Skeletal muscle atrophy is exacerbated by steatotic and fibrotic liver-derived TNF-α in senescence-accelerated mice

Yohei Shirakami, Junichi Kato, Toshihide Maeda, Takayasu Ideta, Kenji Imai, Hiroyasu Sakai, Makoto Shiraki and Masahito Shimizu

Department of Gastroenterology, Graduate School of Medicine, Gifu University, Gifu, Japan

Key words
NASH, Sarcopenia, Skeletal muscle, Steatohepatitis, TNF-α.

Accepted for publication 4 March 2023.

Correspondence
Yohei Shirakami, Department of Gastroenterology, Graduate School of Medicine, Gifu University, 1-1 Yanagido, Gifu 501-1194, Japan.
Email: ys2443@gifu-u.ac.jp

Yohei Shirakami and Junichi Kato contributed equally to this work.

Declaration of conflict of interest: All authors have no conflicts of interest to declare.

Financial support: This work was supported in part by Japan Society for the Promotion of Science (JSPS) in the form of KAKENHI grants (16K09352 and 19K08465 to M. Shimizu and 19K08368 to M. Shiraki).

Abstract

Background and Aim: Although liver diseases, including non-alcoholic steatohepatitis, are associated with skeletal muscle atrophy, the mechanism behind their association has not been fully elucidated. In this study, the effects of aging and non-alcoholic steatohepatitis on the skeletal muscle, and the interaction between the liver and muscle were investigated using a diet-induced non-alcoholic steatohepatitis model in senescence-accelerated mice.

Methods: A total of four groups of senescence-accelerated mice and the control mice were fed either a non-alcoholic steatohepatitis-inducing or control diet, and their livers and skeletal muscles were removed for examinations.

Results: In the senescence-accelerated/non-alcoholic steatohepatitis group, serum level of alanine aminotransferase was markedly elevated and histopathology of non-alcoholic steatohepatitis was significant. Skeletal muscles were also markedly atrophied. The expression of the ubiquitin ligase Murf1 in the muscle was significantly increased with muscle atrophy, while that of Tnfα was not significantly different. In contrast, the hepatic Tnfα expression and serum TNF-α levels were significantly increased in the senescence-accelerated/non-alcoholic steatohepatitis group. These results suggest that liver-derived TNF-α might promote muscle atrophy associated with steatohepatitis and aging through Murf-1. The metabolomic analysis of skeletal muscle indicated higher spermidine and lower tryptophan levels in the steatohepatitis-diet group.

Conclusions: The findings of this study revealed an aspect of liver–muscle interaction, which might be important in developing treatments for sarcopenia associated with liver diseases.

Introduction

In recent years, the loss of skeletal muscle mass, known as sarcopenia, has attracted significant attention. Although sarcopenia was initially defined as skeletal muscle atrophy or depletion which occurs with aging, studies have revealed that it is associated with a poor prognosis for various diseases, including hepatic diseases. In particular, patients with liver cirrhosis have a poor prognosis because sarcopenia and obesity may develop as comorbidities. In addition, non-alcoholic steatohepatitis (NASH) has been reported to correlate with the progression of sarcopenia.

The pathogenesis of sarcopenia is considered multifactorial and is caused by an imbalance between the synthesis and degradation of muscle proteins. Factors regulating muscle mass include exercise, cytokines, myostatin, cellular energy status, and factors concerning the endocrine system, including insulin resistance and the levels of circulating insulin-like growth factor (IGF), corticosteroids, and testosterone. Because patients with chronic liver disease have abnormalities in their nutritional, metabolic, and biochemical status, liver diseases are recognized as common causes of secondary sarcopenia. Although sarcopenia is one of the major complications of chronic liver disease and the interaction between the liver and skeletal muscle has been studied, their association has not been fully understood.

The present study was conducted to elucidate the mechanism underlying NASH-related muscle atrophy using a diet-induced NASH mouse model. In addition, a senescence-accelerated mouse (SAM) model was employed to compare the alterations of disease state in the liver and skeletal muscle between aged and steatohepatitic mice, and to investigate the additive effects of aging on the liver and muscle. Because liver diseases alter the metabolic status of the whole body, metabolome analysis of the skeletal muscle was performed in the mouse model to examine changes in the metabolites under the condition of muscle atrophy.

Materials and methods

Animals and diets. Male SAM prone-8 (SAMP8) and SAM resistant-1 (SAMR1) were purchased from Japan SLC, Inc.
(Shizuoka, Japan) and maintained at the Gifu University Animal Facility under controlled conditions according to the institutional animal care guidelines. Mice were housed in cages with free access to drinking water and maintained with a choline-deficient, L-amino acid-defined, high-fat diet (CDAHFD, #A06071302, Research Diets, Inc., New Brunswick, NJ, USA) or a control diet (#A06071314, Research Diets, Inc.). CDAHFD has been reported as NASH-inducing diet in a previous paper.9

Experimental procedure. Fifteen SAMR1 and 15 SAMP8 aged 8 weeks were separated into several groups. Six SAMR1s in Group 1 and six SAMP8s in Group 2 were fed control diet. Nine SAMR1s and nine SAMP8s were in Groups 3 and 4, respectively, and they were all given CDAHFD. All mice at 20 weeks of age were euthanized. Blood samples were collected and organs and tissues, including the liver, tibialis anterior, and gastrocnemius muscles, were removed. The experimental protocol was approved by the Committee of Institutional Animal Experiments of Gifu University (authorization code 2020-267).

Blood biochemistry. Serum alanine aminotransferase (ALT) levels were determined at a commercial laboratory (SRL, Inc., Waltham, MA, USA), respectively. Serum IGF-1 and TNF-α levels were determined using an ELISA kit (NIKKEN SEIL Co. Ltd., Shizuoka, Japan). Serum IGF-1 and TNF-α levels were measured and analyzed as previously described.16 Briefly, approximately 50 mg of frozen skeletal muscle, gastrocnemius, was cast into 50% (v/v) acetonitrile in Milli-Q water with 20-μM internal standards. The tissue was homogenized, and the homogenate was centrifuged. Subsequently, the upper aqueous layer was filtered by centrifugation to remove the proteins. The filtrate was concentrated and resuspended in Milli-Q water for capillary electrophoresis time-of-flight mass spectrometry (CE-TOFMS) and capillary electrophoresis–triple quadrupole mass spectrometry (CE-QqQMS) analysis using an Agilent system (Agilent Technologies, Santa Clara, CA, USA). Peaks identified in CE-TOF/MS and CE-QqQMS analyses were extracted using automatic integration software (MasterHands version 2.17.1.11, Keio University)17 and MassHunter Quantitative Analysis B.06.00 service pack (Agilent Technologies, respectively).

Histological analysis and immunohistochemistry. For histological evaluation, the liver and muscle tissues, tibialis anterior, were fixed in 10% buffered formalin, embedded in paraffin, and stained with hematoxylin and eosin. Sirius red staining was performed to determine the presence of fibrosis in the liver. Histological features of the liver were evaluated using the non-alcoholic fatty liver disease (NAFLD) activity score (NAS) system.10,11

Metabolome analysis. The metabolome measurements were carried out at Human Metabolome Technologies, Inc. (Tsuruoka, Japan), and the concentrations of the targeted metabolites were measured and analyzed as previously described.16

Statistical analyses. Hierarchical cluster analysis (HCA) and principal component analysis (PCA) were performed using the statistical software developed by Human Metabolome Technologies Inc. To compare specific groups, one-way analysis of variance (ANOVA) was used following normality test. If the ANOVA exhibited significant differences, the Tukey–Kramer multiple comparison test was performed on items to confirm statistical significance. Kruskal–Wallis test and following Steel–Dwass test were performed for non-parametric statistical

### Table 1 Primer sequences

| Target gene | Forward | Reverse |
|-------------|---------|---------|
| Asma        | CTCTTCCAGCACCACCTTCTCAT | TATAGGTGGTTTCGTGGAGTGC |
| Atrogin1    | GCAAACATGCACCATCTTCTCTC | CTTGAGGGGAAATGAGAGCAG |
| Col1a1      | CATGTGACTGTTGGACCTT | GCAGCGTCCTACGAGGATG |
| F4/80       | ACAAGACTGACCAAGACACGGG | TAGCCATCCAGAAGAGCCAG |
| Gapdh       | GACATCAGAAGGTTGGAAGCAG | ATACCAGGAAATGAGCTTGACAA |
| Igf1        | TGCCGCTCATAGTACCCACT | ACGACATATGGTGTATCTTATTG |
| Inos        | CGAAAGCTCCTACTTCACCA | TTAGCTCCATATTGCTTGAGT |
| Murf1       | ACCTGCGTGTGGAAACATC | CTTCGGTCTTCCGACATC |
| Tgfb1       | ACCGGAGAGGCGGATACCA | TATAGGGGCAAGGTCGCCAGA |
| Tnfa        | TGCCCCGACCCCTCAG | ACCCCTCGCTGACCATC |
analysis. Data were presented as mean ± standard deviations and statistical significance was set at \( P < 0.05 \).

**Results**

**General observations.** The body weight change and the liver weight of the mice at the end of the study are shown in Figure 1. The body weights of control diet-fed SAMP8 and CDAHFD-fed SAMR1 were significantly lower than those of control diet-fed SAMR1. The body weights of the mice in CDAHFD-fed SAMP8 were significantly lower than those of the SAMR1 fed the same diet. The relative liver weights of CDAHFD-fed SAMR1 were significantly higher than those of control diet-fed SAMR1. The relative liver weights of CDAHFD-fed SAMP8 were significantly higher than those in control diet-fed SAMP8 and CDAHFD-fed SAMR1. The results indicate that the weights of the body and liver decreased and increased, respectively, due to aging and ingestion of CDAHFD.

**Senescence-accelerated mice develop severe steatohepatitis due to NASH-inducing diet.** The livers of the SAMP8 fed control diet showed slight steatosis (Fig. 2a). However, there was no significant difference in the score between SAMP8 and control mice that were provided with the control diet (Fig. 2b). In contrast, the livers of SAM that were provided with the NASH-inducing diet exhibited massive macrovesicular steatosis, and the NAS was markedly higher than that of control mice fed with the same diet (Fig. 2b).

**Senescence exacerbates hepatic fibrosis following ingestion of NASH-inducing diet.** Hepatic fibrosis caused by the NASH-inducing diet was also enhanced in SAMP8 (Fig. 2a). Regarding the hepatic fibrosis score,\(^1\) the scores of mice fed CDAHFD were significantly higher than those of mice fed control diet (Fig. 2c). In addition, the score was markedly higher in SAMP8 than in control mice, both of which were fed CDAHFD, suggesting that senescence exacerbates hepatic fibrosis. The expression of fibrosis-related genes, namely, \( Asma, \text{Col}1a1, \text{and Tgfb} \), was elevated in mice fed CDAHFD compared with those given control diet, and several genes were also significantly upregulated in SAMP8 (Fig. 2(D)).

**Senescence enhances diet-induced oxidative stress and inflammation in the liver.** The mRNA expression levels of \( \text{Inos} \), which represents the degree of oxidative stress, were markedly higher due to CDAHFD in SAMP8 than control mice. Regarding inflammatory cytokines, the expression of \( F4/80 \) and \( \text{Tnfa} \) was also significantly upregulated following CDAHFD in SAMP8 compared with control (Fig. 3a). The levels of 8-OHdG, a marker of oxidative stress, were significantly higher in the livers of control mice fed CDAHFD compared with control diet, and the levels in SAMP8 fed CDAHFD were markedly higher than those in SAMP8 and SAMR1 fed control diet and CDAHFD, respectively (Fig. 3b). Like the difference in 8-OHdG levels, the serum ALT levels of the mice were markedly higher because both SAMP8 and SAMR1 were fed CDAHFD (Fig. 3c). Although there was no significant difference in the ALT levels between SAMP8 and SAMR1 fed control diet, the levels in SAMP8 administered CDAHFD were significantly elevated compared with control mice. The value of CDAHFD-induced liver damage in this study appeared consistent with the previous report.\(^1\)\(^6\) These results suggest that ingesting CDAHFD causes oxidative stress, hepatic damage, and inflammation, which is enhanced in SAMP8.

**Serum levels of IGF-1 and TNF-α.** The IGF-1 and TNF-α signaling pathways are involved in protein synthesis and skeletal

---

**Figure 1** Body weight change and relative weights of liver. (a) Body weight change of the experimental mice during the study (\( n = 6 \), R1/control diet and P8/control diet; \( n = 9 \), R1/CDAHFD and P8/CDAHFD). Weights are presented as relative to 1 at starting point. (b) Relative weights of liver of the experimental mice at the end of study. Data are the means and standard deviations. *\( P < 0.05 \). CDAHFD, choline-deficient L-amino acid-deficient high fat diet; P8, senescence-accelerated mouse prone (SAMP8, aged mouse); R1, senescence-accelerated mouse resistant (SAMR1, control mouse). (a) R1/control diet; P8/control diet; R1/CDAHFD; P8/CDAHFD.
muscle atrophy, respectively. Therefore, we examined the levels of circulating IGF-1 and TNF-α in the experimental mice. Although there was no significant difference in the serum IGF-1 levels among all groups, the levels of serum TNF-α were markedly elevated in mice fed with CDAHFD compared with those fed with the control diet. In the mice fed with CDAHFD, serum TNF-α concentrations in SAMP8 were significantly higher than those in SAMR1 (Fig. 3d). The results indicated that while aging and consuming an NASH-inducing diet had no effect on serum IGF-1, they led to elevated TNF-α levels.

Senescence and NASH-inducing diet causes skeletal muscle atrophy. We investigated the effects of aging and CDAHFD on the skeletal muscles of mice. Comparing between SAMR1 that were fed control diet and CDAHFD, and between SAMP8 that were fed control diet and CDAHFD, the relative weights of skeletal muscles, tibialis anterior, and gastrocnemius of mice fed CDAHFD were significantly lower than those of mice fed control diet (Fig. 4a). In addition, the muscle weights of SAMP8 that were administered with CDAHFD were markedly decreased compared with those in SAMR1 administered with CDAHFD (Fig. 4a). Examining the muscle fiber areas in the tibialis anterior, smaller muscle fiber areas were observed in both SAMR1 and SAMP8 mice fed CDAHFD (Figs. 4b, c). In addition, the muscle fiber areas of SAMP8 mice fed CDAHFD were significantly smaller than in control mice fed CDAHFD (Figs. 4b, c).

Expression levels of ubiquitin ligases, Igf1, and Tnfa in muscles. The ubiquitin ligases atrogin-1 and muscle RING-finger protein (MuRF)-1 are important regulators of ubiquitin-related protein degradation in skeletal muscles, thereby contributing to muscle atrophy. In this study, the mRNA expression levels of ubiquitin ligases, Atrogin1 and Murfl, growth factor Igf1, and pro-inflammatory cytokine Tnfa in skeletal muscles were determined by qRT-PCR. There was no significant difference in the levels of Atrogin1 among all groups (Fig. 4d). On the other hand, the levels of Murfl were markedly increased in skeletal muscles of mice fed CDAHFD compared with their control groups. In addition, in mice administered with CDAHFD, the levels of Murfl in SAMP8 were significantly higher than those in SAMR1. There was no significant difference in the levels of Igf1 and Tnfa among the groups.
Senescence and NASH-inducing diet causes metabolic alterations in muscles. In previous studies, metabolome analyses using skeletal muscles have been performed to investigate aging-related metabolic alterations in muscles. In this study, we also performed the metabolome analysis of skeletal muscles to examine the effects of aging and NASH diet on the muscles and to investigate whether the metabolic alterations were related to skeletal muscle atrophy. Figures 5a,b and S1 show a principal component analysis (PCA) of metabolomics and a heat map of targeted metabolites in skeletal muscles from all the experimental groups of mice. According to the PCA and heat map, it was suggested that the metabolites in skeletal muscles were altered by aging and consuming CDAHFD. Subsequently, a more detailed analysis of individual metabolites revealed that several metabolites, including spermidine and tryptophan, were affected by aging or consuming CDAHFD (Fig. 5c). Among them, the levels of spermidine and tryptophan were significantly upregulated and downregulated, respectively, due to CDAHFD in SAMR1. The levels of spermidine also tended to be higher in SAMP8 than in control mice fed control diet.

Discussion

Sarcopenia is known as one of the major complications of liver diseases. Recent studies revealed that aging contributes to increased prevalence of NAFLD/NASH and that sarcopenia is associated with progression of NASH; however, detailed mechanisms through which hepatic diseases lead to skeletal muscle atrophy are not fully understood. In this study, the impact of aging and steatohepatitis on skeletal muscle atrophy was investigated using SAM model fed NASH-inducing diet. The findings of this study demonstrated potential mechanism through which TNF-α, produced in the liver showing steatosis and fibrosis, might induce muscle atrophy by enhancing ubiquitin-related pathway.

The most noteworthy aspect of this study was the inter-organ crosstalk between the liver and muscle through an inflammatory cytokine. Another interesting point might be the influence of senescence on steatohepatitis. Because aging is reported as an independent risk factor for NASH progression, researchers have attempted to reveal the interaction between aging and NAFLD/NASH. Senescent cells in the liver have recently attracted attention because cell senescence plays a role in age-associated dysfunction in various organs, including the liver. Using p16 as a marker for senescent cells, these cells were isolated, and specific removal of senescent cells in NAFLD/NASH mouse models resulted in improved hepatic disease states.

Figure 3  Effects of aging and non-alcoholic steatohepatitis-inducing diet on inflammation and oxidative stress in liver and serum insulin-like growth factor (IGF)-1 and tumor necrosis factor (TNF)-α. (a) mRNA expression levels in liver were evaluated among all groups using quantitative real-time reverse transcription-PCR (n = 6). (b) Oxidative stress in the liver evaluated using levels of 8-hydroxy-2′-deoxyguanosine (8-OHdG, n = 6). (c) The values of serum ALT (n = 6). (d) Values of serum IGF-1 and TNF-α measured using ELISA (n = 6). Data are the means and standard deviations. *P < 0.05. ALT, alanine transaminase; CDAHFD, choline-deficient L-amino acid-defined high fat diet; IGF, insulin-like growth factor, P8, senescence-accelerated mouse prone (SAMP8, aged mouse); R1, senescence-accelerated mouse resistant (SAMR1, control mouse).
stress in the liver.\textsuperscript{22} Consistent with the results of the aforementioned study, our study indicated that aged mice fed NASH-inducing diet showed exacerbated steatohepatitis and fibrosis and enhanced oxidative stress in the liver compared with non-aged mice. These results suggest that aged mice are susceptible to the development of diet-induced steatohepatitis.

Metabolome analysis has contributed to novel discoveries in diseases or abnormal states of various organs, including muscle.\textsuperscript{16,19,23,24} In this study, we examined the changes in metabolites in muscle by comprehensive analysis using CE-TOFMS and CE-QqQMS to compare between aged and non-aged mice and between mice fed NASH-inducing diet and control diet. Interestingly, PCA and hierarchical cluster analysis showed metabolic alterations in muscles due to aging and consuming NASH-inducing diet.

Subsequent analyses for individual metabolites, however, revealed that only a few metabolites showed significant differences among the experimental groups, which appeared due to the small number of samples in each group. Among the metabolites exhibiting differences, spermidine and tryptophan were detected with significant difference. Although it has been reported that spermidine, a type of polyamine, is decreased in atrophic muscle in aged mice and supplementation with polyamine may improve sarcopenia,\textsuperscript{19} spermidine in this study was increased in atrophic muscles. This discrepancy was probably caused by the differences in study design and animal model. We could not provide a conclusive answer to why spermidine was increased, but we suspect that polyamine utilization, which is required for muscle growth and maintenance, was impaired. Because details of the relationship between intramuscular polyamine levels and muscle atrophy are not fully understood,\textsuperscript{25} further research is required to investigate exact roles of polyamine in muscles.

Tryptophan is an important amino acid that maintains the skeletal muscle.\textsuperscript{16} In this study, the levels of tryptophan decreased in atrophic muscle, and a marked difference was observed between non-aged mice that were fed NASH-inducing and control diets. There was no significant difference in tryptophan levels among aged mice, regardless of diet. These findings suggested that tryptophan is associated with muscle atrophy induced by CDAHFD in non-aged mice and that aged mice may already be

Figure 4  Effects of aging and non-alcoholic steatohepatitis-inducing diet on skeletal muscle. (a) Relative weights of lower limb muscles, including tibialis anterior and gastrocnemius, of the experimental mice at the end of the study (n = 6, R1/control diet and P8/control diet; n = 9, R1/CDAHFD and P8/CDAHFD). (b) Histopathological alterations in tibialis anterior muscle were evaluated by staining with hematoxylin and eosin. Bars, 100 μm. (c) Cross-section muscle fiber area was measured. (d) mRNA expression levels in the skeletal muscle were evaluated among all groups using quantitative real-time reverse transcription-PCR (n = 6). Data are the means and standard deviations. *P < 0.05. CDAHFD, choline-deficient L-amino acid-defined high fat diet; P8, senescence-accelerated mouse prone (SAMP8, aged mouse); R1, senescence-accelerated mouse resistant (SAMR1, control mouse).
deficient in intramuscular tryptophan and hence CDAHFD administration did not affect the results.

In the present study, skeletal muscle atrophy, which is correlated with the degree of steatohepatitis, was observed, especially in the experimental group of aged mice that were administered NASH-inducing diet. Protein synthesis and degradation in the skeletal muscle are regulated mainly by IGF-1 and TNF-α signaling, where IGF-1 functions for synthesis through PI3K/mTOR and TNF-α induces degradation by enhancing ubiquitin ligase.18,26 Among the ubiquitin ligases, MuRF-1 is regulated by TNF-α, while atrogin-1 is controlled for forkhead box-containing protein (FoxO), which is negatively regulated by IGF-1.18 In this study, there were no significant differences in expression levels of Igf1 in the liver and muscle and the serum IGF-1 concentration among all groups, suggesting that muscle atrophy in this model was not related to IGF-1 signaling. However, the levels of hepatic Tnfa and serum TNF-α were increased in aged and CDAHFD-fed mice, although the Tnfa expression in muscle showed no significant differences among groups. These findings might demonstrate that alteration of Murf1 expression in the muscle is regulated not by Tnfa in muscle but by serum TNF-α in this model, suggesting that skeletal muscle atrophy is induced by TNF-α, which is transported from the liver with steatosis and inflammation.

The role of TNF-α in skeletal muscle atrophy has been extensively investigated.27–32 One study demonstrated that TNF-α produced in fibrotic liver was transported to muscle through the bloodstream, leading to atrophy of skeletal muscle. They also conducted an in vitro study using a muscle cell line and serum from mice with liver fibrosis, indicating an exclusive impact of serum TNF-α on myotube atrophy. Consistent with the findings of the aforementioned study, the results from our study revealed that TNF-α acts as an important cytokine to induce hepatic disease-related skeletal muscle atrophy. The results of muscle fiber area suggested that TNF-α upregulation exacerbates sarcopenia as well as induces sarcopenia directly. Because aged/control diet-fed mice did not show significant sarcopenia compared with non-aged/control diet-fed mice, it can be said that aged mice are susceptible to the development of NASH-derived TNF-α-induced sarcopenia. However, serum TNF-α levels and muscle fiber area appeared to be inversely correlated, which might suggest that NASH-derived TNF-α upregulation has direct effects on induction of sarcopenia rather than exacerbation of sarcopenia.

Figure 5 Metabolome analyses of skeletal muscle. (a) Principal component analysis to compare the metabolites in the gastrocnemius skeletal muscle of the experimental mouse groups (n = 3). (b) Hierarchical cluster analysis (heatmap, n = 3). (c) Comparing individual metabolites among groups (n = 3). * P < 0.05. CDAHFD, choline-deficient L-amino acid-defined high fat diet; P8, senescence-accelerated mouse prone (SAMP8, aged mouse); R1, senescence-accelerated mouse resistant (SAMR1, control mouse). (a) ●, R1-control diet; ○, P8-control diet; ◆, R1-CDAHFD; ◼, P8-CDAHFD.
In summary, the present study indicated that senescent liver is susceptible to steatosis and fibrosis due to enhanced oxidative stress and that TNF-α released from steatotic and fibrotic liver might induce NAFLD/NASH-associated skeletal muscle atrophy (Fig. 6). As was also suggested previously,30 this liver–muscle crosstalk has clinical significance because TNF-α is considered as one of the essential factors for muscle atrophy promoted by hepatic diseases, and this circulating cytokine can be a potential target for the prevention and treatment of NAFLD/NASH-associated muscle atrophy.

Acknowledgments

The authors are grateful to Chiyoko Sano, Akihiro Abe, Toshiki Ohta, Aya Nagai, and Chihiro Shiba for their technical assistance. The authors also would like to thank the Society for Senescence-Accelerated Mouse (SAM) Research for sharing the mice and Editage (www.editage.com) for English language editing.

References

1 Rosenberg IH. Sarcopenia: origins and clinical relevance. J Nutr 1997 May; 127: 990S–1S.
2 Shachar SS, Williams GR, Muss HB, Nishijima TF. Prognostic value of sarcopenia in adults with solid tumours: a meta-analysis and systematic review. Eur J Cancer 2016; 57: 58–67.
3 Ooi PH, Hager A, Mazurak VC, Dajani K, Bhargava R, Gilmour SM, Mager DR. Sarcopenia in chronic liver disease: impact on outcomes. Liver Transpl 2019; 25: 1422–38.
4 Ebadi M, Bhanji RA, Mazurak VC, Montano-Loza AJ. Sarcopenia in cirrhosis: from pathogenesis to interventions. J Gastroenterol Hepatol 2019; 54: 845–59.
5 Hara N, Iwasa M, Sugimoto R et al. Sarcopenia and sarcopenic obesity are prognostic factors for overall survival in patients with cirrhosis. Intern Med 2016; 55: 863–70.
6 Bhanji RA, Narayanan P, Allen AM, Malhi H, Watt KD. Sarcopenia in hiding: the risk and consequence of underestimating muscle dysfunction in nonalcoholic steatohepatitis. Hepatology 2017; 66: 2055–65.
7 Meyer F, Bannert K, Wiese M et al. Molecular mechanism contributing to malnutrition and sarcopenia in patients with liver cirrhosis. Int J Mol Sci 2020; 21: 5357.
8 Hosokawa M. A higher oxidative status accelerates senescence and aggravates age-dependent disorders in SAMP strains of mice. Mech Ageing Dev 2002; 123: 1553–61.
9 Matsumoto M, Hada N, Sakamaki T et al. An improved mouse model that rapidly develops fibrosis in non-alcoholic steatohepatitis. Int J Exp Pathol 2013; 94: 93–103.
10 Kleiner DE, Brunt EM, Van Natta M, Behling C, Contos MJ, Cummings OW et al. Design and validation of a histological scoring system for nonalcoholic fatty liver disease. Hepatology 2005; 41: 1313–21.
11 Lucas C, Lucas G, Lucas N, Krzowska-Firych J, Tomasiwicz K. A systematic review of the present and future of non-alcoholic fatty liver disease. Clin Exp Hepatol 2018; 4: 165–74.
12 Robert F, Mills JR, Agenor A et al. Targeting protein synthesis in a Myc/mTOR-driven model of anorexia–cachexia syndrome delays its onset and prolongs survival. Cancer Res 2012; 72: 747–56.
13 Miyazaki T, Shirakami Y, Mizutani T et al. Novel FXR agonist nelumal A suppresses colitis and inflammation-related colorectal carcinogenesis. Sci Rep 2021; 11: 492.
14 Nagano J, Shimizu M, Hara T et al. Effects of indoleamine 2,3-dioxygenase deficiency on high-fat diet-induced hepatic inflammation. PLoS ONE 2013; 8: e73404.
15 Shirakami Y, Shimizu M. Possible mechanisms of green tea and its constituents against cancer. Molecules 2018; 23: 2284.
16 Ninomiya S, Nakamura N, Nakamura H et al. Low levels of serum tryptophan underlie skeletal muscle atrophy. Nutrients 2020; 12: 978.
17 Sugimoto M, Wong DT, Hirayama A, Soga T, Tomita M. Capillary electrophoresis mass spectrometry-based saliva metabolomics...
identified oral, breast and pancreatic cancer-specific profiles. 
*Metabolomics* 2010; 6: 78–95.
18 Gumucio JP, Mendias CL. Atrogin-1, MuRF-1, and sarcopenia. 
*Endocrine* 2013; 43: 12–21.
19 Uchitomi R, Hatazawa Y, Senoo N et al. Metabolomic analysis of 
skeletal muscle in aged mice. *Sci Rep* 2019; 9: 10425.
20 Omori S, Wang TW, Johmura Y et al. Generation of a p16 reporter 
mouse and its use to characterize and target p16(high) cells in vivo. 
*Cell Metab* 2020; 32: 814–28.e6.
21 Ogrodnik M, Miwa S, Tekhonina T et al. Cellular senescence drives 
age-dependent hepatic steatosis. *Nat Commun* 2017; 8: 15691.
22 Ishizuka K, Kon K, Lee-Okada HC et al. Aging exacerbates high-fat 
diet-induced steatohepatitis through alteration in hepatic lipid 
metabolism in mice. *J Gastroenterol Hepatol* 2020; 35: 1437–48.
23 Saoi M, Britz-McKibbin P. New advances in tissue metabolomics: a 
review. *Metabolites* 2021; 11: 672.
24 Hatazawa Y, Senoo N, Tadaishi M, Ogawa Y, Ezaki O, Kamei Y, Miura 
S. Metabolomic analysis of the skeletal muscle of mice overexpressing 
PGC-1α. *PLoS ONE* 2015; 10: e0129084.
25 Lee NKL, MacLean HE. Polyamines, androgens, and skeletal muscle 
hypertrophy. *J Cell Physiol* 2011; 226: 1453–60.
26 Nakamura S, Sato Y, Kobayashi T et al. Insulin-like growth factor-I is 
required to maintain muscle volume in adult mice. *J Bone Miner Metab* 
2019; 37: 627–35.
27 Patel HJ, Patel BM. TNF-α and cancer cachexia: molecular insights 
and clinical implications. *Life Sci* 2017; 170: 56–63.
28 Thoma A, Lightfoot AP. NF-κB and inflammatory cytokine signalling: 
role in skeletal muscle atrophy. *Adv Exp Med Biol* 2018; 1088: 267–79.
29 Pan L, Xie W, Fu X et al. Inflammation and sarcopenia: a focus on 
circulating inflammatory cytokines. *Exp Gerontol* 2021; 154: 111544.
30 Kurosawa T, Goto M, Kaji N et al. Liver fibrosis-induced muscle 
atrophy is mediated by elevated levels of circulating TNFα. *Cell Death 
Dis* 2021; 12: 11.
31 Kato J, Shirakami Y, Shimizu M. Diabetes mellitus and colon 
carcinogenesis: expectation for inhibition of colon carcinogenesis by 
oral hypoglycemic drugs. *Gastro Dis* 2019; 1: 273–89.
32 Li Y, Zhang F, Modrak S, Little A, Zhang H. Chronic alcohol 
consumption enhances skeletal muscle wasting in mice bearing 
cachectic cancers: the role of TNFα/myostatin axis. *Alcohol Clin Exp 
Res* 2020; 44: 66–77.

**Supporting information**

Additional supporting information may be found online in the 
Supporting Information section at the end of the article.

**Figure S1.** Detailed hierarchical cluster analysis. Expanded 
hierarchical cluster analysis (HCA), compared to Figure 5B, with 
the name of each item. The order of the items is the same as in 
Figure 5B. The numbers mean standardized relative area of 
detected peaks.