8. Zeng P, Han Y, Pang J, Wu S, Gong H, Zhu J, Li J, Zhang T: **Sarcopenia-related features and factors associated with lower muscle strength and physical performance in older Chinese: a cross sectional study**. *BMC Geriatr* 2016, **16**:45.
9. Visser M: **Obesity, sarcopenia and their functional consequences in old age**. *Proc Nutr Soc* 2011, **70**:114-118.
10. Trombetti A, Reid KF, Hars M, Herrmann FR, Pasha E, Phillips EM, Fielding RA: **Age-associated declines in muscle mass, strength, power, and physical performance: impact on fear of falling and quality of life**. *Osteoporos Int* 2016, **27**:463-471.
11. Rizzoli R, Reginster J-Y, Arnal J-F, Bautmans I, Beaudart C, Bischoff-Ferrari H, Biver E, Boonen S, Brandi M-L, Chines A *et al.*: **Quality of life in sarcopenia and frailty**. *Calcif Tissue Int* 2013, **93**:101-120.
12. Srikanthan P, Karlamangla AS: **Relative muscle mass is inversely associated with insulin resistance and prediabetes. Findings from the third National Health and Nutrition Examination Survey**. *J Clin Endocrinol Metab* 2011, **96**:2898-2903.
13. Yang J: **Enhanced skeletal muscle for effective glucose homeostasis**. *Prog Mol Biol Transl Sci* 2014, **121**:133-163.
14. Barzilay J, Cotsonis G: **Insulin resistance is associated with decreased quadriceps muscle strength in nondiabetic adults aged ≥70 years**. *Diabetes Care* 2009, **32**:736-738.
15. Wolfe RR: **The underappreciated role of muscle in health and disease**. *Am J Clin Nutr* 2006, **84**:475-482.

16. Breen L, Phillips SM: **Interactions between exercise and nutrition to prevent muscle waste during ageing**. *Br J Clin Pharmacol* 2013, **75**:708-715.

17. Huber M, Knottnerus JA, Green L, Van der Horst H, Jadad AR, Kromhout D, Leonard B, Lorig K, Loureiro MI, Van der Meer JWM *et al.*: **How should we define health?** *Br Med J* 2011, **343**:d4163.

18. van Ommen B, van der Greef J, Ordovas JM, Daniel H: **Phenotypic flexibility as key factor in the human nutrition and health relationship**. *Genes Nutr* 2014, **9**:1-9.

19. • Stroeve JHM, van Wietmarschen H, Kremer BHA, van Ommen B, Wopereis S: **Phenotypic flexibility as a measure of health: the optimal nutritional stress response test**. *Genes Nutr* 2015, **10**:1-21.
A systematic review of 61 studies applying different nutritional challenge tests to detection early and subtle changes in phenotypic flexibility as a result of nutritional intervention.

20. Krug S, Kastenmüller G, Stückler F, Rist MJ, Skurk T, Sailer M, Raffler J, Römisch-Margl W, Adamski J, Prehn C *et al.*: **The dynamic range of the human metabolome revealed by challenges**. *FASEB J* 2012, **26**:2607-2619.

21. Vis DJ, Hendriks MMWB, Sailer M, Smilde AK, Daniel H, Westerhuis JA: **A technical note on challenge tests in human volunteers for multidimensional phenotyping**. *Chemom Intell Lab Syst* 2014, **136**:81-84.

22. Wopereis S, Bakker G, de Jong-Rubingh C, Dijk-Stroeve A, van den Broek T, van Ommen B, van Erk M: **Ranges of phenotypic flexibility in 100 healthy subjects**. *FASEB J* 2015, **29**:751-755.

23. Kardinaal AFM, Van Erk MJ, Dutman AE, Stroeve JHM, Van De Steeg E, Bijlsma S, Kooistra T, Van Ommen B, Wopereis S: **Quantifying phenotypic flexibility as the response to a high-fat challenge test in different states of metabolic health**. *FASEB J* 2015, **29**:4600-4613.

24. Duivenvoorde LPM, Van Schothorst EM, Swarts HJM, Keijer J: **Assessment of metabolic flexibility of old and adult mice using three noninvasive, indirect calorimetry-based treatments**. *J Gerontol - Ser A Biol Sci Med Sci* 2015, **70**:282-293.

25. Moore DR, Churchward-Venne TA, Witard O, Breen L, Burd NA, Tipton KD, Phillips SM: **Protein ingestion to stimulate myofibrillar protein synthesis requires greater relative protein intakes in healthy older versus younger men**. *J Gerontol - Ser A Biol Sci Med Sci* 2015, **70**:57-62.

26. •• Brook MS, Wilkinson DJ, Phillips BE, Perez-Schindler J, Philp A, Smith K, Atherton PJ: **Skeletal muscle homeostasis and plasticity in youth and ageing: impact of nutrition and exercise**. *Acta Physiol* 2015, **216**:15-41.
Extensive review on metabolic and molecular responses in skeletal muscle to nutrition and exercise in elderly subjects.

27. Wall BT, Gorissen SH, Pennings B, Koopman R, Groen BBL, Verdijk LB, Van Loon LJC: **Aging is accompanied by a blunted muscle protein synthetic response to protein ingestion**. *PLoS One* 2015, **10**:1-13.

28. DeFronzo RA, Ferrannini E, Keen H, Zimmet P: *International textbook of diabetes mellitus*. John Wiley & Sons Ltd; 2005.

29. Lalia AZ, Dasari S, Johnson ML, Robinson MM, Konopka AR, Distelmaier K, Port JD, Glavin MT, Esponda RR, Nair KS *et al.*: **Predictors of whole-body insulin sensitivity across ages and adiposity in adult humans**. *J Clin Endocrinol Metab* 2016, **101**:626-634.

30. Hughes VA, Frontera WR, Roubenoff R, Evans WJ, Singh MAF: **Longitudinal changes in body composition in older men and women: role of body weight change and physical activity**. *Am J Clin Nutr* 2002, **76**:473-481.

31. Cruz-Jentoft AJ, Landi F, Schneider SM, Zúñiga C, Arai H, Boirie Y, Chen LK, Fielding RA, Martin FC, Michel J *et al.*: **Prevalence of and interventions for sarcopenia in ageing adults: a systematic review. Report of the International Sarcopenia Initiative (EWGSOP and IWGS)**. *Age Ageing* 2014, **43**:748-759.

32. Calvani R, Marini F, Cesari M, Tosato M, Anker SD, Von Haehling S, Miller RR, Bernabei R, Landi F, Marzetti E: **Biomarkers for physical frailty and sarcopenia: state of the science and future developments**. *J Cachexia Sarcopenia Muscle* 2015, **6**:278-286.

33. Fougère B, Vellas B, Van Kan GA, Cesari M: **Identification of biological markers for better characterization of older subjects with physical frailty and sarcopenia**. *Transl Neurosci* 2015, **6**:103-110.

34. Kalinkovich A, Livshits G: **Sarcopenia – the search for emerging biomarkers**. *Ageing Res Rev* 2015, **22**:58-71.

35. Ogawa K: **The biomarkers of sarcopenia in elderly people**. *J Phys Fit Sport Med* 2013, **2**:209-214.

36. Palus S, von Haehling S, Springer J: **Muscle wasting: an overview of recent developments in basic research**. *J Cachexia Sarcopenia Muscle* 2014, **5**:193-198.

37. Robb MA, McInnes PM, Califf RM: **Biomarkers and surrogate endpoints: developing common terminology and definitions**. *JAMA* 2016, **315**:1107-1108.

38. Fragala MS, Kenny AM, Kuchel GA: **Muscle quality in aging: a multi-dimensional approach to muscle functioning with applications for treatment**. *Sport Med* 2015, **45**:641-658.

39. Martin-Ruiz C, von Zglinicki T: **Biomarkers of healthy ageing: expectations and validation**. *Proc Nutr Soc* 2014, **73**:422-429.

40. Collino S, Martin FP, Karagounis LG, Horcajada MN, Moco S, Franceschi C, Kussmann M, Offord E: **Musculoskeletal system in the old age and the demand for healthy ageing biomarkers**. *Mech Ageing Dev* 2014, **136-137**:94-100.

41. Cacciatore F, Della-morte D, Basile C, Liguori I, Galizia G, Bonaduce D, Abete P: **Butyryl-cholinesterase is related to muscle mass and strength. A new biomarker to identify elderly subjects at risk of sarcopenia**. *Biomark Med* 2015, **9**:669-678.

42. Watanabe S, Sato K, Hasegawa N, Kurihara T, Matsutani K, Sanada K, Hamaoka T, Fujita S, Iemitsu M: **Serum C1q as a novel biomarker of sarcopenia in older adults**. *FASEB J* 2015, **29**:1003-1010.

43. Lustgarten MS, Price LL, Chale A, Phillips EM, Fielding RA: **Branched chain amino acids are associated with muscle mass in functionally limited older adults**. *J Gerontol - Ser A Biol Sci Med Sci* 2014, **69**:717-724.

44. Bergen HR, Farr JN, Vanderboom PM, Atkinson EJ, White TA, Singh RJ, Khosla S, LeBrasseur NK: **Myostatin as a mediator of sarcopenia versus homeostatic regulator of muscle mass: insights using a new mass spectrometry-based assay**. *Skelet Muscle* 2015, **5**:21-36.

45. Hofmann M, Schober-Halper B, Oesen S, Franzke B, Tschan H, Bachl N, Strasser E-M, Quittan M, Wagner K-H, Wessner B: **Effects of elastic band resistance training and nutritional supplementation on muscle quality and circulating muscle growth and degradation factors of institutionalized elderly women: the Vienna Active Ageing Study (VAAS)**. *Eur J Appl Physiol* 2016, **116**:885-897.

46. • Zierer J, Menni C, Kastenmüller G, Spector TD: **Integration of omics data in aging research: from biomarkers to systems biology**. *Aging Cell* 2015, **14**:933-944.
Substantiating the need for systems biology to integrate data from different experiments in order to explain more of the phenotypic variation during ageing.

47. Keijer J, Hoevenaars FPM, Nieuwenhuizen A, van Schothorst EM: **Nutrigenomics of body weight regulation: a rationale for careful dissection of individual contributors**. *Nutrients* 2014, **6**:4531-4551.

48. Valdes AM, Glass D, Spector TD: **Omics technologies and the study of human ageing**. *Nat Rev Genet* 2013, **14**:601-607.

49. Peters MJ, Joehanes R, Pilling LC, Schurmann C, Conneely KN, Powell J, Reinmaa E, Sutphin GL, Zhernakova A, Schramm K *et al.*: **The transcriptional landscape of age in human peripheral blood**. *Nat Commun* 2015, **6**:8570.

50. •• Pilling LC, Joehanes R, Kacprowski T, Peters M, Jansen R, Karasik D, Kiel DP, Harries LW, Teumer A, Powell J *et al.*: **Gene transcripts associated with muscle strength: a CHARGE meta-analysis of 7,781 persons**. *Physiol Genomics* 2016, **48**:1-11.

A large meta-analysis on the relationship between whole-blood gene expression and hand-grip strength, revealing age- and sex-specific gene expression signatures.

51. • Fazelzadeh P, Hangelbroek RWJ, Tieland M, de Groot LCPGM, Verdijk LB, van Loon LJC, Smilde AK, Alves RDAM, Vervoort J, Muller M *et al.*: **The muscle metabolome differs between healthy and frail older adults**. *J Proteome Res* 2016, **15**:499-509.
This study compared metabolite levels in muscle and plasma in young, healthy older and frail older subjects, and assessed adaptive responses to resistance exercise.

52. • Hangelbroek RWJ, Fazelzadeh P, Tieland M, Boekschoten MV, Hooiveld GJEJ, van Duynhoven JPM, Timmons JA, Verdijk LB, de Groot LCPGM, van Loon LJC *et al.*: **Expression of protocadherin gamma in skeletal muscle tissue is associated with age and muscle weakness**. *J Cachexia Sarcopenia Muscle* 2016 http://dx.doi.org/10.1002/jcsm.12099.
Muscle transcriptome analyses in this study revealed significant remaining plasticity of ageing skeletal muscle to adapt to resistance-type exercise training. They identified a gene cluster that strongly correlated to muscle strength.

53. •• Su J, Ekman C, Oskolkov N, Lahti L, Ström K, Brazma A, Groop L, Rung J, Hansson O: **A novel atlas of gene expression in human skeletal muscle reveals molecular changes associated with aging**. *Skelet Muscle* 2015, **5**:35.
In this study a muscle compendium was created by complete re-annotation of public microarray data from human skeletal muscle biopsies, including nearly 3000 samples. Mitochondrial dysfunction appears a major age-related factor, which can be impacted by physical activity and nutrition.

54. Drewnowski A: **The contribution of milk and milk products to micronutrient density and affordability of the U S. diet**. *J Am Coll Nutr* 2011, **30**:422S-428S.

55. Van Staveren WA, De Groot LPGM: **Evidence-based dietary guidance and the role of dairy products for appropriate nutrition in the elderly**. *J Am Coll Nutr* 2011, **30**:429-437.

56. de Groot LCPGM: **Nutritional issues for older adults: addressing degenerative ageing with long-term studies**. *Proc Nutr Soc* 2016, **75**:169-173.

57. Norton C, Toomey C, McCormack WG, Francis P, Saunders J, Kerin E, Jakeman P: **Protein supplementation at breakfast and lunch for 24 weeks beyond habitual intakes increases whole-body lean tissue mass in healthy older adults**. *J Nutr* 2016, **146**:65-69.

58. Xu Z-R, Tan Z-J, Zhang Q, Gui Q-F, Yang Y-M: **The effectiveness of leucine on muscle protein synthesis, lean body mass and leg lean mass accretion in older people: a systematic review and meta-analysis**. *Br J Nutr* 2015, **113**:25-34.

59. Cermak NM, Res PT, De Groot LC, Saris WHM, Van Loon LJC: **Protein supplementation augments the adaptive response of skeletal muscle to resistance-type exercise training: a meta-analysis**. *Am J Clin Nutr* 2012, **96**:1454-1464.

60. Witard OC, Wardle SL, Macnaughton LS, Hodgson AB, Tipton KD: **Protein considerations for optimising skeletal muscle mass in healthy young and older adults**. *Nutrients* 2016, **8**:1-25.

61. van Vliet S, Burd NA, van Loon LJ: **The skeletal muscle anabolic response to plant- versus animal-based protein consumption**. *J Nutr* 2015, **145**:1981-1991.

62. Radavelli-Bagatini S, Zhu K, Lewis JR, Dhaliwal SS, Prince RL: **Association of dairy intake with body composition and physical function in older community-dwelling women**. *J Acad Nutr Diet* 2013, **113**:1669-1674.

63. Arik G, Ulger Z: **Vitamin D in sarcopenia: understanding its role in pathogenesis, prevention and treatment**. *Eur Geriatr Med* 2016, **7**:207-213.

64. Salles J, Chanet A, Giraudet C, Patrac V, Pierre P, Jourdan M, Luiking YC, Verlaan S, Migné C, Boirie Y *et al.*: **1,25(OH)2-vitamin D3 enhances the stimulating effect of leucine and insulin on protein synthesis rate through Akt/PKB and mTOR mediated pathways in murine C2C12 skeletal myotubes**. *Mol Nutr Food Res* 2013, **57**:2137-2146.

65. Ceglia L, Harris SS: **Vitamin D and its role in skeletal muscle**. *Calcif Tissue Int* 2013, **92**:151-162.

66. Bauer JM, Verlaan S, Bautmans I, Brandt K, Donini LM, Maggio M, McMurdo MET, Mets T, Seal C, Wijers SL *et al.*: **Effects of a vitamin D and leucine-enriched whey protein nutritional supplement on measures of sarcopenia in older adults, the PROVIDE study: A randomized, double-blind, placebo-controlled trial**. *J Am Med Dir Assoc* 2015, **16**:740-747.

67. de Baaij JHF, Hoenderop JGJ, Bindels RJM: **Magnesium in man: implications for health and disease**. *Physiol Rev* 2015, **95**:1-46.

68. Ebert SM, Dyle MC, Bullard SA, Dierdorff JM, Murry DJ, Fox DK, Bongers KS, Lira VA, Meyerholz DK, Talley JJ *et al.*: **Identification and small molecule inhibition of an activating transcription factor 4 (ATF4)-dependent pathway to age-related skeletal muscle weakness and atrophy**. *J Biol Chem* 2015, **290**:25497-25511.

69. • Dyle MC, Ebert SM, Cook DP, Kunkel SD, Fox DK, Bongers KS, Bullard SA, Dierdorff JM, Adams CM: **Systems-based discovery of tomatidine as a natural small molecule inhibitor of skeletal muscle atrophy**. *J Biol Chem* 2014, **289**:14913-14924.
Interesting example of a systems-based strategy to identify a nutritional compound that may inhibit of muscle atrophy.

70. Nicholson JK, Holmes E, Kinross J, Burcelin R, Gibson G, Jia W, Pettersson S: **Host-gut microbiota metabolic interactions**. *Science* 2012, **336**:1262-1267.

71. Jeffery IB, O'Toole PW: **Diet-microbiota interactions and their implications for healthy living**. *Nutrients* 2013, **5**:234-252.

72. Walsh ME, Bhattacharya A, Sataranatarajan K, Qaisar R, Sloane L, Rahman MM, Kinter M, Van Remmen H: **The histone deacetylase inhibitor butyrate improves metabolism and reduces muscle atrophy during aging**. *Aging Cell* 2015, **14**:957-970.

73. •• Zeevi D, Korem T, Zmora N, Israeli D, Rothschild D, Weinberger A, Ben-Yacov O, Lador D, Avnit-Sagi T, Lotan-Pompan M *et al.*: **Personalized nutrition by prediction of glycemic responses**. *Cell* 2015, **163**:1079-1095.
An unique series of studies including continuous monitoring of blood glucose, development and validation of an algorithm based on multiple platforms and a study to demonstrate the benefits of personally tailored dietary interventions.