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Propionate hampers differentiation and modifies histone propionylation and acetylation in skeletal muscle cells

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ABSTRACT

Protein acylation via metabolic acyl-CoA intermediates provides a link between cellular metabolism and protein functionality. A process in which acetyl-CoA and acetylation are fine-tuned is during myogenic differentiation. However, the roles of other protein acylations remain unknown. Protein propionylation could be functionally relevant because propionyl-CoA can be derived from the catabolism of amino acids and fatty acids and was shown to decrease during muscle differentiation. We aimed to explore the potential role of protein propionylation in muscle differentiation, by mimicking a pathophysiological situation with high extracellular propionate which increases propionyl-CoA and protein propionylation, rendering it a model to study increased protein propionylation. Exposure to extracellular propionate, but not acetate, impaired myogenic differentiation in C2C12 cells and propionate exposure impaired myogenic differentiation in primary human muscle cells. Impaired differentiation was accompanied by an increase in histone propionylation as well as histone acetylation. Furthermore, chromatin immunoprecipitation showed increased histone propionylation at specific regulatory myogenic differentiation sites of the Myod gene. Intramuscular propionylcarnitine levels are higher in old compared to young males and females, possibly indicating increased propionyl-CoA levels with age. The findings suggest a role for propionylation and propionyl-CoA in regulation of muscle cell differentiation and ageing, possibly via alterations in histone acylation.

1. Introduction

Post translational modifications of proteins by intermediates of metabolism offers the cell a rapid and integrated mechanism to respond to changes in nutrient availability and adjust consequent cellular decisions. Acyl-CoAs are metabolic intermediates that have an acyl-group that can react with lysine residues on target proteins resulting in protein acylation, thereby regulating protein functionality (Choudhary et al., 2014). For example, acetyl-CoA is a central metabolite in glucose, fatty acid and amino acid metabolism, and levels of this metabolite drive protein acetylation (Pougovkina et al., 2014a). Acetylation of histone proteins serves an important cause as it regulates gene transcription by remodelling of chromatin structure (Sabari et al., 2016). In this way, protein acetylation functions as a metabolic sensor, as it was shown that acetyl-CoA levels are directly linked to acetylation of histone proteins associated with cell growth and proliferation genes (Cai et al., 2011; Wellen et al., 2009).

A process in which acetyl-CoA and acetylation are fine-tuned is during myogenic differentiation. For example, the master regulator of myogenic differentiation, myoblast determination protein 1 (MYOD1 or MYOD), is present in an inactive form in proliferating myoblasts, but following the right cues for differentiation, expression of MYOD is enhanced through acetylation of enhancer regions on the MYOD loci (Hamed et al., 2013). Depleting acetyl-CoA as substrate for acetylation by silencing of the ATP-citrate lyase (ACL) enzyme, resulted in a failed response to upregulate MYOD expression and impaired myogenic

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differentiation, demonstrating the central role of acetylation in this process (Das et al., 2017).

Similar to histone acetylation, histone propionylation may play an important role in differentiation, as propionylation levels have been reported to decrease following monocytic and myogenic differentiation (Liu et al., 2009; Simithy et al., 2017). Propionylation is the covalent binding of a three carbon propionyl-group to lysine residues of proteins and propionyl-CoA, the substrate for propionylation, can be derived from the breakdown of cholesterol, odd-chain fatty acids and the amino acids isoleucine, valine, threonine and methionine (Sbaï et al., 1994). Due to the role of propionyl-CoA in metabolism and because propionylation is likely driven by levels of propionyl-CoA, situations in which propionyl-CoA levels are altered, protein propionylation could be functionally important, for example during protein breakdown (Trefely et al., 2020).

Therefore, the aim of this paper was to explore the potential role of this post-translational modification in muscle differentiation. To study this, we used a pathophysiological metabolic situation similar to patients with an inborn defect in the propionyl-CoA carboxylase gene (PCC). This defect leads to an accumulation of propionyl-CoA and associated metabolites in plasma, urine and other body fluids and tissues, a condition known as propionic acidaemia. Mimicking this pathophysiological situation by exposing the muscle cells to a similar extracellular concentration of propionate (Hommes et al., 1968), increases levels of propionyl-CoA and protein propionylation, rendering it a model to study increased protein propionylation in the cell (Lagerwaard et al., 2020; Pougovkina et al., 2014b). PCC patients, apart from neurological, haematological and hepatic complications, can display myopathic features, including hypotonia and exercise intolerance (Haijes et al., 2019; Saudubray et al., 2016), further suggesting a possible role for propionylation in the skeletal muscle. Therefore, we hypothesised that increased propionylation will compromise normal cellular function. Here, we show that extracellular propionate exposure and consequent increase in propionylation hampers myogenic differentiation in C2C12 myotubes and primary human muscle cells, possibly due to perturbations in histone propionylation as well as acetylation. These findings contribute to the understanding of protein propionylation and its link with propionyl-CoA levels in muscle development and ageing.

2. Materials and methods

2.1. Cell culture

Murine C2C12 myoblasts were routinely cultured at 37 °C with 5% CO_2 in high glucose Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10 % (v/v) fetal bovine serum (FBS), 2 mM glutamine, 25 mM HEPES and 1% (v/v/v) Penicillin/Streptomycin/Amphotericin B. Differentiation was induced by replacing culture medium with high glucose DMEM supplemented with 2% (v/v) horse serum, 2 mM glutamine, 25 mM HEPES and 1% (v/v/v) Penicillin/Streptomycin/Amphotericin B when culture was 90–100 % confluent. Medium was replaced every other day for 5–7 days. Propionate and acetate exposure were induced in culture medium containing 4 mM propionic or acetic acid from a 400 mM pH-balanced stock solution. Medium pH remained within the normal range for culture medium (pH 7.3–7.5)

2.2. SDS –PAGE and Western blotting

Cells were harvested and lysed in TRIS−HCL pH 7.4 with 1% triton X-100 containing protease inhibitors and deacylase inhibitors (1 μM trichostatin A and 20 mM nicotinamide). Lysates were sonicated 5 times 2 s at 40 kHz amplitude on ice. Protein concentrations were determined using Pierce BCA protein assay kit (Thermofisher) and equal protein amounts were loaded on NuPAGE 4–12 % gels (Invitrogen), transferred to nitrocellulose membrane in a transfer tank filled with transfer buffer containing 10 % (v/v) methanol. Membrane was blocked in 3% BSA in PBS with 0.1 % Tween-20 at room temperature and incubated overnight with antibodies in the same buffer at 4 °C. Primary antibodies used: β-actin (#ab8227, 1:5000, Abcam), propionyllysine (#201, 1:1000, PTM biolabs), histone 3 propionyllysine 23 (#613987, 1:2000, Active Motif), histone 3 acetyllysine 23 (#07−355, 1:5000, Millipore), histone 3 acetyllysine 9 (#ab4441, 1:1000, Abcam), histone 4 acetyllysine 8 (#2584, 1:1000, Cell Signalling), murine myosin heavy chain (MF-20, 1:200, DSHB), human myosin heavy chain (ab91506, 1:1000, Abcam), Histone 3 (#ab1791, 1:10000, Abcam). IR-dye based secondary antibodies (LICOR) were used to detect antibody signals using Odyssey scanner (LICOR)

2.3. Immunohistochemistry

C2C12 cells were seeded on glass coverslips. Coverslips were harvested, fixed in 4% formaldehyde and permeabilised with 0.5 % triton. Slips were incubated in blocking buffer Phosphate buffered saline (PBS) with 2%, BSA, 0.02 % HS, 0.1 % Triton X-100, 0.05 % Tween-20, 100 mM glycine), incubated for 1 h at RT in anti-myosin heavy chain (MHC; Developmental Studies Hybridoma Bank MF-20, 1:200). Coverslips were washed with 0,05 % tween-20 in PBS and incubated with secondary anti-body used was Alexa-488 H + L IgG anti-mouse. Nuclear staining was performed by incubating coverslips in a 1 μg/mL 4′,6-diamidino-2-phenylindole (DAPI) staining solution for 5 min. Coverslips were washed in PBS, followed by a PBS:Milli-Q (1:1) wash. Fluormount-G (Southern Biotech) was used to mount coverslips onto glass microscope slides. Representative pictures were taken using a Leica DM6B microscope at 5x magnification

2.4. RNA extraction and semi-quantitative real-time polymerase chain reaction (qPCR)

Cells were washed with 2 mL cold Hanks' Balanced Salt Solution (HBSS) and then directly scraped in 350 μl RLT buffer with 3.5 μl β-mercaptoethanol (β-ME). The cell lysates were homogenized by passing through 21-gauge needle (0.8 mm). RNA was isolated Rneasy Mini Kit (Qiagen, German) according to manufacturer's protocol. The quantity and quality of purified RNA was examined by NanoDrop spectrophotometer (ND-1000). cDNA was synthesized in Eppendorf-Master cycler (5′ 25 °C, 30′ 42 °C, 5′ 85 °C, 10 °C ∞) with ISCRIPT cDNA synthesis kit (Bio-Rad). Measurements of *qPCR* quantified with CFX96 Touch™ Real-Time PCR Detection System (Bio-Rad) and SYBR green master mix (BioRad, USA). The cycling program was set as 95℃ for 30 s, 60℃ for 30 s in 40 cycles. Primers were designed using NCBI primer blast; an overview of primer sequences can be found in Table 1. Normalised expression was calculated according to the ΔΔCq method, by making use of geometric averaging of multiple reference genes using CFX maestro software (Bio-rad). Two housekeeping genes, ribosomal protein S12 (rps12) and ribosomal protein S15 (rps15) were used as reference genes.

2.5. Chromatin immunoprecipitation

C2C12 were seeded in 15 cm dishes, two dishes of cells were combined for each condition in one batch. Cells were harvested at day 0, 1 and 5 after differentiation. ChIP assay was performed according to manufacturer's instructions from SimpleChIP Plus Enzymatic Chromatin IP kit Magnetic Beads (#9005, Cell Signalling). In short, cells were crosslinked by adding 540 μl of 37 % formaldehyde to each dish with 20 mL medium (final concentration 1%) for 10 min at RT with constant agitation. Crosslinking was stopped by adding 2 mL 2.5 M glycine and incubation for 5 min at RT. Cells were washed twice with ice-cold PBS. After that, the cells were scraped in 10 mL ice-cold PBS + 1 tablet EDTA-free Protease Inhibitor Cocktail (Roche, Switzerland) and transferred into 15 mL conical tubes. The cell pellets were collected by centrifuging

Table 1
Primer sequences.

Gene	Accession number	Forward primer sequence	Reverse primer sequence
Myh1	NM_030679.1	TCCCTAAAGGCAGGCTCTCTC	AAGGCTTGTTCTGAGCCTCG
Myf5	NM_008656.5	TGACGGCATGCCTGAATGTA	GCTCGGATGGCTCTGTAGAC
Myod	NM_010866.2	TGCTCTGATGGCATGATGGATT	AGATGCGCTCCACTATGCTG
Myog	NM_031189.2	TCCCAACCCAGGAGATCATTTG	TCAGTTGGGCATGGTTTCGT
Rps12	NM_011295.6	AAGGCATAGCTGCTGGAGGTGTAA	AGTTGGATGCGAGCACACACAGAT
Rps15	NM_009091	CAACGGCAAGACCTTCAACC	TGCTTCACGGGTTTGTAGGT
*Myod*_CER	NC_000073.6	GGGCATTTATGGGTCTTCCT	CTCATGCCTGGTGTTTAGGG
*Myod*_DRR	NC_000073.6	TCAGGACCAGGACCATGTCT	CTGGACCTGTGGCCTCTTAC
*Myod*_PRR	NC_000073.6	GAGTAGACACTGGAGAGGCTTGG	GAAAGCAGTCGTGTCCTGGG
*Myod*_CD1	NC_000073.6	CATCTGACACTGGAGTCGCTTTG	CAAGCAACACTCCTTGTCATCAC
*Myod*_−15 Kb	Non-coding region	TGCCCAGAGCCTAGAATCAT	TCATGCATCCTTGCTGGATA
Rpl30	#7014 Cell signalling	[a]	

[a] Sequence not disclosed.

at 2000 *g* for 5 min at 4℃ and chromatin was isolated and digested by adding 0.5 µl micrococcal nuclease (#10011, Cell Signalling) per sample and incubated for 20 min at 37℃ to digest the chromatin to lengths between 150bp-400bp. Nuclei were pelleted and suspended in ChIP buffer and sonicated 3 × 30 s on ice with 1 min breaks. Chromatin digestion and shearing conditions were optimised and verified in on gel. DNA was purified using SimpleChIP DNA purification and spin columns (#14209, Cell Signalling) according to manufacturer's protocol and was quantified using NanoDrop spectrophotometer (ND-1000). 10 µg chromatin and 2 µl target antibody or 2 µl normal rabbit antibody IgG (# 2729, Cell Signalling Technology, USA) were incubated at 4℃ for 4 h with rotation. Before incubation input samples were collected and stored at -20℃ for later use. 30 µl of protein G magnetic beads (#9006, Cell Signalling) were added to each chromatin-antibody solution and incubated for 2 h at 4℃ with rotation. Antibody-bead-chromatin complexes were washed 3 times with low salt and 1 time with a high salt wash (#9005, Cell Signalling). Chromatin was eluded from antibody-beads for 30 min at 65℃ with agitation in 150 µL elution buffer (#9005, Cell Signalling) and antibody-beads were removed. Immunoprecipitated chromatin and input chromatin were reverse cross-linked by adding 6 µL 5 M NaCl and 2 µL 20 mg/mL Proteinase K and incubated overnight at 65℃. DNA was purified using spin columns and quantified using *qPCR* with SYBR green supermix (Bio-Rad) using *Myod* locus primers (Yang et al., 2011)(Table 1). The results are shown as percentage of total input, or enrichment, which calculated by:

$$Percent\ Input = 2\% \bullet 2^{(C[T]\ 2\%Input\ Sample - C[T]\ IP\ Sample)}$$

where C[T] is the threshold cycle of the *qPCR* reaction.

2.6. Muscle biopsy collection and primary cell culture

Sampling of human material for primary muscle cell culture was part of a study that was approved by the medical ethical committee of Maastricht University (METC azM/UM; NL59895.068.17) and conducted in accordance with the principles of the Declaration of Helsinki (Fortaleza, Brazil 2013) and according to national law (WMO, The Hague, 1998). The study is registered at clinicaltrials.gov under the identifier NCT03666013. Subjects were written and verbally informed on all experimental procedures, including possible risks and discomforts. All subjects provided written informed consent before testing.

A muscle biopsy was taken from the *m. vastus lateralis* under local anesthesia (1.0 % lidocaine without epinephrine) according to the Bergström method modified with suction (Bergström et al., 1967). Human myoblasts were isolated from the vastus lateralis and satellite cells were selected with a CD56 monoclonal antibody (5.1H11; Developmental Studies Hybridoma Bank, Iowa City, USA) using magnetic cell sorting. Satellite cells were grown to 90 % confluency and then initiated to differentiate according to previously published methods (Sparks et al., 2011). Myoblasts were grown in low glucose DMEM containing 0.5 mg/mL bovine serum albumin (BSA), 1 µM dexamethasone, 10 % (v/v) FBS, 1% (v/v) fetuin, 1% (v/v/v) Penicillin/Streptomycin/Amphotericin B, 50 ug/mL gentamycin and 10 ng/mL Human epidermal growth factor and switched to Minimum Essential Medium (α-MEM) differentiation medium upon 90 % confluency containing 2 % (v/v) FBS, 1% (v/v) fetuin and 2 % (v/v) pen/strep for 5 days, changed every other day.

2.7. Muscle carnitine and acylcarnitine measurements

Intramuscular acylcarnitines were determined in *vastus lateralis* biopsies of old and young individuals in a community dwelling population in the northern part of the Netherlands. This study was approved by the Wageningen University Medical Ethical Committee (METC-WUR; 58289.081.16) and was conducted in accordance with the principles of the Declaration of Helsinki (Fortaleza, Brazil, 2013), according to national law (WMO, The Hague, 1998), and is registered in the Dutch Trial Register (NTR6124). Muscle free carnitine and acylcarnitine levels were quantified using liquid chromatography-tandem mass spectrometry as described (Pomar et al., 2019; van der Hoek et al., 2020). We previously reported acylcarnitines in an aggregated manner for young, (fit) old and (pre) frail individuals in relation to gene expression (van der Hoek et al., 2020). Intramuscular acetylcarnitine and propionylcarnitine were not previously analysed individually, which are now reported here for the fit old (79 ± 2.9 years) and young (23 ± 2.0 years) group.

2.8. Data analysis and statistical testing

Statistical analyses were performed using GraphPad Prism v.9 (GraphPad Software, CA, USA). Two-way ANOVA was used to test the main effects of treatment and time points. Post-hoc testing was performed using least significant differences (Fisher's LSD) or Bonferroni test for more than 2 conditions. For muscle carnitine analysis, a two-way ANOVA was performed to test the main effects age and sex. Normality of residuals was checked for Kolmogorov-Smirnov test and equal variances were checked using Spearman's test for heteroscedasticity. Non-normally distributed data were log-transformed. Significance was accepted at $p < 0.05$ and data are presented as mean ± SD.

3. Results

3.1. Exposure to propionate, but not acetate impairs C2C12 myoblast differentiation

To analyse whether propionyl-CoA-derived protein propionylation could play a role in muscle physiology, we first exposed C2C12 myoblasts to 4 mM extracellular propionate, an exposure that is comparable to patients in propionic acidaemia, which was previously shown to induce intracellular protein propionylation (Hommes et al., 1968; Lagerwaard et al., 2020). Indeed, addition of propionate to growth

medium increased levels of protein and histone propionylation in C2C12 cells after 5 days exposure (Fig. 1A). However, protein propionylation levels did not significantly increase globally, yet specific increases in protein propionylation were observed for a protein band at ±50 kDa and for the histone bands at 17 and 14 kDa. This indicated that extracellular propionate exposure is able to induce specific intracellular lysine propionylation.

Next, myoblasts were grown to confluence and differentiation was induced by replacement of culture medium by differentiation medium, either containing 4 mM propionate, 4 mM acetate or control medium (Fig. 1B). An equimolar concentrations acetate was used as a control for, for example, addition of extra substrate and changes in free CoA levels. Moreover, it was previously shown that global histone acetylation decrease during differentiation (Yucel et al., 2019), just as global levels of histone propionylation (Simithy et al., 2017). Immunofluorescence analysis of skeletal myosin heavy chain (MHC), a protein marker exclusively expressed in differentiated myotubes, demonstrated that MHC signals were almost absent in the propionate condition compared to the control and the acetate condition (Fig. 1C), indicating that propionate, but not acetate, inhibited differentiation of myoblasts into myotubes.

We determined the expression of four myogenic regulatory factors using real-time PCR at four different time points after initiation of differentiation, namely day 1, 3, 5 and 7. Myogenic factor 5 (Myf5), a marker for proliferating myoblasts which is expected to decrease following the commitment to differentiation (Tomczak et al., 2004), was decreased upon differentiation in control and acetate conditions, but did not decrease in propionate conditions (Fig. 1D). Myod expression is increased upon differentiation in all conditions but did not further increase in propionate conditions compared to control (trend) and acetate conditions (Fig. 1E). Myod binds to regulatory sites on myogenin, increasing its expression (Deato et al., 2008). Expression of myogenin increased significantly in all conditions following the induction of differentiation, yet the increase in the propionate condition is significantly lower than in the control and the acetate condition (Fig. 1F). Myogenin and Myod work in synergy, inducing transcription of terminal differentiation genes, such as such as Myh1 (Cao et al., 2006). Expression of Myh1 was significantly increased upon differentiation in control and acetate conditions, yet in the propionate condition Myh1 expression was not upregulated (Fig. 1G). Together, these data confirm that exposure to extracellular propionate hampers normal temporal expression of genes responsible for differentiation in a C2C12 muscle model.

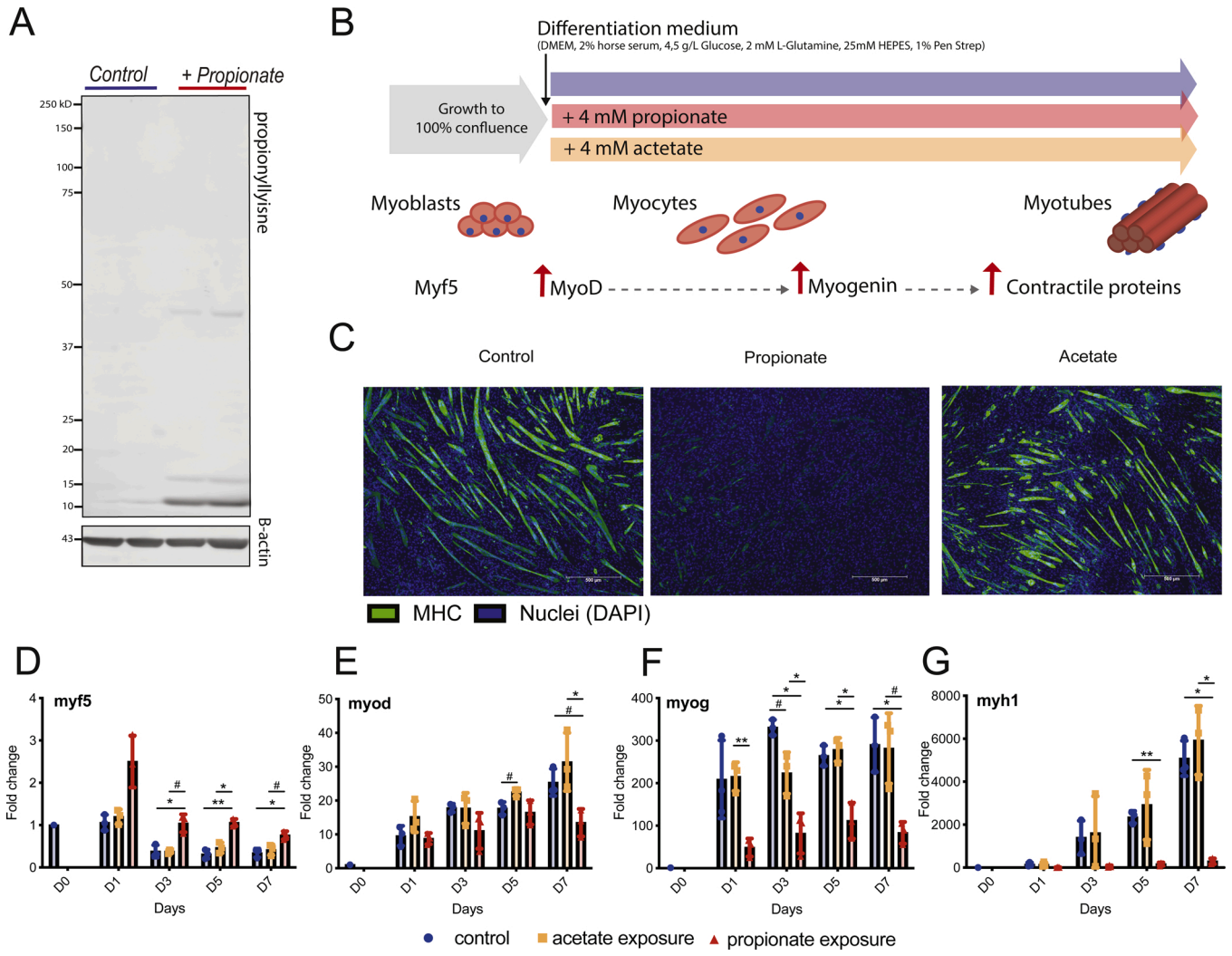

Fig. 1. Exposure to propionate, but not acetate impairs C2C12 myoblast differentiation (A) Western blot analysis of propionyllysine in C2C12 myoblasts exposed to control medium or medium with 4 mM propionate (B) Schematic representation of experimental set up. Myoblasts were grown to confluence and differentiation medium was added with 4 mM propionate or Acetate (C) Immunofluoresence staining after 7 days of differentiation of control, acetate and propionate exposed cells of myosin heavy chain (MHC). Expression of genes involved in myogenic differentiation measured using *q*PCR for Myf5 (D), Myod (E), Myog (F), and Myh1 (G) 3 independent experiments (mean ± SD, # indicates $p < 0.1$ (trend), * indicates $p < 0.05$, ** indicates $p < 0.01$).

3.2. Exposure to propionate causes aberrant acylation patterns on histone proteins

To further elucidate how propionylation impacts C2C12 differentiation, we analysed the effect of propionate exposure on histones protein acylation during differentiation. Since histone propionylation can dynamically interact with histone acetylation, and we have previously shown that histone acetylation increases in rat Fao liver cells exposed to propionate (Lagerwaard et al., 2020). We hypothesised that exposure to propionate would affect acylation levels on histone proteins. To test this, we differentiated C2C12 myoblasts in either control medium or in medium containing 4 mM propionate or 4 mM acetate and determined histone protein acylation using Western blot. In control medium, global histone propionylation and propionylation of histone 3 lysine 23 (H3K23) decreased during myogenic differentiation. On the contrary, in the propionate condition, both global and specific H3K23 propionylation were increased as compared to control (Fig. 2). Next, to validate that propionylation levels were reproducibly different between propionate exposed and control conditions, we performed a new western blotting experiment and included another independent C2C12 differentiation and observed similar differences in total histone propionylation and H3K23 propionylation (Fig S1A) as was shown in Fig. 2. Quantification of the western blot bands demonstrated that the propionylation was higher when normalized to either total histone H3 or B-actin (Fig S1B). Furthermore, protein propionylation in acetate medium was unaltered and followed the same temporal pattern as in control medium (Fig. 2). Exposure to propionate increased global histone acetylation compared to control, while no clear differences in acetylation of H3K23 were observed (Fig. 2). This showed that besides an increase in protein propionylation, levels of protein acetylation were also affected in muscle cells by propionate exposure.

Histone acetyl transferases (HATs) use mainly acetyl-CoA as substrate, but can also use propionyl-CoA as a substrate, albeit to a lesser extent (Kaczmarska et al., 2017; Kebede et al., 2017). Therefore, we explored if our model primarily used enzymatic-driven propionylation or whether the increased propionylation primarily has a non-enzymatic origin. For this, we induced differentiation in C2C12 myoblasts in control medium or medium containing either only propionate, only 10 μM C646, a selective HAT P300 and CBP inhibitor (Bowers et al., 2010), or both propionate and C646. Indeed, exposure to C646 decreased global histone propionylation and acetylation (Fig. 2B). Additionally, C646 decreased overall myogenic differentiation, as was shown by the lower expression of MYH1. Acylation of histones using P300/CBP is thus necessary for differentiation. Exposure to both propionate and C646 increased histone propionylation and acetylation, yet this increase was lower than with propionate alone (Fig. 2B). This implies indeed that P300 and CBP enzymatic activity was, at least partly, used for propionylation of histones, and that the remaining propionylation happens either non-enzymatically or uses other HATs, such as, PCAF (Leemhuis et al., 2008) or MOF (Han et al., 2018).

3.3. Propionate exposure increases propionylation on regulatory promotor regions of MYOD

We showed that exposure to propionate failed to increase expression of the MYOD target myogenin, suggesting that either MYOD transcription or MYOD activation was impaired. Transcription of MYOD is regulated by three regulatory regions that enhance its transcription, namely a core enhancer region (CER) (Goldhamer et al., 1995), a distal regulatory region (DRR) and a proximal regulatory region (PRR) (Chen et al., 2002, 2001; Tapscott et al., 1992). Early differentiation is associated with recruitment of the HAT P300 to these enhancer regions, with subsequent acetylation and consequential increased expression (Hamed et al., 2013). Therefore, we hypothesised that increased propionylation of MYOD regulatory regions could interfere with normal temporal acylation patterns and consequent expression of MYOD, hereby dysregulating differentiation. In order to test if these regions were indeed propionylated, we performed chromatin immunoprecipitation using an antibody against propionylated H3K23 and quantified propionylation in C2C12 myoblasts exposed to either control or propionate differentiation medium on two different time points during differentiation on three MYOD regulatory sites. As controls, we additionally quantified two sites that have not been identified as being regulated for MYOD activation, namely one site 15Kb upstream of the transcription initiation site and one site within the coding region of the *Myod* gene (Fig. 3A).

Propionate exposure significantly increased or had the tendency to increase ($p < 0.1$) propionylation on all sites on both days, expect for the CER after 1 day of differentiation (Fig. 3B). This confirmed that there were indeed increased, albeit low, absolute levels of propionylation of H3K23 on MYOD regulatory sites in the propionate conditions. Still, this increase did not seem to be limited to regulatory sites as it also occurred on the selected non-regulatory MYOD sites. To substantiate this hypothesis, we measured H3K23 propionylation on a gene unrelated to myogenic differentiation, ribosomal protein L30 (*Rpl30*), on day five of differentiation. In three independent experiments, propionylation was on average 7-fold higher (0.023 ± 0.023 vs. 0.16 ± 0.13 % enrichment

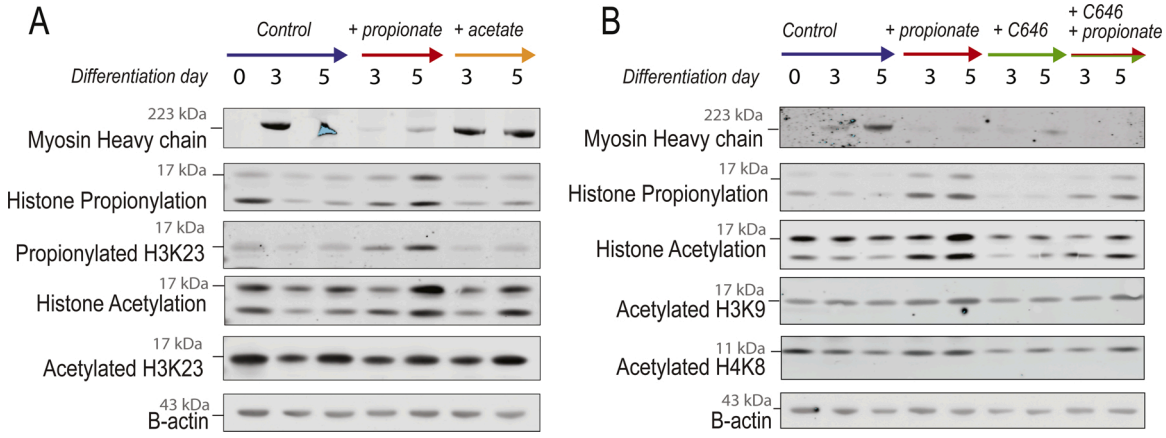

Fig. 2. Exposure to propionate causes aberrant acylation pattern on histone proteins (A) Western blot analysis of myosin heavy chain, histone propionyllysine (pan-propionyllysine antibody), propionylated histone lysine 23 (H3K23), histone acetylation (pan-acetyllysine antibody) and acetylated histone 3 lysine (H3K23) in C2C12 myoblasts exposed to control medium or medium with 4 mM propionate or acetate on 3 and 5 days after initiation of differentiation. (B) Western blot analysis of myosin heavy chain, histone propionyllysine, histone acetyllysine, acetylated histone 3 lysine 9 (H3K9) and acetylated histone 4 lysine 8 (H4K9) in C2C12 myoblasts exposed to control medium or medium with 4 mM propionate with or without P300/CBP inhibitor C646 on 3 and 5 days after initiation of differentiation. The two blots represent two independent experiments.

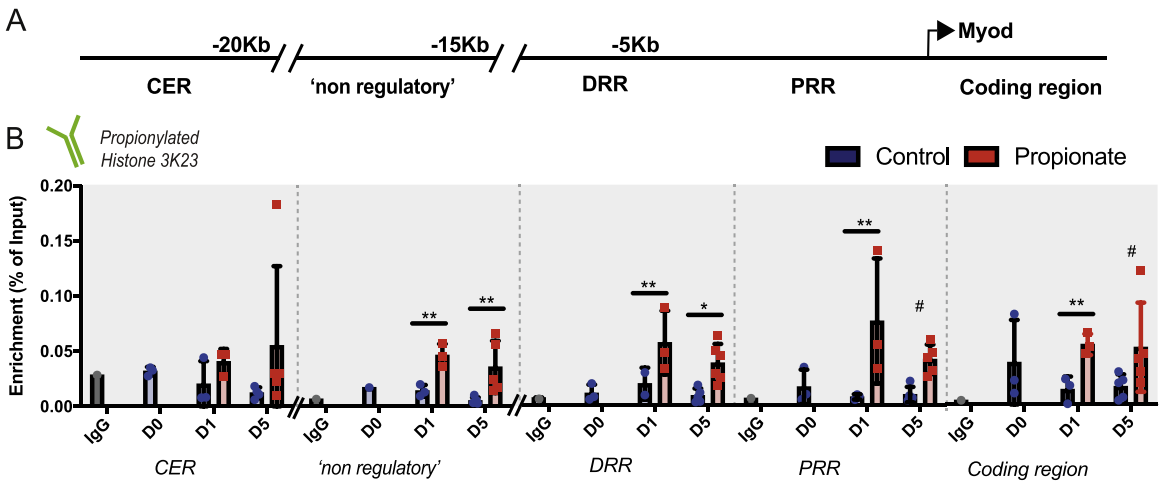

Fig. 3. Propionate exposure increases propionylation and acetylation on regulatory promotor regions of Myod (A) Schematic presentation of regulatory regions on Myod, namely core enhancer region (CER) a distal regulatory region (DRR) and a proximal regulatory region (PRR). (B) Chromatin immunoprecipitation with antibody against propionylated histone 3 lysine 23 (H3K23) and quantified propionylation in C2C12 myoblasts exposed to either control or propionate differentiation medium on day 0 (D0), day 1 (D1) and day 5 (D5) after initiation of differentiation on Myod regulatory sites plus to one site 15Kb upstream and one site on the coding region of the Myod gene (3 independent experiments for D0 and D1, 5 independent experiments for D5) (mean ± SD, * indicates $p < 0.05$, # indicates $p < 0.1$).

of input, $p = 0.134$, data not shown), suggesting that indeed propionylation seems to occur on various loci and is not limited to regulatory regions of *Myod*. Propionate exposure might increase H3K9 acetylation on the regulatory sites, as compared to control, rather than substituting of propionylation for acetylation, propionylation could enhance acetylation on these sites (Figure S2; N = 1).

Exposure to propionate hampers differentiation and affects protein acylation in primary human myocytes

To further validate the observed effect of propionate exposure on myogenic differentiation and acylation in humans, we differentiated primary myocytes isolated from *vastus lateralis* biopsies from five healthy human donors in differentiation medium with and without propionate. Propionate exposure increased protein and histone propionylation in all donor cell lines (Fig. 4), showing that also in human

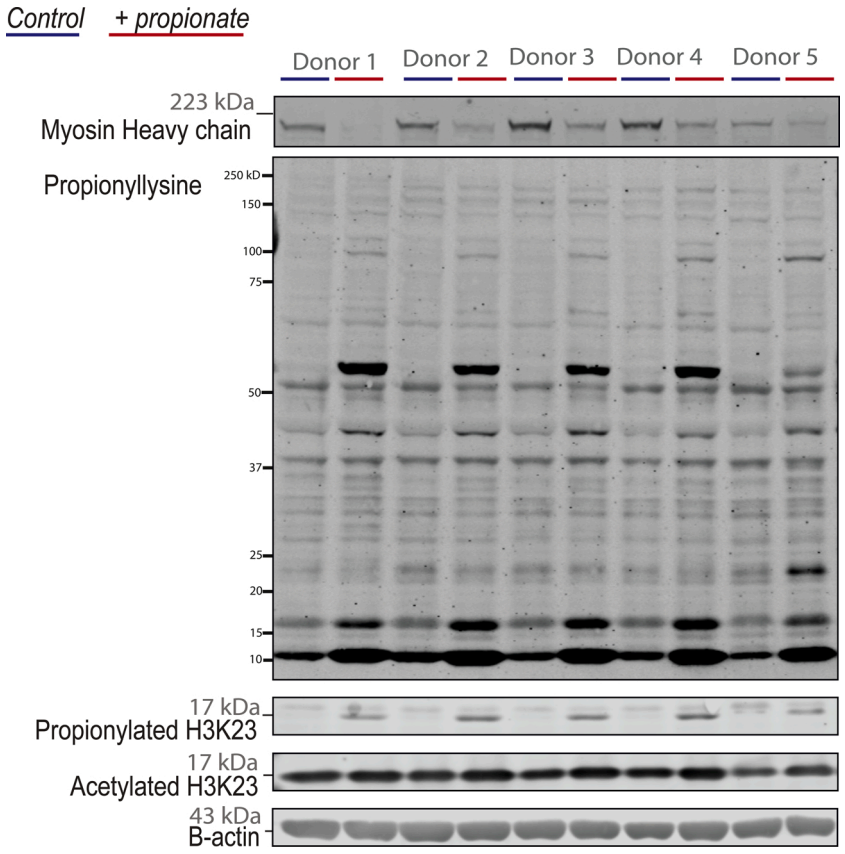

Fig. 4. Exposure to propionate hampers differentiation and affects protein acylation in primary human myocytes Western blot analysis for propionylysine, myosin heavy chain, propionylated histone 3 lysine 23 (H3K23) and acetylated histone 3 lysine 23 (H3K23) primary human myotubes isolated from *vastus lateralis* biopsy of 5 different donors. Cells were differentiated for 5 days on medium with or without 4 mM propionate.

derived myoblasts, propionate was able to induce propionylation. Moreover, as was observed in our C2C12 muscle model, MHC protein expression was absent or markedly lower in all five donors in the propionate compared to the control condition, verifying that exposure to propionate also hampered myogenic differentiation in primary human muscle cells. Furthermore, exposure to propionate during differentiation increased global propionylation as well as propionylation of histones, and specific propionylation of H3K23, and increased acetylation on histones, and specific acetylation of H3K23, compared to differentiation in control medium (Fig. 4). The observation that differentiation is also impaired in human-derived myoblasts upon propionate exposure further advocates for a role of propionate, propionyl-CoA and propionylation in muscle differentiation.

3.4. Increased intramuscular propionylcarnitine in older compared to young individuals

Changes in protein propionylation have been observed in liver of old mice (Baldensperger et al., 2020). Therefore, as a proxy for propionylation, we explored whether metabolic substrates that drive the protein acylation were altered in the ageing human muscle. Since propionylcarnitine levels accurately reflect propionyl-CoA levels (Wikoff et al., 2007), and acylcarnitines can be robustly detected, we studied intramuscular propionylcarnitine in young compared to old individuals. Previously, we identified that intramuscular short-chain acylcarnitines (SCAC; C2-C8:1) were lower in old frail females (van der Hoek et al., 2020). Here, we analyse differences in acetyl- and propionylcarnitine levels between the fit old and young group, between these groups previously no difference in total SCACs was observed (van der Hoek et al., 2020). We show that there was a significant effect of age on intramuscular propionylcarnitine levels (Fig. 5A; $p = < 0.0001$), while there was no significant effect of age on intramuscular acetylcarnitine levels (Fig. 5B; $p = 0.34$), the predominant intramuscular SCAC. The ratio between propionyl- and acetylcarnitine was significantly higher in older individuals (Fig. 5C; $p = 0.0011$), showing that propionylcarnitine levels are relatively increased over acetylcarnitine in older individuals, indicating that intramuscular protein acetylation/propionylation balances could be altered in older individuals.

4. Discussion

The aim of this study was to explore propionate exposure and subsequent alterations in protein and histone protein propionylation in muscle to elucidate the potential role of this post-translational modification in cellular muscle differentiation. We showed that exposure to extracellular propionate, but not acetate, impaired myogenic differentiation in C2C12 myotubes and that exposure to extracellular propionate impaired myogenic differentiation in primary human myotubes isolated from *vastus lateralis* muscle biopsies. The effect of propionate exposure on differentiation was accompanied by an increase in histone propionylation and acetylation, and histone propionylation also specifically increased at regulatory myogenic differentiation sites. Changes in protein propionylation have been observed before during myogenic differentiation and in liver of ageing mice (Simithy et al., 2017). We confirm the changes in protein propionylation during myogenic differentiation and newly suggest that increasing propionylation levels by extracellular exposure to propionate has cellular consequences. Regulation of acylation through metabolism is critical for myogenic differentiation (Bracha et al., 2010; Das et al., 2017; Simithy et al., 2017) and because adult myogenic differentiation from satellite cells is an important mechanism in tissue repair and turnover (Blau et al., 2015), these observations could indicate, besides a role for acetyl-CoA and acetylation, a role for propionylation and propionyl-CoA in muscle physiology.

Additionally, we show increased intramuscular propionylcarnitine in a population of healthy old compared to young males and females, while levels of acetylcarnitines were not significantly different. Propionylcarnitine levels accurately reflect propionyl-CoA levels (Wikoff et al., 2007), and carnitine acyltransferases are known to rapidly establish equilibria between CoA and carnitine derivatives of acyl moieties such as acetate and propionate (Ramsay et al., 2001). Hence, these results indicate increased propionyl-CoA with age. Increased levels of propionyl-CoA have been previously observed in conditions of serum starvation, likely via the catabolism of amino acids, such as isoleucine (Trefely et al., 2020). Therefore, we hypothesise that changes in amino acid metabolism could underly the increase propionylcarnitine in older muscle, but this needs further research. Nevertheless, because levels of propionyl-CoA drive propionylation, increased cellular levels of propionyl-CoA could therefore have unique cellular consequences or signalling roles, possibly via protein and histone propionylation. However, although propionyl-CoA is a distinct metabolite with a distinct metabolic background and despite the observation that only propionate, but not acetate, impaired myogenic differentiation, to date there are no mechanisms on how histone propionylation could be functionally different from histone acetylation. Propionyl-CoA and acetyl-CoA are structurally very similar, likely explaining that propionyl-CoA can use much of the same enzymatic machinery allotted to acetyl-CoA (Han et al., 2018; Kaczmarska et al., 2017) and both acylation neutralise the lysine's positive charge, loosening DNA-histone interactions and facilitate gene transcription (Bannister and Kouzarides, 2011; Gorisch, 2005; Kebede et al., 2017). Additionally, bromodomains, that are known to recognize acetyllysines and transduce the acylation signal by recruitment of transcription factors, also recognize propionyllysine (Flynn et al., 2015), negating a differential mechanistic effects of acetylation and propionylation.

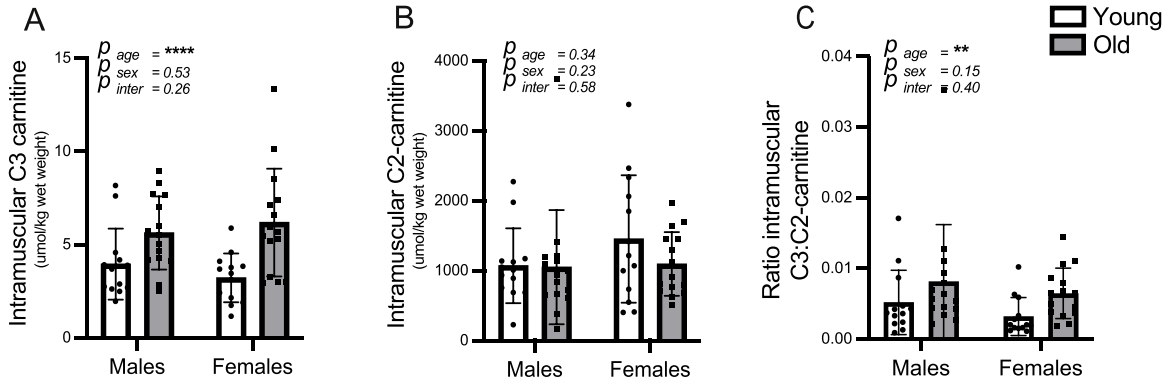

Fig. 5. Increased levels of propionylcarnitine in old compared to young (A) Intramuscular propionylcarnitine (C3) and (B) acetylcarnitine (C2) levels in young (23 ± 2.0 years) and old (79 ± 2.9 years) males and females (C) Ratio intramuscular acetylcarnitine over propionylcarnitine. (mean ± SD, ns indicates not significant, ** indicates $p < 0.01$, ****indicates <0.0001. P_{age} = main age effects, P_{sex} = main sex effects, P_{inter} = main interaction effect (age*sex).

Nevertheless, there are some indications that propionylation and acetylation differ in how they are cellularly localised. For example, the relative abundance of H3K14 and H3K9 propionylation and acetylation was shown to be reasonably similar in basal conditions (Simithy et al., 2017). This is remarkable, as the affinity of HATs for acetyl-CoA is higher than for propionyl-CoA and acetyl-CoA is 8-fold more abundant compared to propionyl-CoA (Han et al., 2018). This suggests that the concentration of propionyl-CoA must be higher in the nucleus, possibly due to propionyl-CoA compartmentalisation. Indeed, it was shown that the acetyl-CoA:propionyl-CoA ratio was approximately 4 in whole cell lysates, while in the nucleus the two acyl-CoAs were found in equimolar concentrations (Trefely et al., 2020). In this way, small changes in cellular propionyl-CoA levels can be amplified due to the compartmentalisation of propionyl-CoA in the nucleus. Hence, the increase in histone propionylation could have impaired differentiation by dysregulation of normal acylation patterns, as myogenic differentiation is characterised by an overall reduction in histone acetylation (Yucel et al., 2019).

The effect of propionate exposure was not limited to an increase in propionylation, as we show that histone acetylation also increases in response to propionate exposure. Interestingly, the increase in acetylation together with propionylation is in contrast with previous research that showed increased propionylation of H3K14 occurred at the expense of acetylation of H3K14 in isolated nuclei following propionyl-CoA exposure (Simithy et al., 2017). One mechanism by which propionate exposure could increase acetylation is via increasing cellular acetyl-CoA levels. Propionyl-CoA is converted into methylmalonyl-CoA, by propionyl-CoA carboxylase, which in turn can be converted into succinyl-CoA, that can used for anaplerosis of the TCA-cycle (Davis et al., 1980; Wongkittichote et al., 2017). Anaplerosis is matched by cataplerosis, for example via cataplerosis of citrate, which can be converted into cytosolic acetyl-CoA via ACL. Although no data exist for skeletal muscle, an intraperitoneal injection with propionate was shown to increase levels of acetylcarnitine in the heart (Wang et al., 2018). These or similar alterations in homeostatic metabolic mechanisms could have interfered with normal control of transcriptional regulation by acetylation and therefore could have contributed to the observed phenotype. Nevertheless, this does not explain why acetate exposure, that also increases cytosolic acetyl-CoA levels (Trefely et al., 2020), did not impair myogenic differentiation. Of course, propionic acid and acetic acid are not identical, as differences exist in, for example density, viscosity, ional potential and PKa, which may result in functional differences propionylation and acetylation. Determination of nuclear concentrations of various Acyl-CoAs in response to acetate, propionate or combined exposures at various concentrations may provide additional insights, especially when related to specific acylation levels. Additionally, another mechanism by which propionyl-CoA could have increased normal cellular acylation levels, is through a direct effect of propionate as a HDAC inhibitor. However, although propionate does possess HDAC activity, it is 10-fold less potent as butyrate in whole cell assays (Waldecker et al., 2008), suggesting that the role of propionate as an HDAC inhibitor might be limited.

The findings that propionate exposure impairs myogenic differentiation in human myoblasts is of particular interest for patients with similar elevated plasma propionate concentrations, such as patients with a defect in the propionyl-CoA carboxylase gene (Hommes et al., 1968). These patients, apart from neurological, haematological and hepatic complications, may display myopathic features, including hypotonia and exercise intolerance (Haijes et al., 2019; Saudubray et al., 2016). Analysis of muscle biopsies from these patients showed a defective mitochondrial respiration, suggesting that mitochondrial dysfunction could contribute to the myopathic pathology (de Keyzer et al., 2009; Schwab et al., 2006). However, exposure to propionate and consequent cellular elevations in propionyl-CoA and propionylation did not induce mitochondrial defects in cultured myotubes or isolated muscle mitochondria, whereas this was induced in cultured liver cells and isolated liver mitochondria (Lagerwaard et al., 2020; Matsuishi et al., 1991). This suggests that in skeletal muscle other mechanisms could contribute to the mitochondrial defects observed in *ex vivo* measurements in muscle biopsies. Therefore, the current data on the effect of propionate exposure on myogenic differentiation could play a role, as differentiated myotubes are more oxidative compared to undifferentiated myoblasts (Remels et al., 2010; Sin et al., 2016), perhaps explaining the mitochondrial defects in patients. Since we show that aberrant acylation might contribute to the differentiation defect, it is of interest to explore strategies that might alleviate the underlying causes. Hence, to decrease the aberrant propionylation we propose to aim for increasing de-propionylation activities. For this, the activation of sirtuin 1 could be of interest, as this enzyme has been shown to de-propionylate proteins in the nucleus, at least *in vitro* (Cheng et al., 2009).

In conclusion, we showed that exposure to extracellular propionate, but not acetate, and the associated increase in propionylation impaired myogenic differentiation. Although the role of propionylation and the mechanism by which increased propionylation hampers myogenic differentiation remains to be elucidated. We show that alterations in histone propionylation, also at regulatory myogenic differentiation sites on de myod gene, might play a role. Additionally, we show that intramuscular propionylcarnitine levels are higher in old compared to young individuals, which suggests higher levels of propionyl-CoA and possibly higher levels of propionylation with ageing. Therefore, these findings provide additional evidence for a role of propionylation and propionyl-CoA in the regulation of muscle differentiation and ageing, which requires further investigation.

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Declaration of Competing Interest

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.mad.2021.111495.

References

Baldensperger, T., Eggen, M., Kappen, J., Winterhalter, P.R., Pfirrmann, T., Glomb, M.A., 2020. Comprehensive analysis of posttranslational protein modifications in aging of subcellular compartments. Sci. Rep. 10, 7596. https://doi.org/10.1038/s41598-020-64265-0.

Bannister, A.J., Kouzarides, T., 2011. Regulation of chromatin by histone modifications. Cell Res. 21, 381–395. https://doi.org/10.1038/cr.2011.22.

Bergström, J., Hermansen, L., Hultman, E., Saltin, B., 1967. Diet, muscle glycogen and physical performance. Acta Physiol. Scand. 71, 140–150. https://doi.org/10.1111/j.1748-1716.1967.tb03720.x.

Blau, H.M., Cosgrove, B.D., Ho, A.T.V., 2015. The central role of muscle stem cells in regenerative failure with aging. Nat. Med. 21, 854–862. https://doi.org/10.1038/nm.3918.

Bowers, E.M., Yan, G., Mukherjee, C., Orry, A., Wang, L., Holbert, M.A., Crump, N.T., Hazzalin, C.A., Liszczak, G., Yuan, H., Larocca, C., Saldanha, S.A., Abagyan, R., Sun, Y., Meyers, D.J., Marmorstein, R., Mahadevan, L.C., Alani, R.M., Cole, P.A., 2010. Virtual ligand screening of the p300/CBP histone acetyltransferase: identification of a selective small molecule inhibitor. Chem. Biol. 17, 471–482. https://doi.org/10.1016/j.chembiol.2010.03.006.

Bracha, A.L., Ramanathan, A., Huang, S., Ingber, D.E., Schreiber, S.L., 2010. Carbon metabolism–mediated myogenic differentiation. Nat. Chem. Biol. 6, 202–204. https://doi.org/10.1038/nchembio.301.

Cai, L., Sutter, B.M., Li, B., Tu, B.P., 2011. Acetyl-CoA induces cell growth and proliferation by promoting the acetylation of histones at growth genes. Mol. Cell 42, 426–437. https://doi.org/10.1016/j.molcel.2011.05.004.

Cao, Y., Kumar, R.M., Penn, B.H., Berkes, C.A., Kooperberg, C., Boyer, L.A., Young, R.A., Tapscott, S.J., 2006. Global and gene-specific analyses show distinct roles for Myod and Myog at a common set of promoters. EMBO J. 25, 502–511. https://doi.org/10.1038/sj.emboj.7600958.

Chen, J.C.J., Love, C.M., Goldhamer, D.J., 2001. Two upstream enhancers collaborate to regulate the spatial patterning and timing ofMyoD transcription during mouse development. Dev. Dyn. 221, 274–288. https://doi.org/10.1002/dvdy.1138.

Chen, J.C.J., Ramachandran, R., Goldhamer, D.J., 2002. Essential and redundant functions of the MyoD distal regulatory region revealed by targeted mutagenesis. Dev. Biol. 245, 213–223. https://doi.org/10.1006/dbio.2002.0638.

Cheng, Z., Tang, Y., Chen, Y., Kim, S., Liu, H., Li, S.S.C., Gu, W., Zhao, Y., 2009. Molecular characterization of propionyllysines in non-histone proteins. Mol. Cell Proteomics 8, 45–52. https://doi.org/10.1074/mcp.M800224-MCP200.

Choudhary, C., Weinert, B.T., Nishida, Y., Verdin, E., Mann, M., 2014. The growing landscape of lysine acetylation links metabolism and cell signalling. Nat. Rev. Mol. Cell Biol. 15, 536–550. https://doi.org/10.1038/nrm3841.

Das, S., Morvan, F., Morozzi, G., Jourde, B., Minetti, G.C., Kahle, P., Rivet, H., Brebbia, P., Toussaint, G., Glass, D.J., Fornaro, M., 2017. ATP Citrate Lyase Regulates Myofiber Differentiation and Increases Regeneration by Altering Histone Acetylation. Cell Rep. 21, 3003–3011. https://doi.org/10.1016/j.celrep.2017.11.038.

Davis, E.J., Spydevold, O., Bremer, J., 1980. Pyruvate carboxylase and Propionyl-CoA carboxylase as anaplerotic enzymes in skeletal muscle mitochondria. Eur. J. Biochem. 110, 255–262. https://doi.org/10.1111/j.1432-1033.1980.tb04863.x.

de Keyzer, Y., Valayannopoulos, V., Benoist, J.-F., Batteux, F., Lacaille, F., Hubert, L., Chrétien, D., Chadefeaux-Vekemans, B., Niaudet, P., Touati, G., Munnich, A., de Lonlay, P., 2009. Multiple OXPHOS deficiency in the liver, kidney, heart, and skeletal muscle of patients with methylmalonic aciduria and propionic aciduria. Pediatr. Res. 66, 91–95. https://doi.org/10.1203/PDR.0b013e3181a7c270.

Deato, M.D.E., Marr, M.T., Sottero, T., Inouye, C., Hu, P., Tjian, R., 2008. MyoD targets TAF3/TRF3 to activate myogenin transcription. Mol. Cell 32, 96–105. https://doi.org/10.1016/j.molcel.2008.09.009.

Flynn, E.M.M., Huang, O.W.W., Poy, F., Oppikofer, M., Bellon, S.F.F., Tang, Y., Cochran, A.G.G., 2015. A subset of human bromodomains recognizes butyryllysine and crotonyllysine histone peptide modifications. Structure 23, 1801–1814. https://doi.org/10.1016/j.str.2015.08.004.

Goldhamer, D.J., Brunk, B.P., Faerman, A., King, A., Shani, M., Emerson, C.P., 1995. Embryonic activation of the myoD gene is regulated by a highly conserved distal control element. Development 121, 637–649.

Gorisch, S.M., 2005. Histone acetylation increases chromatin accessibility. J. Cell. Sci. 118, 5825–5834. https://doi.org/10.1242/jcs.02689.

Haijes, H.A., Jans, J.J.M., Tas, S.Y., Verhoeven-Duif, N.M., Hasselt, P.M., 2019. Pathophysiology of propionic and methylmalonic acidemias. Part 1: complications. J. Inherit. Metab. Dis. 42, 730–744. https://doi.org/10.1002/jimd.12129.

Hamed, M., Khilji, S., Chen, J., Li, Q., 2013. Stepwise acetyltransferase association and histone acetylation at the Myod1 locus during myogenic differentiation. Sci. Rep. 3, 2390. https://doi.org/10.1038/srep02390.

Han, Z., Wu, H., Kim, S., Yang, X., Li, Q., Huang, H., Cai, H., Bartlett, M.G., Dong, A., Zeng, H., Brown, P.J., Yang, X.J., Arrowsmith, C.H., Zhao, Y., Zheng, Y.G., 2018. Revealing the protein propionylation activity of the histone acetyltransferase MOF (males absent on the first). J. Biol. Chem. 293, 3410–3420. https://doi.org/10.1074/jbc.RA117.000529.

Hommes, F.A., Kuipers, J.R.G., Elema, J.D., Jansen, J.F., Jonxis, J.H.P., 1968. Acidosis propionic acid hereditary disease propionyl coenzyme liver, fatty A carboxylase metabolic disease Propionicacidemia, a New Inborn Error of Metabolism. Pediat. Res.

Kaczmarska, Z., Ortega, E., Goudarzi, A., Huang, H., Kim, S., Márquez, J.A., Zhao, Y., Khochbin, S., Panne, D., 2017. Structure of p300 in complex with acyl-CoA variants. Nat. Chem. Biol. 13, 21–29. https://doi.org/10.1038/nchembio.2217.

Kebede, A.F., Nieborak, A., Shahidian, L.Z., Le Gras, S., Richter, F., Gómez, D.A., Baltissen, M.P., Meszaros, G., Magliarelli, H., de, F., Taudt, A., Margueron, R., Colomé-Tatché, M., Ricci, R., Daujat, S., Vermeulen, M., Mittler, G., Schneider, R., 2017. Histone propionylation is a mark of active chromatin. Nat. Struct. Mol. Biol. 24, 1048–1056. https://doi.org/10.1038/nsmb.3490.

Lagerwaard, B., Pougovkina, O., Bekebrede, A.F., Brinke, H., Wanders, R.J.A., Nieuwenhuizen, A.G., Keijer, J., Boer, V.C.J., 2020. Increased protein propionylation contributes to mitochondrial dysfunction in liver cells and fibroblasts, but not in myotubes. J. Inherit. Metab. Dis. jimd. 12296. https://doi.org/10.1002/jimd.12296.

Leemhuis, H., Packman, L.C., Nightingale, K.P., Hollfelder, F., 2008. The human histone acetyltransferase P/CAF is a promiscuous histone propionyltransferase. Chem. Bio. Chem. 9, 499–503. https://doi.org/10.1002/cbic.200700556.

Liu, B., Lin, Y., Darwanto, A., Song, X., Xu, G., Zhang, K., 2009. Identification and characterization of propionylation at histone H3 lysine 23 in mammalian cells. J. Biol. Chem. 284, 32288–32295. https://doi.org/10.1074/jbc.M109.045856.

Matsuishi, T., Stumpf, D.A., Seliem, M., Eguren, L.A., Chrislip, K., 1991. Propionate mitochondrial toxicity in liver and skeletal muscle: acyl CoA levels. Biochem. Med. Metab. Biol. 45, 244–253.

Pomar, C.A., Kuda, O., Kopecky, J., Rombaldova, M., Castro, H., Picó, C., Sánchez, J., Palou, A., 2019. Alterations in plasma acylcarnitine and amino acid profiles may indicate poor nutrition during the suckling period due to maternal intake of an unbalanced diet and may predict later metabolic dysfunction. FASEB J. 33, 796–807. https://doi.org/10.1096/fj.201800327RR.

Pougovkina, O., te Brinke, H., Ofman, R., van Cruchten, A.G., Kulik, W., Wanders, R.J.A., Houten, S.M., de Boer, V.C.J., 2014a. Mitochondrial protein acetylation is driven by acetyl-CoA from fatty acid oxidation. Hum. Mol. Genet. 23, 3513–3522. https://doi.org/10.1093/hmg/ddu059.

Pougovkina, O., te Brinke, H., Wanders, R.J.A., Houten, S.M., de Boer, V.C.J., 2014b. Aberrant protein acylation is a common observation in inborn errors of acyl-CoA metabolism. J. Inherit. Metab. Dis. 37, 709–714. https://doi.org/10.1007/s10545-014-9684-9.

Ramsay, R.R., Gandour, R.D., van der Leij, F.R., 2001. Molecular enzymology of carnitine transfer and transport. Biochim. Biophys. Acta - Protein Struct. Mol. Enzymol. 1546, 21–43. https://doi.org/10.1016/S0167-4838(01)00147-9.

Remels, A.H.V., Langen, R.C.J., Schrauwen, P., Schaart, G., Schols, A.M.W.J., Gosker, H. R., 2010. Regulation of mitochondrial biogenesis during myogenesis. Mol. Cell. Endocrinol. 315, 113–120. https://doi.org/10.1016/j.mce.2009.09.029.

Sabari, B.R., Zhang, D., Allis, C.D., Zhao, Y., 2016. Metabolic regulation of gene expression through histone acylations. Nat. Rev. Mol. Cell Biol. 18, 90–101. https://doi.org/10.1038/nrm.2016.140.

Saudubray, J.-M., Baumgartner, M.R., Walter, J., 2016. In: Saudubray, J.-M., Baumgartner, M.R., Walter, J. (Eds.), Inborn Metabolic Diseases. Springer Berlin Heidelberg, Berlin, Heidelberg, p. 658. https://doi.org/10.1007/978-3-662-49771-5.

Sbaï, D., Narcy, C., Thompson, G.N., Mariotti, A., Poggi, F., Saudubray, J.M., Bresson, J. L., 1994. Contribution of odd-chain fatty acid oxidation to propionate production in disorders of propionate metabolism. Am. J. Clin. Nutr. 59, 1332–1337. https://doi.org/10.1093/ajcn/59.6.1332.

Schwab, M.A., Sauer, S.W., Okun, J.G., Nijtmans, L.G.J., Rodenburg, R.J.T., van den Heuvel, L.P., Dröse, S., Brandt, U., Hoffmann, G.F., Ter Laak, H., Kölker, S., Smeitink, J.A.M., 2006. Secondary mitochondrial dysfunction in propionic aciduria: a pathogenic role for endogenous mitochondrial toxins. Biochem. J. 398, 107–112. https://doi.org/10.1042/BJ20060221.

Simithy, J., Sidoli, S., Yuan, Z.-F., Coradin, M., Bhanu, N.V., Marchione, D.M., Klein, B.J., Bazilevsky, G.A., McCullough, C.E., Magin, R.S., Kutateladze, T.G., Snyder, N.W., Marmorstein, R., Garcia, B.A., 2017. Characterization of histone acylations links chromatin modifications with metabolism. Nat. Commun. 8, 1141. https://doi.org/10.1038/s41467-017-01384-9.

Sin, J., Andres, A.M., Taylor, D.J.R., Weston, T., Hiraumi, Y., Stotland, A., Kim, B.J., Huang, C., Doran, K.S., Gottlieb, R.A., 2016. Mitophagy is required for mitochondrial biogenesis and myogenic differentiation of C2C12 myoblasts. Autophagy 12, 369–380. https://doi.org/10.1080/15548627.2015.1115172.

Sparks, L.M., Moro, C., Ukropcova, B., Bajpeyi, S., Civitarese, A.E., Hulver, M.W., Thoresen, G.H., Rustan, A.C., Smith, S.R., 2011. Remodeling lipid metabolism and improving insulin responsiveness in human primary myotubes. PLoS One 6, e21068. https://doi.org/10.1371/journal.pone.0021068.

Tapscott, S.J., Lassar, A.B., Weintraub, H., 1992. A novel myoblast enhancer element mediates MyoD transcription. Mol. Cell. Biol. 12, 4994–5003. https://doi.org/10.1128/MCB.12.11.4994.

Tomczak, K.K., Marinescu, V.D., Ramoni, M.F., Sanoudou, D., Montanaro, F., Han, M., Kunkel, L.M., Kohane, I.S., Beggs, A.H., 2004. Expression profiling and identification of novel genes involved in myogenic differentiation. FASEB J. 18, 1–23. https://doi.org/10.1096/fj.03-0568fje.

Trefely, S., Huber, K., Liu, J., Singh, J., Doan, M., Lovell, C.D., Noji, M., Krusenstiern, Evon, Jiang, H., Bostwick, A., Izzo, L., Zhao, S., Bedi, K.C., Rame, J.E., Bogner-Strauss, J.G., Mesaros, C., Wellen, K.E., Snyder, N.W., 2020. Quantitative Sub-cellular acyl-CoA Analysis Reveals Distinct Nuclear Regulation. bioRxiv. https://doi.org/10.1101/2020.07.30.229468.

van der Hoek, M.D., Nieuwenhuizen, A.G., Kuda, O., Bos, P., Paluchová, V., Verschuren, L., van den Hoek, A.M., Kleemann, R., Veeger, N.J.G.M., van der Leij, F. R., Keijer, J., 2020. Intramuscular short-chain acylcarnitines in elderly people are decreased in (pre-)frail females, but not in males. FASEB J. 34, 11658–11671. https://doi.org/10.1096/fj.202000493R.

Waldecker, M., Kautenburger, T., Daumann, H., Busch, C., Schrenk, D., 2008. Inhibition of histone-deacetylase activity by short-chain fatty acids and some polyphenol metabolites formed in the colon. J. Nutr. Biochem. 19, 587–593. https://doi.org/10.1016/j.jnutbio.2007.08.002.

Wang, Y., Christopher, B.A., Wilson, K.A., Muoio, D., McGarrah, R.W., Brunengraber, H., Zhang, G.-F., 2018. Propionate-induced changes in cardiac metabolism, notably CoA trapping, are not altered by L-carnitine. Am. J. Physiol. Metab. 315, E622–E633. https://doi.org/10.1152/ajpendo.00081.2018.

Wellen, K.E., Hatzivassiliou, G., Sachdeva, U.M., Bui, T.V., Cross, J.R., Thompson, C.B., 2009. ATP-citrate lyase links cellular metabolism to histone acetylation. Science (80-.) 324, 1076–1080. https://doi.org/10.1126/science.1164097.

Wikoff, W.R., Gangoiti, J.A., Barshop, B.A., Siuzdak, G., 2007. Metabolomics identifies perturbations in human disorders of propionate metabolism. Clin. Chem. 53, 2169–2176. https://doi.org/10.1373/clinchem.2007.089011.

Wongkittichote, P., Ah Mew, N., Chapman, K.A., 2017. Propionyl-CoA carboxylase – a review. Mol. Genet. Metab. 122, 145–152. https://doi.org/10.1016/j.ymgme.2017.10.002.

Yang, J.-H., Song, Y., Seol, J.-H., Park, J.Y., Yang, Y.-J., Han, J.-W., Youn, H.-D., Cho, E.-J., 2011. Myogenic transcriptional activation of MyoD mediated by replication-independent histone deposition. Proc. Natl. Acad. Sci. 108, 85–90. https://doi.org/10.1073/pnas.1009830108.

Yucel, N., Wang, Y.X., Mai, T., Porpiglia, E., Lund, P.J., Markov, G., Garcia, B.A., Bendall, S.C., Angelo, M., Blau, H.M., 2019. Glucose metabolism drives histone acetylation landscape transitions that dictate muscle stem cell function. Cell Rep. 27, 3939–3955. https://doi.org/10.1016/j.celrep.2019.05.092 e6.