JCSM Communications

WILEY

ORIGINAL ARTICLE OPEN ACCESS

Atorvastatin Impairs Pathways Associated With Skeletal Muscle Function and Health in Myoblasts From Older Adults

Maisha Chaudhery[1,2] | Mark A. Burton[1,3] | Hanan Y. Sharkh[1,2] | Leo D. Westbury[4] | Elaine M. Dennison[4] | Nicholas C. Harvey[3,4] | Cyrus Cooper[3,4,5] | Harnish P. Patel[3,4,6] | Keith M. Godfrey[1,3,4] | Karen A. Lillycrop[1,2,3,†] | Emma S. Garratt[1,3]

[1]Human Development and Health Academic Unit, Faculty of Medicine, University of Southampton, Southampton, UK | [2]Biological Sciences, University of Southampton, Southampton, UK | [3]NIHR Southampton Biomedical Research Centre, University of Southampton and University Hospital Southampton NHS Foundation Trust, Southampton, UK | [4]MRC Lifecourse Epidemiology Centre, University of Southampton, Southampton, UK | [5]NIHR Oxford Biomedical Research Centre, University of Oxford, Oxford, UK | [6]Academic Geriatric Medicine, Faculty of Medicine, University of Southampton, Southampton, UK

Correspondence: Maisha Chaudhery (m.chaudhery@soton.ac.uk)

Received: 11 September 2025 | **Revised:** 22 December 2025 | **Accepted:** 26 December 2025

Keywords: atorvastatin | myoblast | sarcopenia | skeletal muscle

ABSTRACT

Background: Statins are widely prescribed to reduce low-density lipoprotein (LDL) cholesterol to decrease the risk of cardiovascular disease. However, there are ongoing concerns surrounding the frequently reported skeletal muscle side effects. These include muscle pain, weakness and reduced function and are defined as statin-associated muscle symptoms (SAMS). This study aimed to characterise the biological processes, which underlie SAMS through analysing in vitro muscle cell phenotypic and transcriptomic effects of atorvastatin, the most prescribed statin, using human myoblasts from older adults.

Methods: Human myoblasts were isolated from *vastus lateralis* biopsies of 11 female older adult participants (average age 78.4 years) who were not on statin therapy from the Hertfordshire Sarcopenia Study extension (HSSe). Myoblasts were treated with 1, 5, or 10 μM atorvastatin for 4 days in proliferating or differentiating cultures. In proliferating cells, cytotoxicity, senescence and proliferation were measured using LDH cytotoxicity, β-galactosidase (β-gal) and 5-ethynyl-2′-deoxyuridine (EdU) assays. To understand the influence of atorvastatin treatment across myoblast differentiation, immunocytochemistry (ICC) was undertaken analysing Myogenic Differentiation 1 (MyoD), Myogenin (MyoG) and Myosin Heavy Chain (MyHC). RNA sequencing (RNA-seq) was performed on a subset of 10 differentiating myoblast cultures treated with 10 μM atorvastatin followed by gene ontology and protein–protein interaction (PPI) pathway analysis (Metascape).

Results: Atorvastatin treatment was not significantly toxic to myoblasts at any of the concentrations tested (1 μM $p = 0.32$, 5 μM $p = 0.21$ or 10 μM $p = 0.76$). Senescence increased with atorvastatin at 5 μM ($p = 1.95 \times 10^{-4}$) and 10 μM ($p = 9.77 \times 10^{-4}$). Myoblast proliferation decreased at all of 1 μM ($p = 1.86 \times 10^{-2}$), 5 μM and 10 μM ($p = 9.77 \times 10^{-4}$) concentrations. In differentiating cells, ICC identified MyoD significantly decreased with 10 μM atorvastatin; decreased MyoG at 1, 5, and 10 μM atorvastatin ($p = 9.77 \times 10^{-4}$); and decreased MyHC at 1 μM ($p = 3.2 \times 10^{-2}$), 5 μM ($p = 2.93 \times 10^{-3}$) and 10 μM atorvastatin ($p = 1.37 \times 10^{-2}$). RNA-Seq analysis following 10 μM atorvastatin treatment in differentiating myoblast cultures revealed

Harnish P. Patel, Keith M. Godfrey, Karen A. Lillycrop and Emma S. Garratt are joint senior authors.

[†]Deceased

822 genes upregulated and 888 genes downregulated in expression (false discovery rate [FDR] < 0.05). Pathway and MCODE analysis identified key networks downregulated, including muscle contraction and cell cycle process, and upregulated pathways implicated in cholesterol and fatty acid synthesis.

Conclusions: These findings show that atorvastatin treatment negatively impacts skeletal muscle at the cellular level by disrupting many key gene regulatory pathways involved in muscle maintenance, function and health. Identification of such disruption, which likely underpins SAMS, provides novel molecular mechanisms, which could be targeted through pharmaceutical/nutraceutical interventions to reduce the negative effects of statins on skeletal muscle health.

1 | Introduction

Statins are a class of medications prescribed to reduce low-density lipoprotein (LDL) cholesterol by inhibiting 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGCR), a key enzyme in the mevalonate pathway that is essential for cholesterol biosynthesis. Through reduced cholesterol synthesis, statins function to decrease cardiovascular disease risk and associated complications [1]. The National Institute for Health and Care Excellence estimates that approximately 5.3 million people in England were prescribed statins in 2023/2024, a figure that has almost tripled since 2015/2016, with the proportion of those aged 70+ prescribed statins increasing from 22.3% in 2009 to 35.6% in 2021 [2, 3]. Atorvastatin is the most prescribed statin for reducing LDL cholesterol and acts through its high lipophilicity property to easily penetrate cell membranes and enhance the inhibition of HMGCR [4].

Although statins are generally well tolerated, muscle side effects including muscle fatigue, weakness, pain and inflammation, defined as statin-associated muscle symptoms (SAMS), are commonly reported. These affect up to 29% of people on statin treatment and are a major reason for treatment withdrawal [4, 5]. Skeletal muscle accounts for 30% and 40% of total body mass in women and men, respectively. Aside from its principal role in locomotion, skeletal muscle has physiological functions including glucose uptake, metabolism and thermogenesis, giving it a crucial role in maintaining health and function [6]. Several studies have proposed potential pathways and contributing factors, but the precise mechanisms through which statins affect skeletal muscle have not been fully elucidated. Research by Macchi et al. [7] showed mice on a high fat and cholesterol diet given atorvastatin for 12 weeks had lower muscle mitochondrial biogenesis and reduced ATP synthesis. In another study with rhabdomyosarcoma cells, pitavastatin treatment reduced the number of viable cells and increased apoptosis through the upregulation of caspase-3 and caspase-7 [8]. Grunwald et al. [9] showed a 72-h simvastatin or rosuvastatin treatment in human myoblasts reduced proliferation, and simvastatin reduced myoblast differentiation. Other researchers have demonstrated treatment of atorvastatin in rats increased reactive oxygen species (ROS) by 60% in the plantaris muscle, which can contribute to cellular damage and reduce exercise capacity [10].

Older adults are particularly susceptible to SAMS due to age-related changes in skeletal muscle. Reductions in skeletal mass, reduced regenerative capacity and low-grade inflammation all contribute to a decline in muscle adaptability with ageing [11]. This progressive decline in muscle health can lead to sarcopenia, the accelerated age-related loss of skeletal muscle mass, strength and function in the older population, increasing the risk of falls, fractures and metabolic disease [12]. The pathophysiology of sarcopenia is multifactorial and complex involving, but not limited to, a decline in myoblast pool that regenerates the muscle, hormonal changes, mitochondrial dysregulation and lifestyle and genetic factors that collectively contribute to muscle atrophy [11, 13]. Sarcopenic muscles display decreased muscle fibre size, changes in muscle fibre composition and a diminished ability to regenerate and repair the muscle following injury. These alterations are further exacerbated by impaired mitochondrial function and increased oxidative stress, which can impact muscle endurance and performance [14]. Because statins may affect pathways overlapping with sarcopenia and muscle health, statin therapy may drive the muscle toward a sarcopenic phenotype or induce characteristics associated with sarcopenia, potentially accelerating functional decline in this population. Risk factors for SAMS include being female, aged over 80 and frailty, all of which further overlap with risk factors for sarcopenia [15, 16]. In a case–control study of 368 older adults, Herghelegiu et al. [4] found that long-term statin treatment increases the risk of sarcopenia development and was positively correlated with the dosage level. This further emphasises the need to investigate the pathways altered in the older female population with statin treatment.

Studies have investigated several statins across various cell types and model organisms. However, in older individuals, there is limited research into the effects of atorvastatin, the most prescribed statin, on human myoblasts, key progenitor cells that differentiate to muscle fibres. This study specifically aimed to understand and identify the mechanism of SAMS through phenotypic and transcriptomic changes induced by atorvastatin in human myoblasts from older female adults—a population with a high prevalence of statin use, higher susceptibility to SAMS and with preexisting age-related muscle impairment [15, 16].

2 | Methods

2.1 | Study Design

This study used myoblasts derived from muscle biopsies from female participants of the Hertfordshire Sarcopenia Study extension (HSSe). The HSSe is a cohort study, part of the Hertfordshire Cohort Study (HCS), aimed to explore life course influences on skeletal muscle health in community dwelling older adults from Hertfordshire, United Kingdom. The study was approved by the Hertfordshire Research Ethics Committee (07/Q02024/68).

With participant consent, anthropometric measurements, grip strength, appendicular lean mass index (ALMi) and gait speed were measured (details in the Supporting Information) and muscle biopsies taken from the *vastus lateralis*, as described in [17, 18].

2.2 | Myoblast Isolation and Culture

Details of myoblast isolation, culture and purity are described in [19]. Proliferating myoblasts were treated with 1, 5, or 10 μM atorvastatin for 4 days. Atorvastatin concentrations were selected based on prior in vitro studies investigating the effects of statins. Concentrations of 1, 5, and 10 μM are within the range shown to produce physiologically relevant responses [20–22]. Differentiating cultures were treated with the same concentrations of atorvastatin from differentiation Day 0 (D0) to D4.

2.3 | Cytotoxicity

Cytotoxicity was performed using the LDH-Cytotoxicity Assay Kit (Abcam) as per the manufacturer's instructions.

2.4 | Senescence Detection

Senescence-associated β-galactosidase (β-gal) activity was measured in actively proliferating cells using the Abcam senescence detection kit according to the manufacturer's instructions. Cells were imaged with the ImageXpress Automated Imager (molecular devices) (details in the Supporting Information).

2.5 | EdU Staining

Proliferation was measured using the Click-iT EdU assay kit (Invitrogen) as per the manufacturer's recommendations. Cells were imaged with the ImageXpress Automated Imager (molecular devices) (details in the Supporting Information).

2.6 | Immunocytochemistry

For immunocytochemistry (ICC), differentiating cells were stained at D4 for Myogenic Differentiation 1 (MyoD) (Abcam), Myogenin (MyoG) (Cell Signalling Technology), Myosin Heavy Chain (MyHC) (DSHB MF-20), for muscle cell marker PAX7 (DSHB) and for 4′,6-diamidino-2-phenylindole (DAPI) (1 μg/mL). The fusion index was calculated as the percentage of nuclei in myotubes within the total area imaged determined using CellReporterXpress on the ImageXpress Automated Imager (molecular devices).

2.7 | RNA Extraction

RNA was extracted using Trizol (Sigma-Aldrich) according to the manufacturer's instructions. RNA concentrations were quantified with the Quibit Fluorometer 2.0 (ThermoScientific) and assessed for quality with the NanoDrop 1000 Spectrophotometer (ThermoScientific) and Bioanalyzer (Agilent) to obtain RNA integrity numbers (RIN). All RNA had a RIN score of >7.

2.8 | RNA Sequencing

For RNA sequencing (RNA-Seq), ≥ 200 ng of total RNA was used for library preparation (PolyA enrichment) and paired-end sequencing undertaken with a read length of 150 bp and depth of 20 million reads per sample (Illumina X Plus). Reads were aligned to the human genome (GRCh38) with HISAT2 [22]. Gene expression was quantified with featureCounts, and genes with lower than 10 reads were filtered out [23]. DESeq2 was used for a paired gene differential expression analysis [24]. Differentially expressed genes were analysed on Metascape for pathway enrichment analysis, which included analysis of Gene Ontology (GO) biological processes, KEGG and Reactome pathways. MCODE analysis was also performed on Metascape to identify protein–protein interaction (PPI) networks (FDR <0.05) [25].

2.9 | Quantitative Real-Time PCR

Quantitative real-time PCR (qRT-PCR) was performed using the LightCycler 480 Real-Time PCR System (Roche, United Kingdom). Five-hundred nanograms of total RNA was treated with DNase (Sigma) and reverse-transcribed into cDNA with the Moloney Murine Leukaemia Virus (M-MLV) reverse transcriptase (Promega) according to the manufacturer's instructions. cDNA amplification was carried out with Quatitect (Qiagen) or KicqStart primers (Sigma) with QuantiFast SYBR Green (Qiagen) as per the manufacturer's recommendations. Each sample was measured in duplicate where technical replicates had a standard deviation (SD) <1 *Ct*. *Ct* values of samples were normalised to two endoengously expressed housekeeping genes, Peptidylproyl Isomerase A (*PPIA*) and Cytochrome C1 (*CYC1*) determined through GeNorm analysis (Primer Design). Data were analysed using the comparative ΔΔCT method.

2.10 | Identification of Candidate Nutraceutical Interventions

To identify genes with potential therapeutic relevance, gene lists from the Top 5 significantly downregulated enriched GO terms were queried against the Comparative Toxigenic Database (CTD) to identify genes with nutraceutical annotations that can increase expression [26]. Genes present in at least two of these pathways were prioritised. Nutraceuticals were then filtered to include only those targeting two or more of these genes. This approach enabled identification of compounds most likely to impact multiple pathways to maximise effects for future follow-up work.

2.11 | Statistical Analysis

All statistical analyses were performed on R (Version 4.2.2). Wilcoxon signed rank test or Students *T* test were used to

analyse the effect of treatments in comparison to the vehicle control dimethyl sulfoxide (DMSO). Each participant contributed three technical replicates per treatment condition, which were then averaged to generate a single value. Boxplots show the median, interquartile range (IQR) and whiskers extending to 1.5 times the IQR. Individual data points represent all measured samples for each treatment. Bar chart error bars represent standard error. Graphs were generated using the ggplot2 package (Version 3.3.6). A significance level of $p < 0.05$ was used for all data analysis.

3 | Results

3.1 | Participant Characteristics

Table 1 shows the characteristics of 11 female participants (mean age 78.2 years) used to analyse phenotypic effects on myoblasts in the first phase of the study. Participants had a mean grip strength of 22.36 kg, gait speed of 0.98 m/s, ALMi of 5.76 kg/m^2 and BMI of 26.23 kg/m^2. Table 2 summarises characteristics of the 10 participants (selected based on RNA availability) used for the second phase of the study to investigate transcriptomic effects; they had a mean age of 79.1 years, grip strength of 20.1 kg, gait speed of 1.02 m/s, ALMi of 5.27 kg/m^2 and BMI of 23.74 kg/m^2. Sarcopenia was characterised by the European Working Group on Sarcopenia in Older People (EWGSOP) criteria [13].

TABLE 1 | HSSe participant characteristics: Phenotypic experiments.

Characteristic	$n = 11$
Age (years)	78.22 ± 2.14
Sex	F
Grip strength (kg)	22.36 ± 6.56
Gait speed (m/s)	0.98 ± 0.22
ALMi (kg/m^2)	5.76 ± 0.64
BMI (kg/m^2)	26.23 ± 3.79

Note: Values are mean ± standard deviation (SD).
Abbreviations: ALMi, appendicular lean mass index; BMI, body mass index.

TABLE 2 | HSSe participant characteristics: RNA sequencing.

Characteristic	$n = 10$
Age (years)	79.07 ± 2.38
Sex	F
Grip strength (kg)	20.1 ± 7.23
Gait speed (m/s)	1.02 ± 0.22
ALMi (kg/m^2)	5.27 ± 0.60
BMI (kg/m^2)	23.74 ± 1.94

Note: Values are mean ± standard deviation (SD).
Abbreviations: ALMi, appendicular lean mass index; BMI, body mass index.

3.2 | Atorvastatin Treatment Was Not Significantly Toxic to Myoblasts

To examine potential toxic effects of statin treatment at the cellular level, cytotoxicity in statin-treated cells was measured (Figure 1a). Atorvastatin treatment showed no significant toxicity at any statin concentration (1 μM $p = 0.32$, 5 μM $p = 0.21$ and 10 μM $p = 0.76$).

3.3 | Cellular Senescence Increased With Treatment

Because cellular senescence increases with age and can impair myoblast differentiation and SAMS includes muscle pain and weakness, this prompted us to investigate the effect of atorvastatin on cellular senescence [27]. The β-galactosidase assay was carried out after a 4-day atorvastatin treatment on proliferating myoblasts and revealed senescence increased with 5 μM ($p = 1.95 \times 10^{-4}$) and 10 μM ($p = 9.77 \times 10^{-4}$) (Figure 1b).

3.4 | Cellular Proliferation Decreased in Statin Treated Myoblasts

Given that previous work has revealed simvastatin can reduce proliferation in human muscle cells, the EdU assay was carried out to observe if the same effects occur with atorvastatin [9]. Atorvastatin reduced proliferation at 1 μM ($p = 1.86 \times 10^{-2}$), 5 μM ($p = 9.77 \times 10^{-4}$) and 10 μM ($p = 9.77 \times 10^{-4}$) (Figure 1c).

3.5 | Statin Treatment Impaired the Cellular Muscle Differentiation Pathway

As myogenic differentiation is essential for muscle function and maintenance, ICC was used to stain for the early differentiation marker MyoD and the later differentiation markers MyoG and MyHC [28]. Ten-micromolar atorvastatin decreased MyoD ($p = 9.77 \times 10^{-4}$), *MyoG* expression decreased with atorvastatin (all concentrations $p = 9.77 \times 10^{-4}$) and MyHC decreased in a dose dependent manner with atorvastatin treatment (1 μM $p = 3.20 \times 10^{-2}$, 5 μM $p = 2.93 \times 10^{-3}$ and10 μM $p = 1.37 \times 10^{-2}$) (Figure 2a–c).

3.6 | Atorvastatin Treatment Significantly Alters Gene Expression of Differentiating Myoblast Cultures

To understand the global transcriptomic effects of 10 μM atorvastatin treatment on differentiating myoblast cultures, RNA-seq was carried out on cultures from the participants shown in Table 2. Ten micromolar was selected as an upper-limit concentration to allow us to capture the maximal cellular response. Principal component analysis (PCA) showed clearly distinct groups between the vehicle control (red) and atorvastatin (blue) treated cell cultures (Figure 3a), with Principal Component 1 (PC1) accounting for 63% of the variance and Principal Component 2 (PC2) accounting for an additional 10%. RNA-seq analysis with false discovery rate (FDR) < 0.05 identified 1710 differentially expressed genes (DEGs), with 822 genes upregulated and

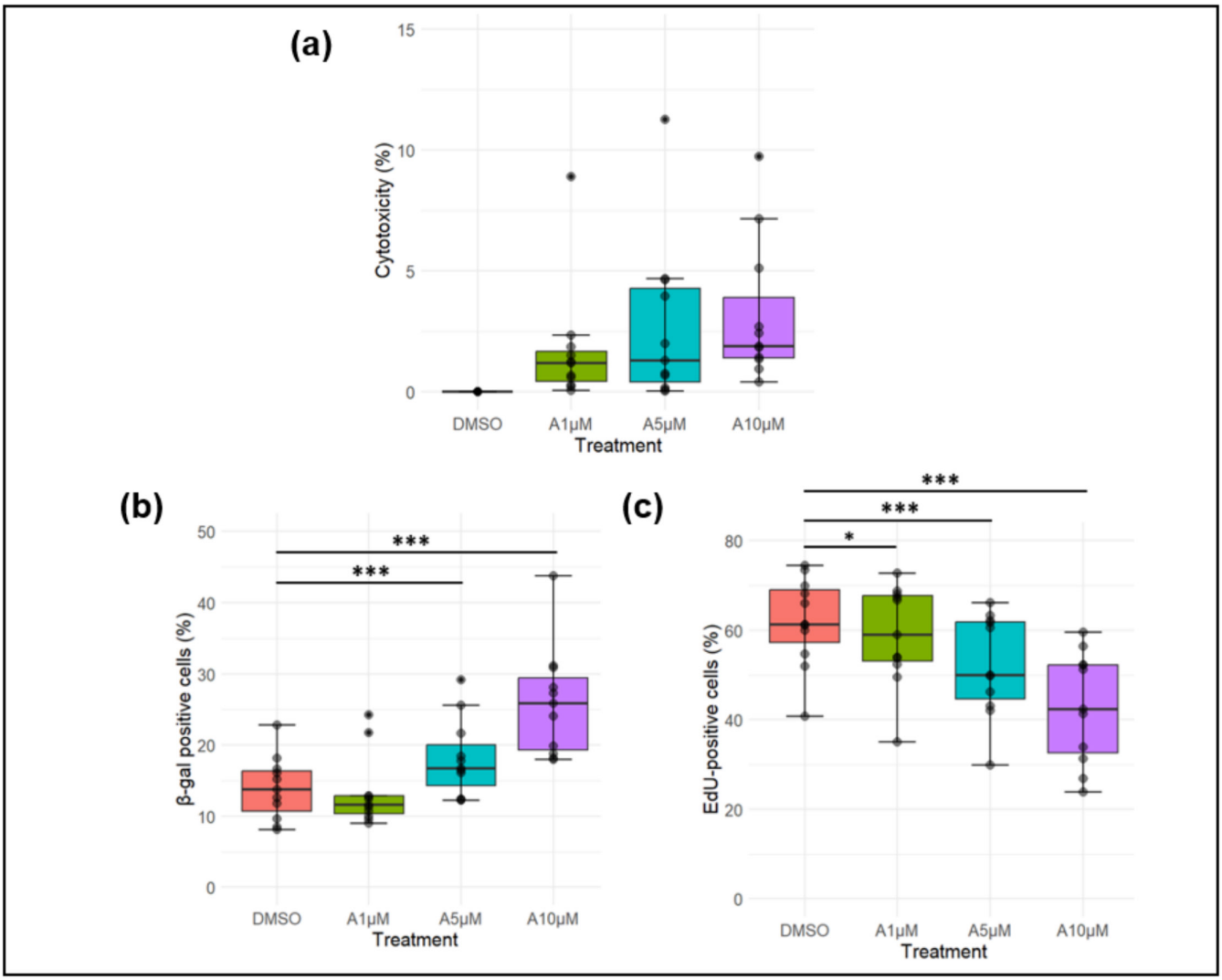


FIGURE 1 | Boxplots of percentages of (a) cytotoxicity, (b) senescent (β-gal) cells and (c) proliferation (EdU). A1μM, A5μM and A10μM represent atorvastatin at 1, 5, and 10 μM concentrations. Boxplots show the median, interquartile range (IQR) and whiskers extending to 1.5 times the IQR. Individual data points represent all measured samples for each treatment. $*p < 0.05$, $***p < 0.001$.

888 genes downregulated (Figure 3b/c). The Top 2 upregulated genes included Transmembrane Protein 97 (*TMEM97*) (FDR 2.10×10^{-103}, log fold change = 3.24) and Squalene Epoxidase (*SQLE*) (FDR 9.65×10^{-97}, log fold change = 1.15). ATP Binding Cassette Transporter A1 (*ABCA1*) (FDR = 1.17×10^{-25}, log fold change = −2.74) and Importin 5 (IPO5) (FDR = 2.24×10^{-17}, log fold change = −0.41) were the top 2 downregulated (Table S1).

The top upregulated gene, *TMEM97*, and the top downregulated gene, *ABCA1*, were validated using qRT-PCR (Figure 4). qRT-PCR revealed *TMEM97* was upregulated ($p = 9.80 \times 10^{-4}$) with atorvastatin treatment and *ABCA1* was downregulated ($p = 4.90 \times 10^{-4}$) with atorvastatin treatment, consistent with the RNA-seq findings.

3.7 | Atorvastatin Induced Changes in Key Cellular Pathways at the Transcriptional Level

Pathway analysis was carried out to identify biological regulatory pathways associated with significant DEGs. The top upregulated pathway was cholesterol metabolism with Bloch and Kandutsch Russel pathways (logp = −31.07), with several other pathways being upregulated including response to virus (logp = −19.46), fatty acid metabolic process (logp = −12.64) and cholesterol biosynthesis with skeletal dysplasia (logp = −11.33). All upregulated pathways are summarised in Figure 5a. The top pathway downregulated was ribonucleoprotein complex biogenesis (logp = −41.72), with notable downregulated pathways including muscle contraction (logp = −16.55), cell cycle (logp = −14.16), translation (logp = −27.42), VEGFA VEGFR2 signalling (logp = −23.79), protein folding (logp = −16.41) and intracellular protein transport (logp = −15.07). All downregulated pathways are summarised in Figure 6a. To explore protein–protein interaction (PPI) networks with the DEGs, molecular complex detection (MCODE) analysis was also carried out on Metascape. Twelve clusters were detected with upregulated DEGs (Figure 5b) including fatty acid metabolic processes (logp = −8.9), clathrin-mediated endocytosis (logp = −8.7) and innate immune response (logp = −4.8). MCODE1 was assigned to sterol biosynthetic processes (logp = −24.2)

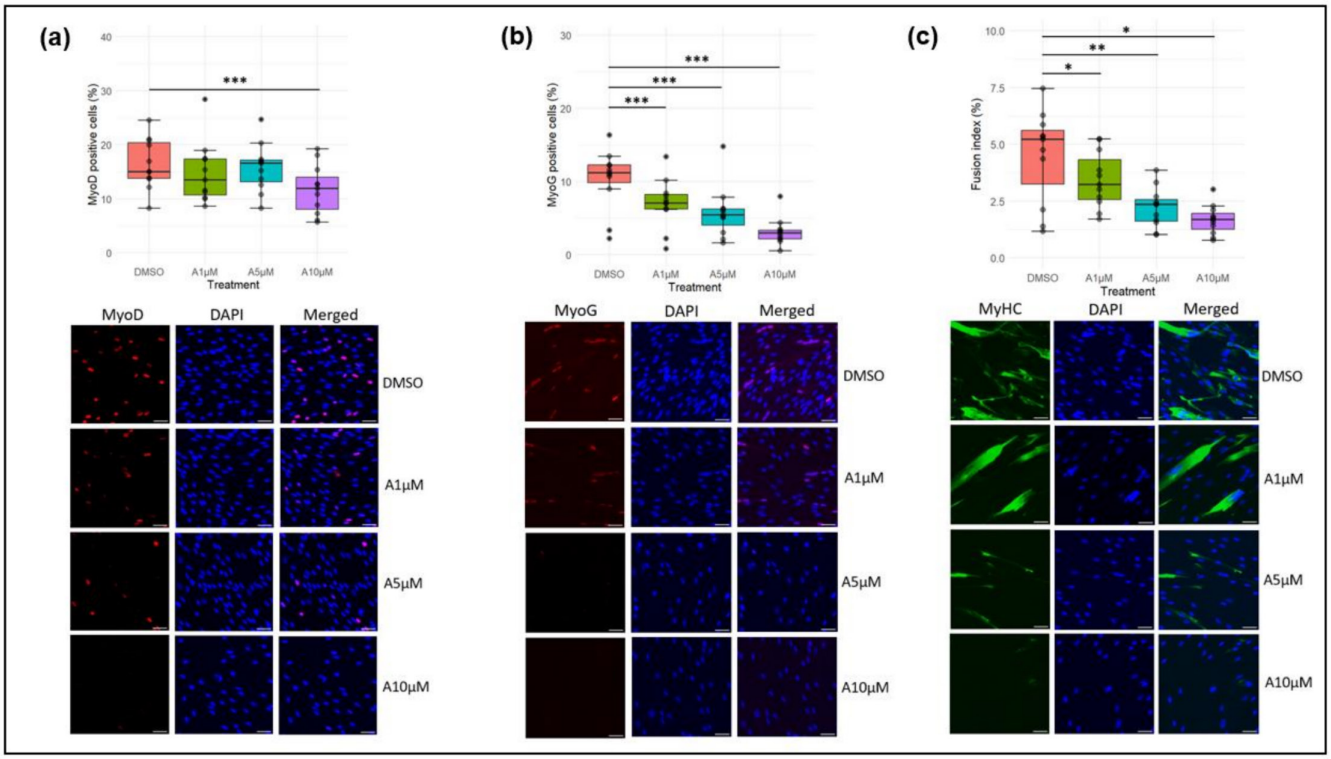


FIGURE 2 | Boxplots and ICC in primary myotubes of (a) MyoD, (b) MyoG and (c) MyHC. A1μM, A5μM, and A10μM represent atorvastatin at 1, 5, and 10 μM concentrations. Boxplots show the median, interquartile range (IQR) and whiskers extending to 1.5 times the IQR. Individual data points represent all measured samples for each treatment. Scale bar represents 50 μm. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

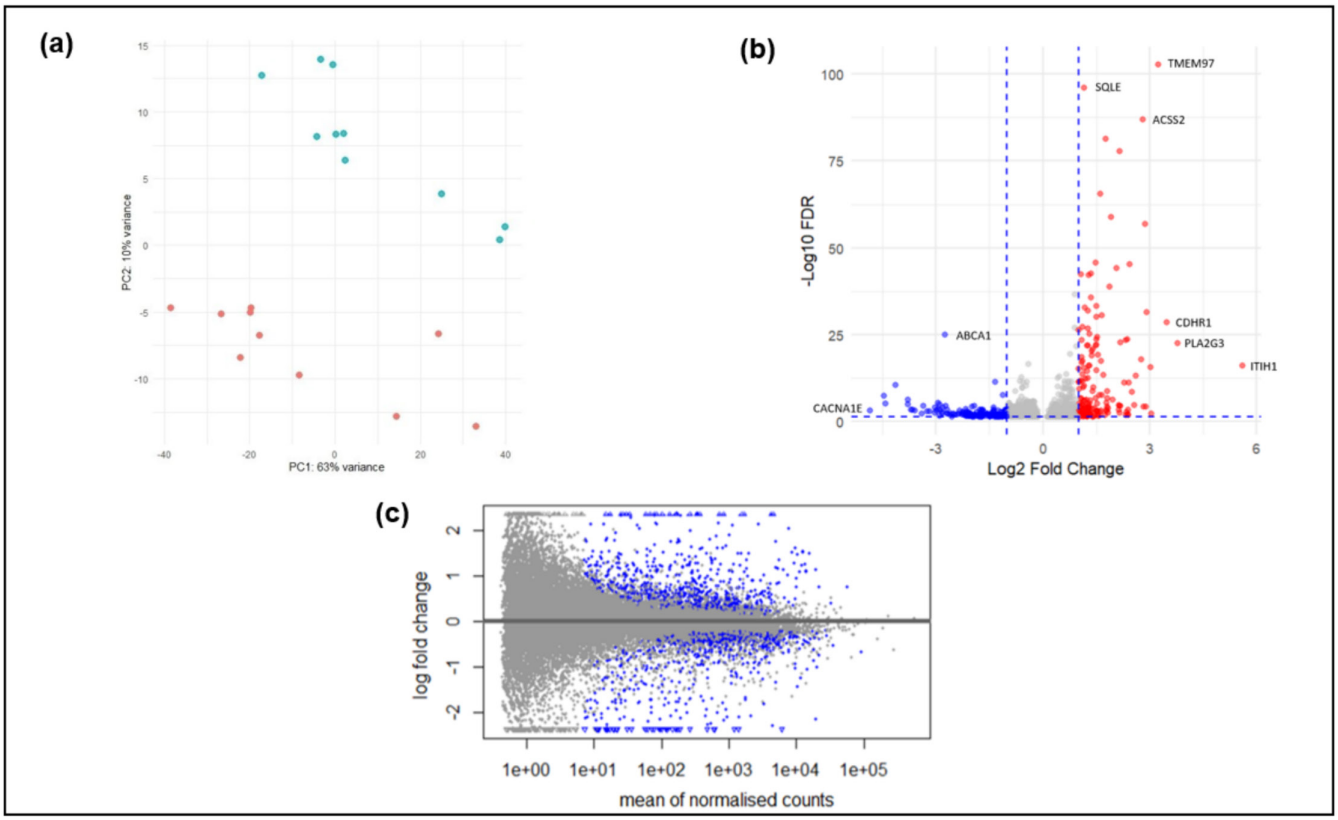


FIGURE 3 | (a) Principal components analysis (PCA) plot of RNA-sequencing samples. Myotubes treated with DMSO (vehicle control) highlighted in red and atorvastatin 10-μM treated samples in blue. (b) Volcano plot of gene expression false discovery rate (FDR) versus log2 fold change with red showing upregulated genes and blue showing downregulated genes. (c) Mean average (MA) plot of log fold change against mean of normalised counts. Blue denotes a significant gene FDR < 0.05.

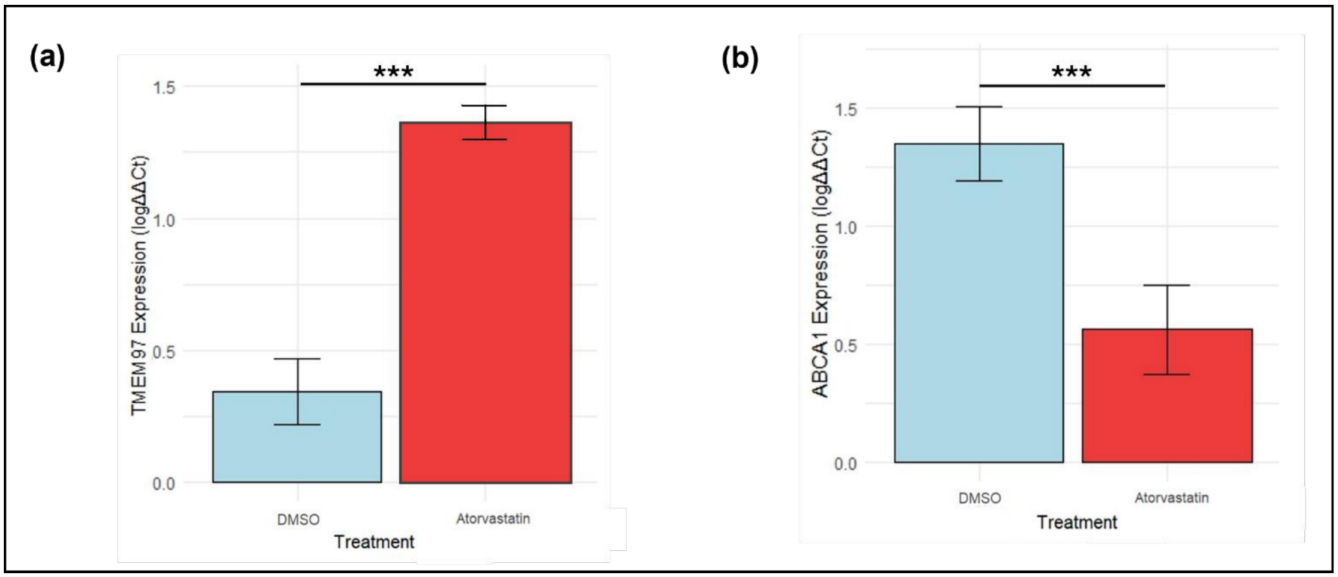
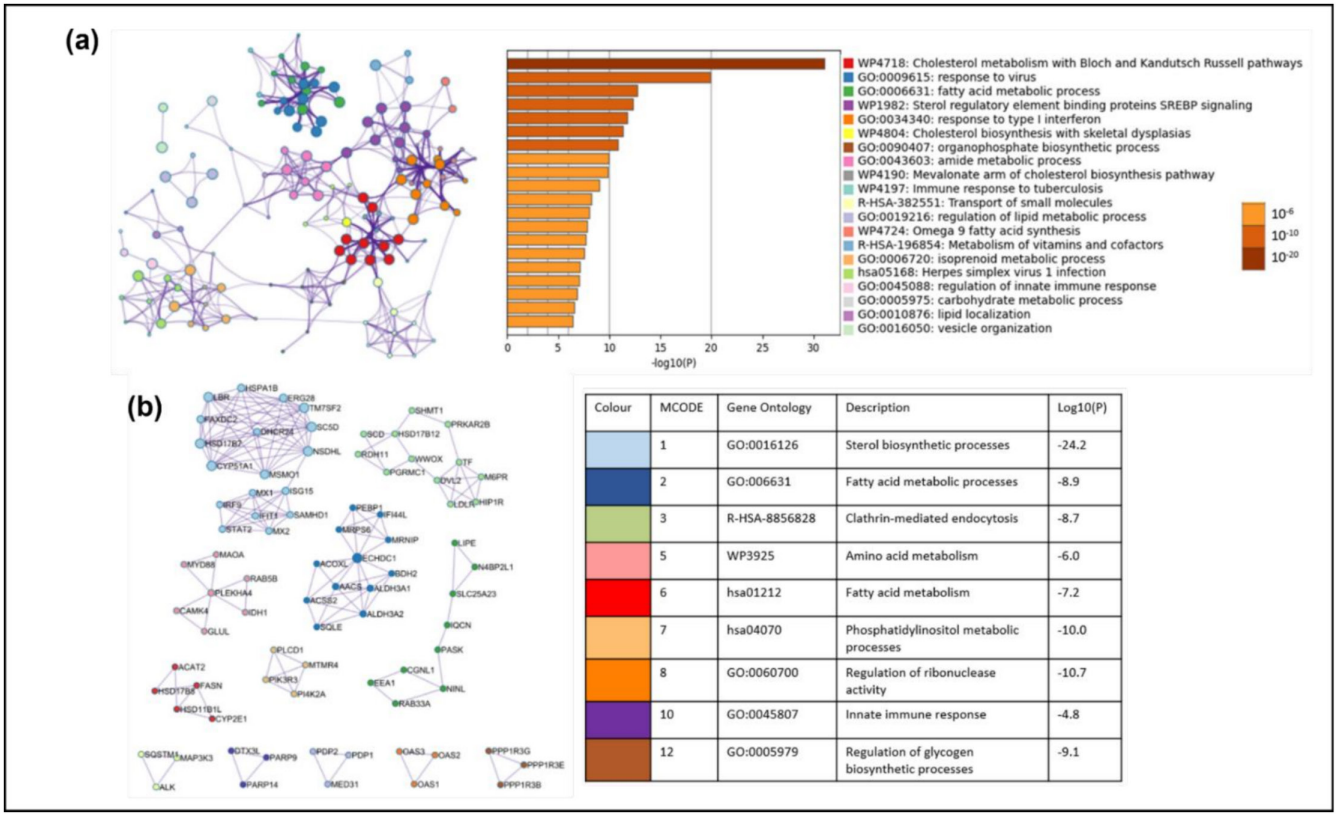


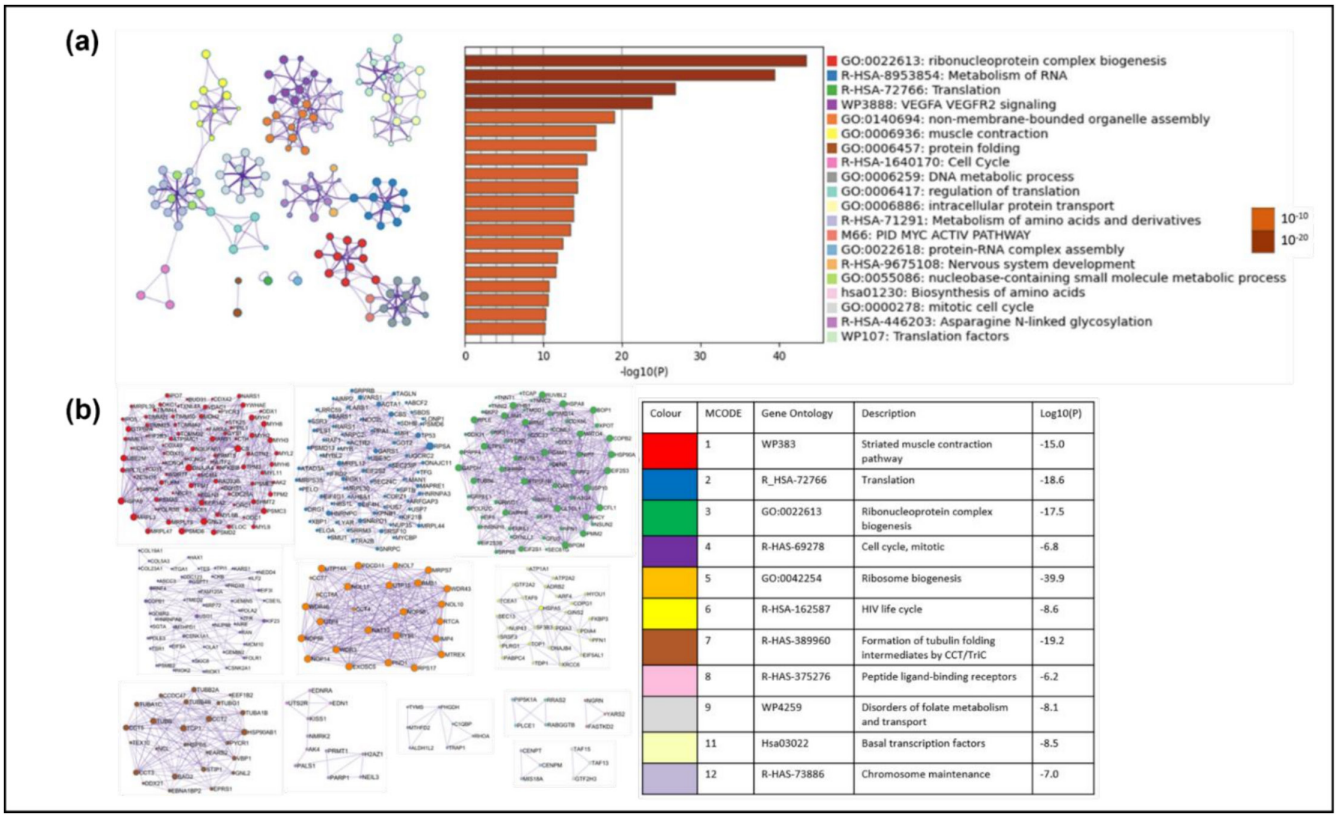
FIGURE 4 | Bar charts showing validation of RNA-sequencing results by quantitative real-time PCR (qRT-PCR) of (a) the top upregulated gene *TMEM97* and (b) top downregulated gene *ABCA1*. Bar chart error bars represent standard error. ***$p < 0.001$.



Colour	MCODE	Gene Ontology	Description	Log10(P)
	1	GO:0016126	Sterol biosynthetic processes	-24.2
	2	GO:006631	Fatty acid metabolic processes	-8.9
	3	R-HSA-8856828	Clathrin-mediated endocytosis	-8.7
	5	WP3925	Amino acid metabolism	-6.0
	6	hsa01212	Fatty acid metabolism	-7.2
	7	hsa04070	Phosphatidylinositol metabolic processes	-10.0
	8	GO:0060700	Regulation of ribonuclease activity	-10.7
	10	GO:0045807	Innate immune response	-4.8
	12	GO:0005979	Regulation of glycogen biosynthetic processes	-9.1

FIGURE 5 | Pathway and MCODE analysis was performed on Metascape with false discovery rate (FDR) < 0.05 upregulated genes. (a) Network and bar graph of functionally grouped terms, coloured by cluster ID, with terms sharing similar genes connected by edges. (b) Protein–protein interaction (PPI) networks of differentially expressed genes (DEGs) with corresponding MCODE and gene ontology (GO) description.

involving genes including Ergosterol biosynthesis 28 homologue (*ERG28*), Sterol-C5-Desaturase (*SC5D*) and NAD(P) dependent 3-beta-hydroxysteroid dehydrogenase (*NSDHL*). Thirteen clusters were detected with downregulated DEGs, with actin-myosin filament sliding assigned to MCODE1 (logp = −17.5) involving genes *MyHC3* and Myosin Light chain 2 (*MyL2*). Other downregulated PPI networks included translation (logp = −16.3), ribosome biogenesis (logp = −11.8) and mitotic cell cycle (logp = −6.8), with a full list summarised in Figure 6b.



Colour	MCODE	Gene Ontology	Description	Log10(P)
	1	WP383	Striated muscle contraction pathway	-15.0
	2	R_HSA-72766	Translation	-18.6
	3	GO:0022613	Ribonucleoprotein complex biogenesis	-17.5
	4	R-HAS-69278	Cell cycle, mitotic	-6.8
	5	GO:0042254	Ribosome biogenesis	-39.9
	6	R-HSA-162587	HIV life cycle	-8.6
	7	R-HAS-389960	Formation of tubulin folding intermediates by CCT/TriC	-19.2
	8	R-HAS-375276	Peptide ligand-binding receptors	-6.2
	9	WP4259	Disorders of folate metabolism and transport	-8.1
	11	Hsa03022	Basal transcription factors	-8.5
	12	R-HAS-73886	Chromosome maintenance	-7.0

FIGURE 6 | Pathway and MCODE analysis was performed on Metascape with false discovery rate (FDR) < 0.05 downregulated genes. (a) Network and bar graph of functionally grouped terms, coloured by cluster ID, with terms sharing similar genes connected by edges. (b) Protein–protein interaction (PPI) networks of differentially expressed genes (DEGs) with corresponding MCODE and gene ontology (GO) description.

3.8 | Nutraceutical Annotations for Genes Across the Top Downregulated GO Pathways

In order to identify potential druggable hits, genes that were involved in more than two of the Top 5 GO pathways were cross-referenced with the CTD to find nutraceutical targets (Table S2). Table S3 summarises compounds that target two or more of the prioritised genes. The top nutraceutical compound was resveratrol which had targeted 10 genes (*BCCIP*, *DDX1*, *DKC1*, *EXOSC5*, *FASTKD2*, *MRTO4*, *NGRN*, *NIP7*, *RPF2* and *ZPR1*) across four GO terms (ribonucleoprotein complex biogenesis, nonmembrane-bounded organelle assembly, protein folding and DNA metabolic process).

4 | Discussion

Here, we report that atorvastatin treatment altered the human myoblast phenotype, driving increased senescence and reduced proliferation. Furthermore, treatment impaired differentiation capacity, downregulating key myogenic markers, MyoD, MyoG and MyHC. RNA-seq analysis revealed widespread transcriptional changes with 822 genes upregulated and 888 downregulated. Pathway analysis highlighted upregulation of cholesterol metabolism, with key downregulated processes including ribonucleoprotein complex biogenesis, RNA metabolism, translation and muscle contraction. MCODE analysis revealed multiple clusters of PPI networks associated with these pathways. These data provide novel insights into the gene regulatory pathways in human myoblasts through which atorvastatin exerts effects that may underlie the mechanisms associated with SAMS that patients can experience on statin treatment.

Atorvastatin treatment led to a significant reduction in myoblast proliferation, aligning with findings of Grunwald et al. [9], who also identified a decrease in myoblast proliferation with simvastatin and rosuvastatin treatment. Myoblast proliferation is essential for muscle regeneration and in maintaining the muscle stem cell pool [29]. The observed decrease in proliferation may be explained by atorvastatin's inhibition of HMGCR in the mevalonate pathway, which is essential in cholesterol synthesis. Cholesterol is necessary for cell proliferation because it is required for membrane biogenesis and cell cycle progression. Cholesterol is a major component in cell membranes as it helps maintain their fluidity, elasticity and permeability, which modulate the activity of ion channels, receptors and transporters. During the cell cycle, cells committed to divide activate the mevalonate pathway and upregulate the expression of HMGCR, which is required for regulation of the S-phase. The mevalonate pathway also synthesises isoprenoids. Isoprenoids including farnesyl pyrophosphate (FPP) and geranylgeranyl phosphatase (GGPP) carry out posttranslational modification and protein prenylation, essential for the activation of small GTPases like Ras and Rho involved in cell cycle regulation [30–32]. Disruption of these biological pathways by atorvastatin may impair cell cycle progression, leading to cell cycle arrest, thereby reducing myoblast proliferation and potentially driving cells into a senescent state. Although

HMGCR is the primary enzymatic target of statins, statin-treated cells typically respond by upregulating the expression of *HMGCR*. This is consistent with previous literature on statin treatment, where there is well-established compensatory feedback of *HMGCR* when it is pharmacologically inhibited [33–35]. Other cholesterol and mevalonate genes were also upregulated including *HMGCS1*, *MVK*, *MVD*, *SQLE*, *FDFT1*, *DHCR2*, *DHCR24*, *INSIG1* and *SREBF2* and also additional cholesterol-associated pathways. This could also explain why there was also increased expression of several Ras and Rho related genes including *ARHGEF3*, *ARHGEF9*, *RAB33A*, *RAB5B*, *RAB27B*, *RAB29* and *RGL2*. Hence, the increase we observe in the RNA-seq dataset likely reflects cellular compensation, rather than enhanced pathway activity.

Senescence, a state of irreversible growth arrest in cells, is a hallmark of ageing. Senescent cells accumulate over time and develop a senescence associated secretory phenotype (SASP), characterised by the release of proinflammatory cytokines and chemokines. During ageing, there is inefficient clearance and resistance to apoptosis of senescent cells, leading to an accumulation of these proinflammatory markers that may impair muscle regeneration and promote inflammation. Here, atorvastatin treatment increased senescence in myoblasts. This may translate to impaired muscle function, potentially exacerbating muscle ageing in patients undergoing statin therapy. Senescence has also been previously documented to affect muscle function. In mice, transplantation of senescence cells resulted in reduced walking speed and hanging endurance, which suggests a decrease in muscle function [36]. Additionally, with the inhibition of *HMGCR* and the reduction of cholesterol and isoprenoid intermediates, lipid homeostasis may be disrupted, which could compromise membrane integrity and subsequently cellular functions, resulting in stress-induced senescence. This could accelerate the build-up of senescence cells, which may drive muscle dysfunction further. To further support the increase in senescence shown, RNA-seq analysis revealed the upregulation of senescence and inflammatory associated genes, for example, *CD9*, *MYD88* and *CTSS*. *CD9* has previously been recognised as a key senescence regulator that increases during ageing, and *MYD88* signalling is linked to increased inflammation and elevated in stressed muscle [37, 38]. *CTSS* has been shown to increase oxidative stress and inflammation in the muscles of mice, and elevated *CTSS* is linked to decreased grip strength [39].

With a higher percentage of senescent cells and lower percentage of proliferating cells, this decreases the pool of active myoblasts and may have contributed to the reduction in the number of differentiating cells. MyoD, MyoG and MyHC are essential transcription factors in myogenesis. MyoD directs cells into differentiation, MyoG is involved in regulating myoblast fusion to form multinucleated myotubes and MyHC is involved in muscle fibre contraction. As atorvastatin treatment downregulated all these markers, this suggests atorvastatin disrupts myogenesis at multiple timepoints during differentiation, which may prevent the regeneration of skeletal muscle. With MyHC being a major contractile protein within the thick filament of the sarcomere, responsible for muscle contraction, its downregulation not only affects myoblast differentiation but also sarcomere assembly and contractile function [40]. This could translate to the development of weaker and less functional muscle fibres, ultimately resulting in diminished muscle function and muscle pain. In line with the ICC data, the RNA-seq dataset further reinforced that atorvastatin treatment reduces myogenic capacity. Several genes involved in myogenesis including *MYL6B*, *MYL2*, *MYH6*, *MYH7*, *MYL11*, *MYH8*, *MYH2*, *MYH3* and *MYL9* exhibited large log fold changes and were significantly downregulated with atorvastatin treatment. The coordinated suppression of multiple MyHC and light chain isoforms indicates broad impairment on various aspects of muscle structure and integrity. This widespread disruption of contractile gene expression is likely to compromise greater the ability for progression toward mature myotube formation and consequently muscle function. However, these findings should be interpreted within the broader context of the cellular phenotype, where there is a heterogenous mix of proliferating, differentiating and fused cells. We have shown that proliferation decreases and senescence increases with treatment, which are both pathways that can also affect differentiation; the reduction in differentiation markers likely reflects the combination of direct and indirect effects of atorvastatin.

Consistent with these findings, RNA-seq data revealed pathways including ribonucleoprotein complex biogenesis, cell cycle, PID MYC ACTIV pathway and translation, all to be significantly downregulated, which influence proliferation, senescence and differentiation. Ribonucleoprotein complex biogenesis is a cellular process referring to the biosynthesis and assembly of ribonucleoproteins, which regulate RNA processing and genome stability [41]. Because this is necessary for RNA metabolism and protein production, their downregulation by atorvastatin treatment would also have knock-on effects to pathways involved in translation, cell growth and differentiation. The PID MYC ACTIV pathway refers to the signalling pathway centred around transcription factor MYC. MYC impacts multiple processes influencing cellular functions including DNA damage response, apoptosis, cell cycle, differentiation and ribosome biogenesis. Downregulation of MYC suggests cells may exit the cell cycle and instead enter a senescent state or trigger a stress response, which over time may lead to muscle wasting and a loss in muscle function and health [42–44]. Additionally, downregulation of intracellular protein transport and protein folding implies that atorvastatin therapy may disrupt cellular homeostasis and protein turnover. Aligned with this, multiple ubiquitin-proteasome genes (*UBE3C*, *UBE2N*, *UBE4B* and *RNF41*), proteosome assembly factors (*PSMD2*, *PSMD8*, *PSMA5*, *PSMB2* and *PSMC3*), autophagy (*ATG101*) and protein folding genes (*HSPA5*, *DNAJB4* and *PDIA3*) were downregulated with atorvastatin treatment, further suggesting impaired proteostasis. This would subsequently result in a reduced number of proteins and more misfolded proteins, which may impair the function of sarcomeres, leading to muscle weakness, reduced muscle regeneration and contractile efficiency [45, 46]. Another pathway significantly downregulated was VEGFA VEGFR2 signalling. VEGFA is essential in muscle maintenance and recovery but has been documented to significantly decline during ageing. Research has shown that knockdown of VEGFA in C2C12 cells resulted in decreased muscle differentiation, resulting in poor muscle regeneration. It is also important in promoting angiogenesis and blood flow within muscles, vital for hypertrophy following injury and muscle maintenance. Downregulation of this would

suggest limited nutrient supply to muscle, which may result in reduced energy availability in fibres and muscle atrophy due to potentially insufficient oxygen and nutrient delivery for metabolism and repair [47]. MCODE analysis highlighting the top PPI networks further reinforces findings from the pathway analysis, emphasising pathways and processes including striated muscle contraction pathway, translation, ribonucleoprotein complex biogenesis and cell cycle. This strengthens the argument that these pathways are biologically significant, and the proteins identified within these networks may represent key molecular players which are selectively affected or highly sensitive to atorvastatin. Exploring their roles further in atorvastatin treatment could provide targets in developing cotreatments to prevent or ameliorate SAMS.

RNA-seq further revealed *ABCA1* as the top downregulated gene and *TMEM97* as the top upregulated gene. Both *ABCA1* and *TMEM97* are involved in cholesterol processes, with *ABCA1* being a key mediator of cholesterol efflux and lipid homeostasis and *TMEM97* being involved in the uptake of LDL and cholesterol trafficking. The suppression of *ABCA1* may contribute to the reduced intracellular levels of cholesterol through reduction of the LXR/RXR and SREBP-2 pathway where *ABCA1* is a direct target and is activated to respond to changes in cholesterol. Consistent with this interpretation, several LXR/RXR and SREBP dependent genes were downregulated in the RNA-seq data set. These included transcription factor encoding gene *SREBF1*, which is involved in regulating and responding to cholesterol and fatty acid biosynthesis. *VLDR* and *ABCD1* were also downregulated genes; these mediate the uptake of VLDL-derived lipids into skeletal muscle and maintain lipid homeostasis, respectively [48, 49]. Together, this suggests coordinated suppression of networks involved in lipid and cholesterol handling. In contrast, the upregulation of *TMEM97* implies a potential compensatory mechanism since cholesterol is still required for cellular processes. As *TMEM97* interacts with the LDL receptor, this may enhance LDL uptake, subsequently leading to the increase of cholesterol in the cell [50–52]. This upregulation therefore highlights how myotubes may attempt to offset the effects of atorvastatin induced cholesterol depletion. Pathway enrichment analysis further reinforced the impact of atorvastatin on cholesterol since pathways including cholesterol metabolism and cholesterol biosynthesis with skeletal dysplasia were upregulated. The link to skeletal dysplasia further emphasises the connection to SAMS.

With atorvastatin treatment, myoblast proliferation decreased, cellular senescence increased and myogenesis was impaired. RNA-seq analysis also revealed that muscle contraction and other pathways important in muscle health, maintenance and function were downregulated. In support of this, key genes involved in muscle structure such as *ACTA1*, *TUBA1B*, *MYH7* and *TPM1* were downregulated, suggesting diminished contractile capacity of the muscle. Markers of mitochondrial metabolism and cellular energy homeostasis were also suppressed including *TIMM23*, *PCK2*, *GOT2* and *ATP5F1B*, which aligns with the reduced oxidative capacity observed in sarcopenic muscle [11]. Taken together with the reduced expression in protein quality and proteostasis markers, this provides molecular evidence that these mechanisms align with what is observed in sarcopenic muscle [38]. As atorvastatin mirrors key cellular mechanisms underlying sarcopenia, this suggests that atorvastatin treatment may drive muscle to a sarcopenic phenotype or display sarcopenic characteristics such as reduced muscle grip strength. Given that statins are commonly prescribed to older people who are at risk of sarcopenia, these findings raise important concerns about their potential to exacerbate muscle decline in an already vulnerable group.

A key strength of this study is that it is the first to examine the effects of atorvastatin in muscle cells from exclusively older individuals, providing new insights into the mechanisms underlying its effects on muscle. Previous studies have either used different statins or been undertaken in different cell types or using model organisms. Kobayashi et al. [8] treated rhabdomyosarcoma cells with pitavastatin and reported an increase in apoptosis and a decrease in the number of viable cells, which may be indicative of enhanced cellular stress. Although we did not directly assess apoptosis, our RNA-seq data highlighted the downregulation of many key survival pathways including translation and pathways involved in RNA processing and metabolism; with the increase in senescence, this may shift cells to a more compromised cellular state and reduce the number of functional cells. In line with this, Grunwald et al. [9] showed that simvastatin treatment in human muscle cells reduced proliferation, which further supports our data. We found a decrease in proliferation but also a downregulation in key pathways involved in cell proliferation including cell cycle, the PID MYC ACTIV pathway and translation-associated processes.

Another strength of this study is that it looked at the global changes atorvastatin can induce rather than only analysing selected pathways of interest; this revealed pathways and processes that may play important roles in SAMS but would have otherwise been overlooked. A limitation of this work is that RNA-seq analysis only shows effects of the transcriptome and not the protein levels that ultimately determine functional changes. As mRNA levels do not always directly correlate with protein abundance due to additional posttranscriptional processes, the full extent of atorvastatin's effects is not fully understood. Future studies should include proteomic work such as western blotting or liquid chromatography mass spectrometry to verify changes in expression. Additionally, the work was carried out on mixed differentiating myoblast cultures rather than purified myotubes. However, this approach did allow us to capture the atorvastatin induced effect across myogenesis, which is relevant for understanding how statin treatment may interfere with muscle regeneration and better reflects what occurs in a heterogenous environment that contains proliferating, differentiating and fusing cells [53]. Because the cultures used were mixed differentiating myoblast cultures (rather than isolated myotubes) and the ICC data showed a downregulation in key myogenesis markers, some transcriptomic changes detected may reflect variation in differentiation status and reduced myotube abundance and not solely a direct response from atorvastatin treatment. Nevertheless, the transcriptomic response was broader than only affecting myogenic markers and pathways independent from myotube abundance, including significant alterations in unfolded protein response, cholesterol regulation and proteasome function. This shows atorvastatin directly interrupts and induces a broader cellular response, rather than a passive consequence of reduced myotube fusion; if the differentiation state alone was driving the changes in the

transcriptome, these would be expected to remain confined to myogenic pathways. As MyoD, MyoG and MyHC display dynamic and temporal expression patterns across myogenesis, a time-course analysis would provide added insight into whether statin treatment directly influences myoblast differentiation, acts indirectly through decreased proliferation and increased senescence or involves a combination of both direct and indirect responses. Further limitations of this work are that we did not study individuals with SAMS and that we only studied myoblasts from female participants. No men were analysed as they are less susceptible to SAMS, but there may be sex-specific muscle differences that are unaccounted for with statin treatment. Women and men exhibit distinctive muscle differences due to hormonal regulation, metabolic pathways and physical composition [54]. This could result in muscle cells from females responding to atorvastatin differently to muscle cells from males. The current study also focuses exclusively on muscle cells from older adults, who are most at risk of SAMS. Future work could focus on comparing muscle cells from older and younger participants to determine whether age modifies cellular susceptibility to statin exposure. Importantly, this study provides a framework for follow-up studies. Candidate nutraceutical compounds were identified from the top GO pathways, which yielded a prioritised compound list for future work (summarised in the Table S3). Resveratrol emerged as the top compound that targeted 10 genes that were involved in 4 of the top 5 downregulated GO pathways. This broad coverage is advantageous because it indicates the ability to target multiple processes dysregulated by atorvastatin. Resveratrol is a naturally occurring plant-derived polyphenol typically found in nuts, berries and grapes and has been widely studied for its potential beneficial effects on health. In a previous study, resveratrol treated mice displayed higher expression of key myogenic markers including MyoD, MyoG and Pax7, along with increased mitochondrial DNA content and upregulation of pathways related to differentiation and microtubule motor activity [55]. Another study carried out in resveratrol treated mice further reported enhanced proliferation, differentiation and mitochondrial biogenesis in the muscle cells [56]. This supports its relevance in targeting multiple processes central to improving skeletal muscle health and function.

5 | Conclusions

Collectively, these results provide novel insights into the molecular mechanisms that may underpin SAMS in older individuals. Atorvastatin treatment negatively impacts skeletal muscle health, function and maintenance by disrupting many important key cellular pathways in human muscle cells. Future research is required to explore targeted use of statins or strategies to mitigate these muscle side effects while preserving their cardiovascular benefits.

Author Contributions

Maisha Chaudhery: conceptualization (supporting), formal analysis (lead), methodology (equal), writing – original draft (lead), writing – review and editing (lead). **Mark A. Burton:** conceptualization (supporting), formal analysis (supporting), methodology (supporting), writing – original draft (supporting), writing – review and editing (supporting). **Hanan Y. Sharkh:** formal analysis (supporting), methodology (supporting), writing – review and editing (supporting). **Leo D. Westbury:** data curation (supporting), writing – review and editing (supporting). **Elaine M. Dennison:** conceptualization (supporting), writing – review and editing (supporting). **Nicholas C. Harvey:** conceptualization (supporting), writing – review and editing (supporting). **Cyrus Cooper:** conceptualization (supporting), funding acquisition (supporting), methodology (supporting), writing – review and editing (supporting). **Harnish P. Patel:** conceptualization (supporting), funding acquisition (supporting), methodology (supporting), writing – review and editing (supporting). **Keith M. Godfrey:** conceptualization (equal), funding acquisition (equal), methodology (supporting), supervision (supporting), writing – review and editing (supporting). **Karen A. Lillycrop:** conceptualization (equal), funding acquisition (equal), methodology (supporting), supervision (lead), writing – review and editing (supporting). **Emma S. Garratt:** conceptualization (supporting), formal analysis (supporting), methodology (equal), supervision (supporting), writing – original draft (supporting), writing – review and editing (supporting).

Acknowledgements

This work was supported by funding from the University of Southampton and the Rank Prize. K.M.G. is supported by the UK Medical Research Council (MC_UU_20/4), the National Institute for Health Research (as an NIHR Senior Investigator [NF-SI-055-0042] and through the NIHR Southampton Biomedical Research Centre [NIHR203319]), the Rosetrees Trust, Wessex Medical Trust, Gerald Kerkut Charitable Trust and Alzheimer's Research UK (ARUK-PG2022A-008). K.A.L. was supported by the Rosetrees Trust, Wessex Medical Trust and Rank Prize. H.P.P. is supported by the National Institute for Health Research through the NIHR Southampton Biomedical Research Centre. http://N.C.H.is supported by the UK Medical Research Council (MC_PC_21003 and MC_PC_21001), the National Institute for Health Research (as a NIHR Senior Investigator [NIHR305844] and through the NIHR Southampton Biomedical Research Centre [NIHR203319]). This report is independent research, and the views expressed in this publication are those of the authors and not necessarily those of the NHS, the NIHR or the Department of Health. The grant funders had no role in the design, collection, analysis and interpretation of data; writing of the paper; or decision to submit for publication. For the purpose of Open Access, the author has applied a Creative Commons Attribution (CC BY) licence to any Author Accepted Manuscript version arising from this submission.

Funding

This study was supported by the University of Southampton, the Rank Prize, the UK Medical Research Council (MC_UU_20/4, MC_PC_21003 and MC_PC_21001), the National Institute for Health Research (NF-SI-055-0042, NIHR203319 and NIHR305844), Rosetrees Trust, Wessex Medical Trust, Gerald Kerkut Charitable Trust and Alzheimer's Research UK (ARUK-PG2022A-008).

Ethics Statement

The authors of this manuscript certify that they comply with the ethical guidelines for authorship and publishing in the *Journal of Cachexia, Sarcopenia and Muscle*. The study was approved by the Hertfordshire Research Ethics Committee (07/Q0204/68) and has therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments. All participants gave their written informed consent prior to their inclusion in the study.

Conflicts of Interest

K.M. Godfrey and H.P. Patel have received reimbursement for speaking at conferences sponsored by companies selling nutritional products. C. Cooper has received consultancy fees and honoraria from Amgen, Danone, Eli Lilly, GlaxoSmithKline, Medtronic, Merck, Nestlé, Novartis, Pfizer, Roche, Servier, Shire, Takeda and UCB. N.C. Harvey has received consultancy/lecture fees/honoraria/grant funding from

Alliance for Better Bone Health, Amgen, MSD, Eli Lilly, Radius Health, Servier, Shire, UCB, Consilient Healthcare, Kyowa Kirin, Theramex and Internis Pharma. M.A. Burton, E.S. Garratt, K.M. Godfrey and K.A. Lillycrop are part of academic research programmes that have received research funding from BenevolentAI Bio Ltd., Nestec, Bayer and Boehringer Ingelheim. The other authors declare no conflicts of interest.

References

1. H. Mollazadeh, E. Tavana, G. Fanni, et al., "Effects of Statins on Mitochondrial Pathways," *Journal of Cachexia, Sarcopenia and Muscle* 12, no. 2 (2021): 237–251.

2. R. Muzambi, K. Bhaskaran, H. Strongman, T. van Staa, L. Smeeth, and E. Herrett, "Trends and Inequalities in Statin Use for the Primary and Secondary Prevention of Cardiovascular Disease Between 2009 and 2021 in England," *medRxiv*, (2024).

3. National Institute for Health and Care Excellence (NICE), "More People Are Benefitting From NICE-Recommended Statins to Reduce Heart Attacks and Strokes," [Online], https://www.nice.org.uk/news/articles/more-people-are-benefitting-from-nice-recommended-statins-to-reduce-heart-attacks-and-strokes.

4. A. Herghelegiu, G. Prada, R. Nacu, A. Kozma, and I. Alexa, "Statins Use and Risk of Sarcopenia in Community Dwelling Older Adults," *Famacia* 66, no. 4 (2018): 202–702.

5. E. Stroes, P. D. Thopson, A. Corsini, et al., "Statin-Associated Muscle Symptoms: Impact on Statin Therapy—European Atherosclerosis Society Consensus Panel Statement on Assessment, Aetiology and Management," *European Heart Journal* 36, no. 17 (2015): 1012–1022.

6. O. Mashinchian, A. Pisconti, E. Moal, and C. F. Bentzinger, "Chapter Two—The Muscle Stem Cell Niche in Health and Disease," *Current Topics in Developmental Biology* 126 (2018): 23–65.

7. C. Macchi, V. Bonalume, M. F. Greco, et al., "Impact of Atorvastatin on Skeletal Muscle Mitochondrial Activity, Locomotion and Axonal Excitability—Evidence From ApoE-/- Mice," *International Journal of Molecular Sciences* 23, no. 10 (2022): 5415.

8. M. Kobayashi, I. Chisaki, K. Narumi, et al., "Association Between Risk of Myopathy and Cholesterol-Lowering Effect: A Comparison of All Statins," *Life Sciences* 82, no. 17–18 (2008): 969–975.

9. S. A. Grunwald, O. Popp, S. Haafke, et al., "Statin-Induced Myopathic Changes in Primary Human Muscle Cells and Reversal by a Prostaglandin F2 Alpha Analogue," *Scientific Reports* 10, no. 1 (2020): 2158.

10. J. Bouitbir, A.-L. Charles, L. Rasseneur, et al., "Atorvastatin Treatment Reduces Exercise Capacities in Rats: Involvement of Mitochondrial Impairments and Oxidative Stress," *Journal of Applied Physiology* 111, no. 5 (2011): 1477–1483.

11. E. Migliavacca, S. K. Tay, H. P. Patel, et al., "Mitochondrial Oxidative Capacity and NAD+ Biosynthesis Are Reduced in Human Sarcopenia Across Ethnicities," *Nature Communications* 10, no. 1 (2019): 5808.

12. T. N. Kim and K. M. Choi, "Sarcopenia: Definition, Epidemiology, and Pathophysiology," *Journal of Bone Metabolism* 20, no. 1 (2013): 1–10.

13. A. Cruz-Jentoft, J. Baeyens, J. Bauer, et al., "Sarcopenia: European Consensus on Definition and Diagnosis," *Age and Ageing* 39, no. 4 (2010): 412–423.

14. L. Larsson, H. Degens, M. Li, et al., "Sarcopenia: Aging-Related Loss of Muscle Mass and Function," *Physiological Reviews* 99, no. 1 (2018): 427–511.

15. L. Campins, M. Camps, A. Riera, E. Pleguezuelos, J. Yebenes, and M. Serra-Prat, "Oral Drugs Related With Muscle Wasting and Sarcopenia. A Review," *Karger* 99 (2017): 1–8.

16. D. V. Sutil, A. N. Parentoni, L. A. Da Costa Teixeira, et al., "Prevalence of Sarcopenia in Older Women and Level of Agreement Between the Diagnostic Instruments Proposed by the European Working Group on Sarcopenia in Older People 2 (EWGSOP2)," *BMC Musculoskeletal Disorders* 24, no. 1 (2023): 182.

17. H. P. Patel, H. E. Syddall, H. J. Martin, C. E. Stewart, C. Cooper, and A. A. Sayer, "Hertfordshire Sarcopenia Study: Design and Methods," *BMC Geriatrics* 10 (2010): 1–7.

18. L. D. Westbury, R. M. Dodds, H. E. Syddall, et al., "Associations Between Objectively Measured Physical Activity, Body Composition and Sarcopenia: Findings From the Hertfordshire Sarcopenia Study (HSS)," *Calcified Tissue International* 103 (2018): 237–245.

19. E. Antoun, E. S. Garratt, A. Taddei, et al., "Epigenome-Wide Association Study of Sarcopenia: Findings From the Hertfordshire Sarcopenia Study (HSS)," *Journal of Cachexia, Sarcopenia and Muscle* 13, no. 1 (2021): 240–253.

20. B. Buranrat, L. Senggunprai, A. Prawan, and V. Kukongviriyapan, "Simvastatin and Atorvastatin as Inhibitors of Proliferation and Inducers of Apoptosis in Human Cholangiocarcinoma Cells," *Life Sciences* 153 (2016): 41–49.

21. C. Stormo, M. K. Kringen, R. Lyle, et al., "RNA-Sequencing Analysis of HepG2 Cells Treated With Atorvastatin," *PLoS ONE* 9, no. 8 (2014): e105836.

22. D. Kim, J. M. Paggi, C. Park, C. Bennett, and S. L. Salzberg, "Graph-Based Genome Alignment and Genotyping With HISAT2 and HISAT-Genotype," *Nature Biotechnology* 37 (2019): 907–915.

23. Y. Liao, G. K. Smyth, and W. Shi, "featureCounts: An Efficient General Purpose Program for Assigning Sequence Reads to Genomic Features," *Bioinformatics* 30, no. 7 (2014): 923–930.

24. M. I. Love, W. Huber, and S. Anders, "Moderated Estimation of Fold Change and Dispersion for RNA-seq Data With DESeq2," *Genome Biology* 15, no. 12 (2014): 550.

25. Y. Zhou, B. Zhou, L. Pache, et al., "Metascape Provides a Biologist-Oriented Resource for the Analysis of Systems-Level Datasets," *Nature Communications* 10, no. 1 (2019): 1523.

26. A. P. Davis, T. C. Wiegers, D. Sciaky, et al., "Comparative Toxicogenomics Database's 20th Anniversary: Update 2025," *Nucleic Acids Research* 53, no. D1 (2025): D1328–D1334.

27. Y. Saito and T. S. Chikenji, "Diverse Roles of Cellular Senescence in Skeletal Muscle Inflammation, Regeneration, and Therapeutics," *Frontiers in Pharmacology* 12 (2021): 739510.

28. D. Yu, Z. Cai, D. Li, et al., "Myogenic Differentiation of Stem Cells for Skeletal Muscle Regeneration," *Stem Cells International* 1 (2021): 8884283.

29. M. V. Gugliuzza and C. Crist, "Muscle Stem Cell Adaptations to Cellular and Environmental Stress," *Skeletal Muscle* 12, no. 1 (2022): 5.

30. A. Musso, M. R. Zocchi, and A. Poggi, "Relevance of the Mevalonate Biosynthetic Pathway in the Regulation of Bone Marrow Mesenchymal Stromal Cell-Mediated Effects on T-Cell Proliferation and B-Cell Survival," *Haematologica* 96, no. 1 (2010): 16.

31. M. Delgado-Ramírez, S. Sánchez-Armass, U. Meza, and A. A. Rodríguez-Menchaca, "Regulation of Kv7.2/Kv7.3 Channels by Cholesterol: Relevance of an Optimum Plasma Membrane Cholesterol Content," *Biochimica et Biophysica Acta (BBA) - Biomembranes* 1860, no. 5 (2018): 1242–1251.

32. F. R. Danesh, M. M. Sadeghi, N. Amro, et al., "3-Hydroxy-3-Methylglutaryl CoA Reductase Inhibitors Prevent High Glucose-Induced Proliferation of Mesangial Cells via Modulation of Rho GTPase/p21 Signaling Pathway: Implications for Diabetic Nephropathy," *Proceedings of the National Academy of Sciences* 99, no. 12 (2002): 8301–8305.

33. O. Bjarnadottir, M. Feldt, M. Inasu, et al., "Statin Use, HMGCR Expression, and Breast Cancer Survival—The Malmö Diet and Cancer Study," *Scientific Reports* 10, no. 1 (2020): 558.

34. Y. Zhou, J. Tashiro, S. Kamatani, et al., "HMG-CoA Reductase Degrader, SR-12813, Counteracts Statin-Induced Upregulation of HMG-CoA Reductase and Augments the Anticancer Effect of Atorvastatin," *Biochemical and Biophysical Research Communications* 677 (2023): 13–19.

35. A. Göbel, D. Breining, M. Rauner, L. C. Hofbauer, and T. D. Rachner, "Induction of 3-Hydroxy-3-Methylglutaryl-CoA Reductase Mediates Statin Resistance in Breast Cancer Cells," *Cell Death & Disease* 10, no. 2 (2019): 91.

36. M. Xu, T. Pirtskhalava, J. N. Farr, et al., "Senolytics Improve Physical Function and Increase Lifespan in Old Age," *Nature Medicine* 24, no. 8 (2018): 1246–1256.

37. J. H. Cho, E.-C. Kim, Y. Son, et al., "CD9 Induces Cellular Senescence and Aggravates Atherosclerotic Plaque Formation," *Cell Death and Differentiation* 27, no. 9 (2020): 2681–2696.

38. O. S. Kwon, R. Tanner, K. M. Barrows, et al., "MyD88 Regulates Physical Inactivity-Induced Skeletal Muscle Inflammation, Ceramide Biosynthesis Signaling, and Glucose Intolerance," *American Journal of Physiology - Endocrinology and Metabolism* 309, no. 1 (2015): E11–E21.

39. Y. Wan, L. Piao, S. Xu, et al., "Cathepsin S Activity Controls Chronic Stress-Induced Muscle Atrophy and Dysfunction in Mice," *Cellular and Molecular Life Sciences* 80, no. 9 (2023): 254.

40. M. Agarwal, A. Sharma, P. Kumar, et al., "Myosin Heavy Chain-Embryonic Regulates Skeletal Muscle Differentiation During Mammalian Development," *Development* 147, no. 7 (2020): dev184507.

41. J. Ule, "Ribonucleoprotein Complexes in Neurologic Diseases," *Current Opinion in Neurobiology* 18, no. 5 (2008): 516–523.

42. H.-R. Lu, L.-H. Meng, M. Huang, H. Zhu, Z.-H. Miao, and J. Ding, "DNA Damage, c-myc Suppression and Apoptosis Induced by the Novel Topoisomerase II Inhibitor, Salvicine, in Human Breast Cancer MCF-7 cells," *Cancer Chemotherapy and Pharmacology* 55, no. 3 (2005): 286–294.

43. B. M. Iritani and R. N. Eisenman, "c-Myc Enhances Protein Synthesis and Cell Size During B Lymphocyte Development," *National Academy of Sciences* 96, no. 23 (1999): 13180–13185.

44. S. O. Freytag, "Enforced Expression of the c-myc Oncogene Inhibits Cell Differentiation by Precluding Entry Into a distinct predifferentiation state in G0/G1," *Molecular and Cellular Biology* 8, no. 4 (1988): 1614–1624.

45. C. Etard, O. Armant, U. Roostalu, V. Gourain, M. Ferg, and U. Strähle, "Loss of Function of Myosin Chaperones Triggers Hsf1-Mediated Transcriptional Response in Skeletal Muscle Cells," *Genome Biology* 16, no. 1 (2015): 267.

46. B. Joureau, J. de Winter, S. Conijn, et al., "Dysfunctional Sarcomere Contractility Contributes to Muscle Weakness in ACTA1-Related Nemaline Myopathy (NEM3)," *Annals of Neurology* 83, no. 2 (2018): 269–282.

47. Y. Endo, C. D. Hwang, Y. Zhang, et al., "VEGFA Promotes Skeletal Muscle Regeneration in Aging," *Advanced Biology* 7, no. 10 (2023): 2200320.

48. J. R. Goudriaan, S. M. Espirito Santo, P. J. Voshol, et al., "The VLDL Receptor Plays a Major Role in Chylomicron Metabolism by Enhancing LPL-Mediated Triglyceride Hydrolysis," *Journal of Lipid Research* 45, no. 8 (2004): 1475–1481.

49. A. Buda, S. Forss-Petter, R. Hua, et al., "ABCD1 Transporter Deficiency Results in Altered Cholesterol Homeostasis," *Biomolecules* 13, no. 9 (2023): 1333.

50. A. Riad, C. Zeng, C.-C. Weng, et al., "Sigma-2 Receptor/TMEM97 and PGRMC-1 Increase the Rate of Internalization of LDL by LDL Receptor Through the Formation of a Ternary Complex," *Scientific Reports* 8, no. 1 (2018): 16845.

51. D. Ebrahimi-Fakhari, L. Wahlster, F. Bartz, et al., "Reduction of TMEM97 Increases NPC1 Protein Levels and Restores Cholesterol Trafficking in Niemann-Pick Type C1 Disease Cells," *Human Molecular Genetics* 25, no. 16 (2016): 3588–3599.

52. P. He, I. C. Gelissen, and A. J. Ammit, "Regulation of ATP Binding Cassette Transporter A1 (ABCA1) Expression: Cholesterol-Dependent and -Independent Signaling Pathways With Relevance to Inflammatory Lung Disease," *Respiratory Research* 21, no. 1 (2020): 250.

53. S. N. Oprescu, F. Yue, J. Qiu, L. F. Brito, and S. Kuang, "Temporal Dynamics and Heterogeneity of Cell Populations During Skeletal Muscle Regeneration," *IScience* 23, no. 4 (2020): 100993.

54. B. Glenmark, M. Nilsson, H. Gao, J.-A. Gustafasson, K. Dahlman-Wright, and H. Westerblad, "Difference in Skeletal Muscle Function in Males vs. Females: Role of Estrogen Receptor-β," *American Journal of Physiology - Endocrinology and Metabolism* 287, no. 6 (2004): E1125–E1131.

55. X. Qin, W. Niu, K. Zhao, et al., "Resveratrol Enhances Post-Injury Muscle Regeneration by Regulating Antioxidant and Mitochondrial Biogenesis," *Current Research in Food Science* 10 (2025): 100972.

56. W. Niu, H. Wang, B. Wang, X. Mao, and M. Du, "Resveratrol Improves Muscle Regeneration in Obese Mice Through Enhancing Mitochondrial Biogenesis," *Journal of Nutritional Biochemistry* 98 (2021): 108804.

Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Table S1:** Top 20 upreg/downreg genes (FDR ranked) **Table S2:** Genes from the top 5 enriched GO pathways which are associated with at least 2 GO terms **Table S3:** Prioritised list of nutraceutical hits of downregulated genes involved in at least 2 of the top 5 GO pathways