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Ameliorative Effects of dbcAMP and des (1-3) IGF-1 on Myogenic and Adipogenic mRNA Expression in Transdifferentiated C2C12 Myogenic Cells

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ABSTRACT

The metabolic modifiers, including beta agonist (BA) and growth hormone (GH), were extensively used in animal production as they increase protein synthesis and reduce lipid synthesis in animals, particularly in muscles. However, studies on their effects at the molecular level are limited. This study examined the effects of dibutyryl cyclic adenosine monophosphate (dbcAMP), a mimic of BA, and des (1-3) insulin-like growth factor-1 (des (1-3) IGF-1), an analogue for GH, on myogenic and adipogenic mRNA expression in transdifferentiated-myogenic C2C12 cell lines. Following treatment, lipid droplet formation was observed alongside the expression of myogenic and adipogenic mRNAs, indicating the coexistence of myotubes and adipocytes in the culture and reflecting a model resembling intramuscular fat deposition in vivo. Lipid droplet accumulation was significantly reduced ($P < 0.001$) by dbcAMP and des(1-3) IGF-1 treatments. Moreover, dbcAMP exerted a significant ($P < 0.05$) stimulatory effect on myogenesis-associated genes, while downregulating lipogenic and adipogenic mRNA expression ($P < 0.05$). Meanwhile, des (1-3) IGF-1 appeared to promote adipogenic differentiation, with no effect on lipogenic and lipolytic gene expression, suggesting different pathways of dbcAMP and IGF-1 induction on myogenesis and adipogenesis in vitro.

Keywords: Adipogenic, C2C12, dbcAMP, des (1-3) IGF-1, lipogenic, lipolytic, myogenic

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INTRODUCTION

Metabolic modifiers, including beta-agonist (BA) and growth hormone (GH), were utilised for feed efficiency enhancement

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via improving animal growth performance and increasing lean tissue content, and thereby increasing profitability (Dunshea et al., 2016; Dikeman, 2007). Generally, their mode of action involves promoting protein accretion while reducing fat deposition (Chikani & Ho, 2013; Hostrup & Onslev, 2022; Pfau et al., 2024; 2023). These metabolic modifiers are often described as repartitioning agents, as they redirect nutrient utilisation away from adipose stores and the gastrointestinal tract towards skeletal muscle for hypertrophy and mammary glands for milk production (Brown et al., 2018; Bao et al., 2016; Scascia et al., 2015; Hemmings et al., 2015). Regarding lipid metabolism, BA are known to stimulate lipolysis while suppressing lipogenesis (Pfau et al., 2023; de Almeida et al., 2012; Dunshea et al., 2005; Mersmann, 2002). GH exerts similar observations, as it also reduced fat accretion (Dunshea et al., 2016). Thus, this may explain their role in modifying the composition of fat and subsequent impacts on meat tenderness and palatability (Dikeman, 2007; Ladeira et al., 2016; Warriss et al., 1990). As fat content in meat is considered unhealthy, the use of metabolic modifiers could benefit consumers by producing healthier products (Gebre et al., 2012). However, the improvement in lean meat production by these metabolic modifiers has been shown to negatively affect meat quality, likely through reduced intramuscular fat (IMF) (Dunshea et al., 2005).

Although the metabolic modifiers have been demonstrated to enhance performance and carcass characteristics, most in vitro studies have primarily focused on myogenesis, with far less attention given to how these agents modulate lipid metabolic processes at the molecular level. In muscle, BA appear to mediate activation of beta-adrenergic receptors and subsequently stimulate protein kinase A (PKA) activity (Johnson et al., 2014; Milioto et al., 2017). Meanwhile, the GH actions are exerted through insulin-like growth factor-1 (IGF-1), as indicated by the elevated levels of IGF-1 in the plasma after GH stimulation (Gahete et al., 2013; Yamamoto & Bando, 2023). Thus, the current study aimed to ascertain how BA and GH influence lipid-related processes, including adipogenesis, lipogenesis, and lipolysis, alongside their effects on myogenesis in cultured muscle cells.

The cyclic AMP analogue, dibutyryl cyclic adenosine monophosphate (dbcAMP), which mimics the effect of BA, and the analogue for IGF-1, des (1-3) IGF-1, which mimics the effect of GH, were used and their effects on several mRNA expression related to myogenesis, lipogenesis, lipolysis and adipogenesis were assessed on the transdifferentiated myogenic cells, that acquired 'adipocyte-like' characteristics in this study. The C2C12 cells were used as a skeletal muscle model and induced to undergo transdifferentiation in adipogenic medium, leading to the formation of adipocyte-like cells containing lipid droplets, representing an in vitro model of intramuscular fat, before the treatments with the agents. The response of these agents towards the expression of the key metabolic genes was assessed to explore the possible mechanisms underlying lipid metabolism in muscle tissue. We hypothesised that C2C12 incubated in adipogenic media would respond to dbcAMP

or des (1-3) IGF-1 by altering the transcription of myogenic, adipogenic, lipogenic, and lipolytic genes, which are associated with reduced fat deposition.

MATERIALS AND METHODS

C2C12 Culture Conditions and Treatment with dbcAMP and des (1-3) IGF-1

The mouse-derived C2C12 myoblast cell lines were grown in Dulbecco's modified Eagle's growth medium (DMEM) (Sigma-Aldrich, UK), and supplemented with 10% (v/v) heat-inactivated fetal bovine serum (hi-FBS) (Gibco, UK) and 1% (v/v) penicillin and streptomycin (P/S) (Sigma-Aldrich, UK), under maintenance at 5% CO_2 at 37°C. Once the cells reached approximately 70-80% confluence, the culture medium was replaced with adipogenic induction medium, consisting of DMEM with 10% (v/v) hi-FBS, 1% (v/v) P/S, 0.5 mM isobutylmethylxanthine (IBMX), 1 µM dexamethasone (DEX), 850 nM insulin, 10 nM triiodothyronine (T3) and 1 µM rosiglitazone (peroxisome proliferator activated receptor γ (PPARγ) agonist). This stage was designated as day 0 of differentiation. After two days, the induction medium was replaced with adipogenic differentiation medium, containing DMEM with 10% (v/v) hi-FBS, antibiotics, 850 nM insulin, 10 nM T3, and 1 mM rosiglitazone. The medium changes were performed every 48 hours for nutrient replenishment until the experiment was concluded. On day 6 of differentiation, the cells were exposed to either 100 ng/ml des (1-3) IGF-1 (DES) (Autogen Bioclea, Bioscience UK Ltd) or 1 mM dibutyryl cyclic adenosine monophosphate (dbcAMP) (DB) (Sigma-Aldrich, UK) that were added to the adipogenic differentiation media, while the control was unsupplemented. This point was marked as day 0 of treatment. The cells were scraped on days 1, 2, and 3 for the RNA extraction, whilst the lipid droplet quantification was performed on day 3 of treatment.

Lipid Droplets Quantification

Lipid droplet accumulation was assessed in C2C12 cells using Oil Red O staining and haematoxylin counterstaining, as described by Ryan et al. (2013). After staining, the cells were visualised and imaged using an Olympus SZH10 microscope (Olympus, Southend-on-Sea, UK). For each treatment replicate, ten random field-of-view images were taken and processed using ImageJ Software (version 1.50I, National Institutes of Health, USA). using the application of colour threshold on the stained regions, and the percentage of stained area for each image was quantified.

RNA Extraction, cDNA Synthesis, and Quantitative Real-Time PCR

The total RNA was extracted from cultured C2C12 cells using the High Pure RNA Isolation Kit (Roche, UK) according to the manufacturer’s guidelines. Subsequently, 500 ng RNA

was used to synthesise the complementary DNA (cDNA) using RevertAid First Strand cDNA Synthesis Kit (Thermo Scientific, UK) in accordance with the manufacturer's protocols. Quantitative real-time PCR (qPCR) was carried out using the SYBR-green method as outlined by Brown et al. (2012). Transcript abundances were quantified against a standard curve and normalised against a total cDNA content using the OliGreen® reagent (Life Technologies, Oregon, USA) as described by Rhinn et al. (2008) and established by Brown et al. (2012). The primer sequences were designed using the mouse reference genome (GRCm38), and their specificity was confirmed by melting curve analysis. The sequences are displayed in Table 1.

Analysis of Data

The data was analysed using Genstat for Windows 17th Edition (VSN International Ltd., UK). A one-way ANOVA was applied for lipid droplets quantification, and a two-way ANOVA for the effects of the duration of treatment and effects of the treatments (dbcAMP, des (1-3) IGF-1 or control) on the mRNA expressions. Where applicable, Bonferroni's post hoc was conducted. All data are displayed as mean ± standard error of mean (SEM), with $P < 0.05$ being considered significant.

RESULTS

Lipid Droplets Quantification

The exposure of C2C12 cells to the adipogenic conditions (incubation with adipogenic induction medium and adipogenic differentiation medium) appeared to induce adipogenic differentiation. During the treatment period, the cells were incubated in the adipogenic differentiation medium, supplemented with treatments: dbcAMP, and des (1-3) IGF-1, with an unsupplemented control. By the end of the treatment, noticeable lipid droplets were observed in all groups (Figure 1). However, the lipid droplet accumulation was significantly ($P < 0.001$) reduced in the dbcAMP- and des (1-3) IGF-1-treated cells, with higher accumulation in the control (Figure 2), suggesting that dbcAMP and des (1-3) IGF-1 significantly reduced lipid deposition in this model.

The Effects of dbcAMP and des (1-3) IGF-1 on the mRNA Expression Related to Myogenic Markers

Previously, it has been demonstrated that the C2C12 incubated in adipogenic conditions resulted in the formation of lipid droplets, while maintaining their capacity to differentiate into myotubes (Ryan et al., 2013). Likewise, the myogenic regulatory factors (MRFs) were expressed in the adipogenic condition and during the exposure to the treatments, as observed in this study. Across the 3-day treatment period, a significant ($P < 0.05$) reduction in Myf5 mRNA expression (Figure 3A, and a significant ($P < 0.01$) reduction of

Table 1
Quantitative PCR primers for mouse transcripts

Type	Target Gene	Sequence of the primer (5' to 3')
Myogenic Markers		
Myogenic Regulatory Factors	Myogenic Factor 5 (Myf5)	F: CAGCCCCACCTCCAACTG
		R: GCAGCACATGCATTTGATACATC
	Myogenic Differentiation 1 (MyoD)	F: CGTGGCAGCGAGCACTAC
		R: TGTAATCCATCATGCCATCAGA
	Myogenin	F: CCCATGGTGCCCAGTGAA
		R: GCAGATTGTGGGCGTCTGTA
Muscle Differentiation Marker	Creatine Kinase (CK)	F: GCACTGGCCGCAGCAT
		R: AGGGTAGTACTTGCCCTTGAACTC
Myofibrillar Protein Markers	Myosin Heavy Chain I (MyHCI)	F: CTCAAGCTGCTCAGCAATCTATTT
		R: GGAGCGCAAGTTTGTCATAAGT
	Myosin Heavy Chain IIa (MyHCIIa)	F: AGGCGGCTGAGGAGCACGTA
		R: GCGGCACAAGCAGCGTTGG
	Myosin Heavy Chain IIb (MyHCIIb)	F: CAATCAGGAACCTTCGGAACAC
		R: GTCCTGGCCTCTGAGAGCAT
	Myosin Heavy Chain IIx (MyHCIIx)	F: GAGGGACAGTTCATCGATAGCAA
		R: GGGCCAACTTGTCATCTCTCAT

Table 1 (*continued*)

Type	Target Gene	Sequence of the primer (5' to 3')
Lipid Turnover and Adipogenic Markers		
Lipogenic	Acetyl CoA-Carboxylase (ACC)	F: CCATTGGTATTGGGGCTTAC
		R: CCGACCAAGGACTTTGTTG
	Fatty Acid Synthase (FAS)	F: CGGAAACTTCAGGAAATGTCC
		R: TCAGAGACGTGTCACTCCTGG
Lipolytic	Adipose Triglyceride Lipase (ATGL)	F: CAACGCCACTCACATCTAC
		R GGACACCTCAATAATGTTG
	Hormone Sensitive Lipase (HSL)	F: GCGGCTGTCTAATGTCTTTGCA
		R: CCTTCATCACCCTCGAAGAAGA
Adipocyte Transcription Factors	Peroxisome Proliferator Activated Receptor γ (PPARγ)	F: GCATGGTGCCTTCGCTGA
		R: TGGCATCTCTGTGTCAACCATG
	Cluster Determinant 36 (CD/36)	F: AAACCCAGATGACGTGGC
		R: AAGATGGCTCCATTGGGC
	CCAAT/Enhancer Binding Protein Alpha (CEBPα)	F: GCCAAACTGAGACTCTTC
		R: GGAAGCCTAAGTCTTAGC
	Fatty Acid Binding Protein-4 (FABP4)	F: AAGTGGGAGTGGGCTTTGC
		R: TGGTGACCAAATCCCCATTT

Note. F, forward; R, reverse; all primers are listed 5' to 3'

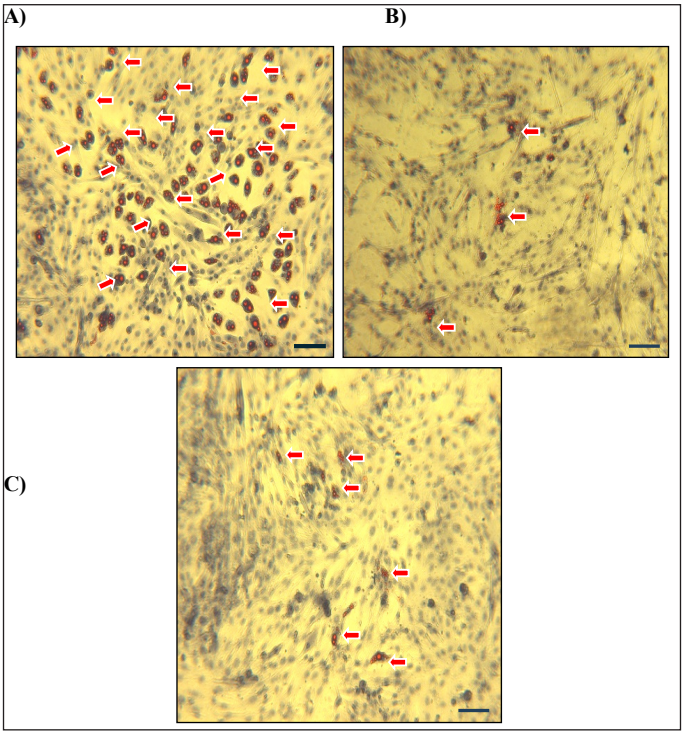


Figure 1. Effects of (A) control, (B) dbcAMP or (C) des (1-3) IGF-1 on lipid droplet accumulation (red arrows) in C2C12 muscle cells under adipogenic conditions. Scale bar = 100 µm

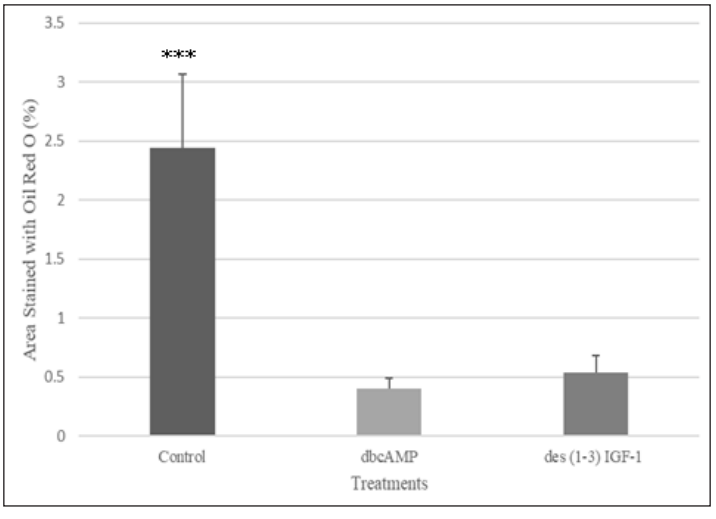


Figure 2. Effects of control, dbcAMP or des (1-3) IGF-1 on the percentage area stained with Oil Red O, with n = 3 per treatment group.
Note. Asterisks (***) indicate P <0.001 significant effect of treatment

both MyoD (Figure 3B) and Myogenin (Figure 3C) mRNA expressions were observed after 3 days of treatment, with no significant effect of treatment. In addition, no significant interaction was observed between the two factors on the mRNA expression of those MRFs.

In addition to the mRNA expression of myogenic transcription factors, C2C12 cells also showed significant creatine kinase (CK) mRNA expression, which is categorised as a myogenic differentiation marker. The cells treated with dbcAMP showed a significant ($P < 0.01$) increase in CK mRNA expression, as compared with other treatments (Figure 4). However, similar to the earlier observations on MRFs, there was no observation of any significant interaction between the two factors involved in the CK mRNA expression.

Meanwhile, the mRNA expression of all isoforms for the myosin heavy chain (MyHC), which are categorised as myofibrillar markers, was observed during treatments. The overall expression of MyHCI ($P < 0.001$) (Figure 5A), MyHCIIa ($P < 0.01$) (Figure 5B), and MyHCIIx mRNA ($P < 0.05$) (Figure 5D) were significantly reduced across the treatment

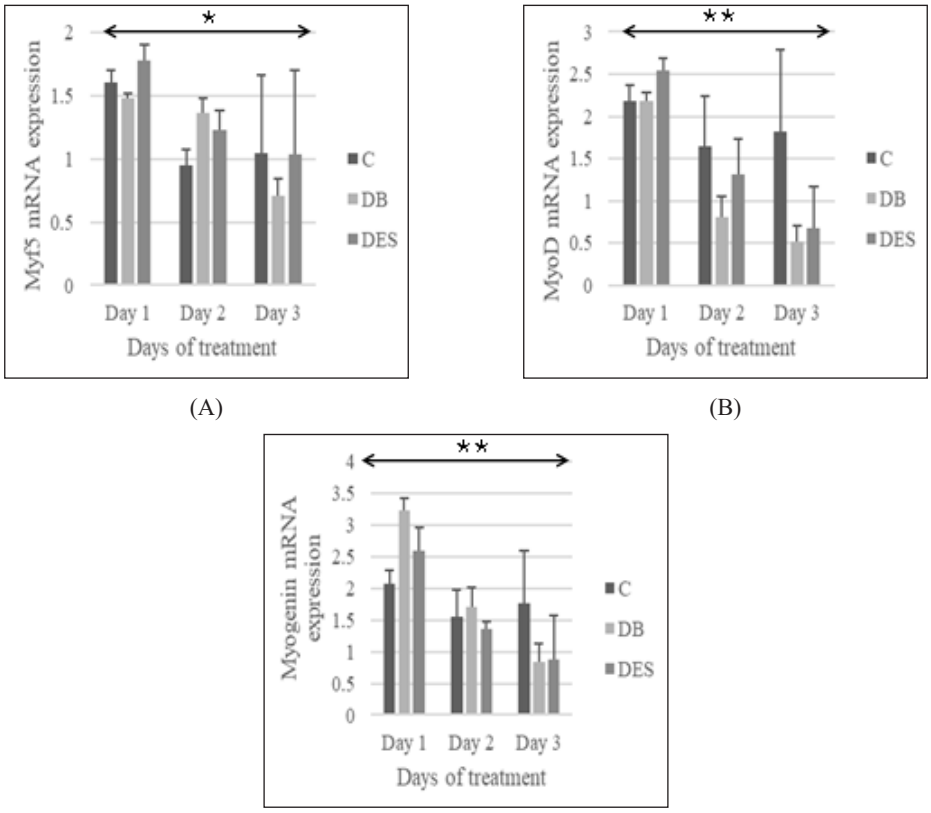


Figure 3. Effects of control (C), dbcAMP (DB) or des (1-3) IGF-1 (DES) on myogenic regulatory transcription factor mRNA expression in C2C12 muscle cells under adipogenic conditions. (A) Myf5, (B) MyoD, and (C) Myogenin mRNA, with n=3 per treatment group

Note. Asterisks (*) indicate a $P < 0.05$ or (**) indicate a $P < 0.01$ effect of days of treatment. The error bars are ± SEM

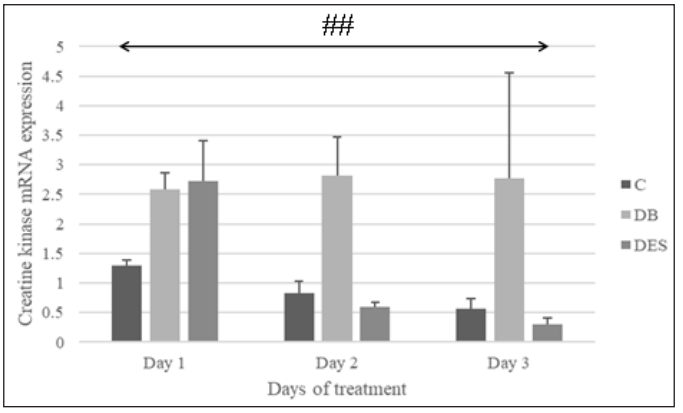
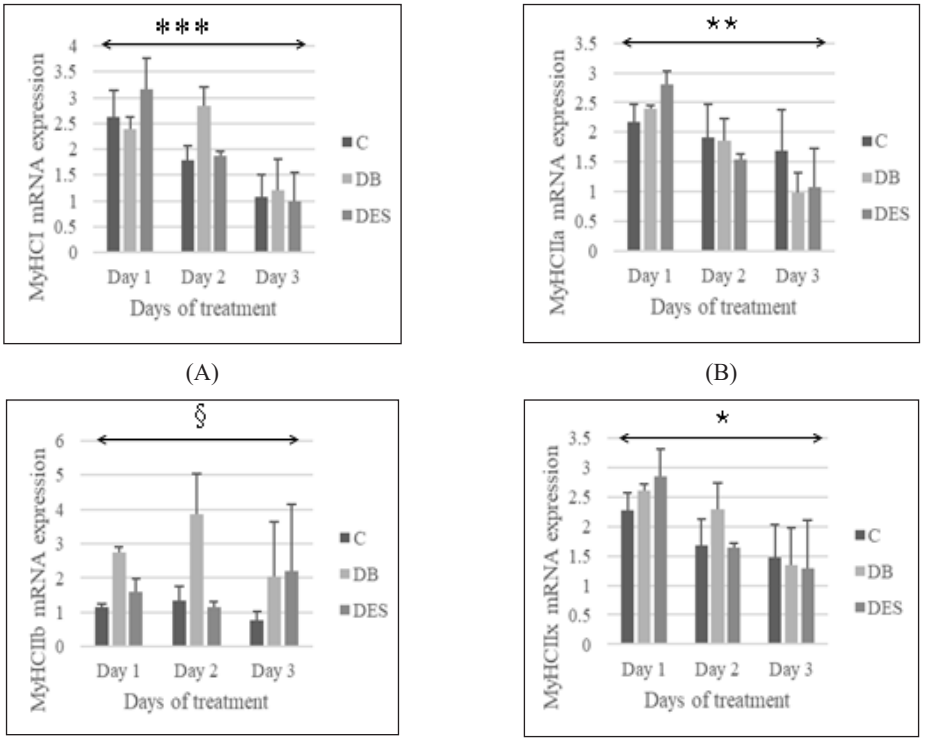


Figure 4. Effects of control (C), dbcAMP (DB) or des (1-3) IGF-1 (DES) on creatine kinase (CK) mRNA expression in C2C12 muscle cells under adipogenic conditions, with n=3 per treatment group
Note. Symbol (##) indicates a P <0.01 effect of treatment. The error bars are ± SEM



(A) (B)

(C) (D)

Figure 5. Effects of control (C), dbcAMP (DB) or des (1-3) IGF-1 (DES) on myosin heavy chain (MyHC) isoform mRNA expression in C2C12 muscle cells under adipogenic conditions. (A) MyHCI, (B) MyHCIIa, (C) MyHCIIb, and (D) MyHCIIx mRNA, with n=3 per treatment group
Note. Asterisks (*) indicate P <0.05, (**) indicate P <0.01, or (***) indicate P <0.001 for the effect of days of treatment, or (§) indicate a trend (P <0.10) for the effect of treatment. The error bars are ± SEM

period, while the expressions were not affected by the treatments. However, there was an observation of a trend toward the influence of treatment (P = 0.088), with dbcAMP tended to elevate the mRNA expression of MyHCIIb compared with other treatments (Figure 5C). Generally, neither factor signified any interaction in affecting all the mRNA expression of those myofibrillar markers.

The Effects of dbcAMP and des (1-3) IGF-1 on the mRNA Expression Related to Lipid Turnover and the Adipogenic Markers

ACC and FAS are two important lipogenic genes, while ATGL and HSL are lipolytic genes. For ACC mRNA expression, duration of treatment and the treatment had no effect on its expression (Figure 6A). In contrast, the prolonged duration of the treatment significantly (P <0.05) reduced the FAS mRNA expression, with the lowest levels being observed on day 3.

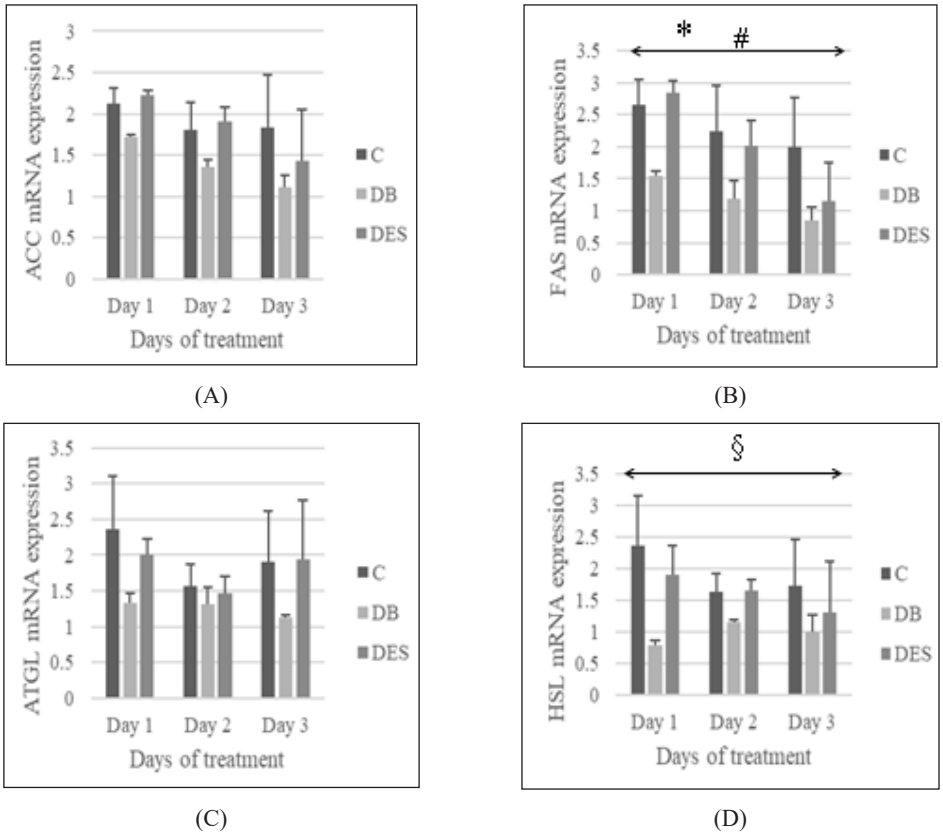


Figure 6. Effects of control (C), dbcAMP (DB) or des (1-3) IGF-1 (DES) on the lipid turnover-related mRNA expressions in C2C12 muscle cells under adipogenic conditions. (A) ACC, (B) FAS, (C) ATGL and (D) HSL mRNA, with n=3 per treatment group

Note. Symbol (§) indicate P <0.10 for the trend and (#) indicate P <0.05 for the effect of treatment, whilst (*) indicates P <0.05 for the effect of days of treatment. The error bars are ± SEM

In addition, the dbcAMP-treated cells displayed significantly lower FAS mRNA expression ($P < 0.05$) than other treatments (Figure 6B). Meanwhile, both factors had no effect on the ATGL mRNA expression (Figure 6C). However, for HSL mRNA expression, there was a trend ($P = 0.091$) for an effect of treatment, with dbcAMP-treated cells tending to have the lowest HSL mRNA expression (Figure 6D). For all lipid turnover markers, the significant interaction between both factors was not observed.

During adipogenesis, the differentiation of adipocytes is controlled by two regulators: PPARγ and CEBPα. Meanwhile, FABP4 is involved in the adipogenic activity inside the cells. The PPARγ also activates another target gene, including CD36, during preadipocyte differentiation into mature adipocytes. In the dbcAMP-treated cells, the FABP4 mRNA expression was significantly ($P < 0.05$) lower compared with other treatments (Figure 7A).

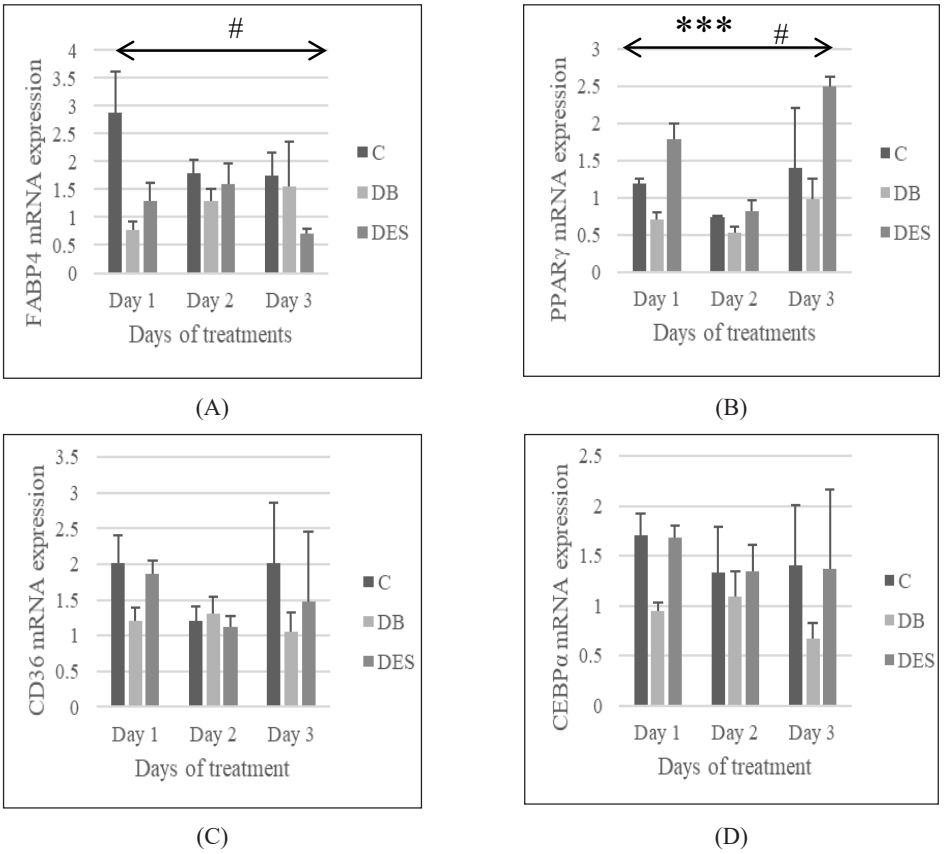


Figure 7. Effects of control (C), dbcAMP (DB) or des (1-3) IGF-1 (DES) on adipocyte differentiation transcription markers mRNA expression in C2C12 muscle cells under adipogenic conditions. (A) FABP4, (B) PPARγ, (C) CD36, and (D) CEBPα mRNA, with n = 3 per treatment group

Note. Symbol (#) indicate $P < 0.05$ for the effect of treatment, whilst (***) indicates $P < 0.001$ for the effect of days of treatment. The error bars are ± SEM

For PPARγ, the des (1-3) IGF-1 treatment led to a significantly ($P < 0.05$) higher expression compared with other treatments (Figure 7B). In addition, the PPARγ mRNA expression was significantly ($P < 0.001$) higher at day 3 (Figure 7B). Meanwhile, there were no effects of any factors, and any interactions were found for the CD36 and C/EBPα mRNA expression (Figure 7C and 7D). Moreover, no significant interaction between the duration of the treatment and the treatments in affecting the mRNA expression in all measured adipogenic markers.

DISCUSSION

This study demonstrated the influence of dbcAMP and des (1-3) IGF-1 on the expression of genes involving myogenesis and adipogenesis in C2C12 myoblast cells, with dbcAMP showing a stronger transcriptional response on both myogenic and adipogenic activities, while des (1-3) IGF-1 was mainly involved in adipogenic-associated gene expression, as summarised in Figure 8. In terms of myogenic development and regulations, dbcAMP

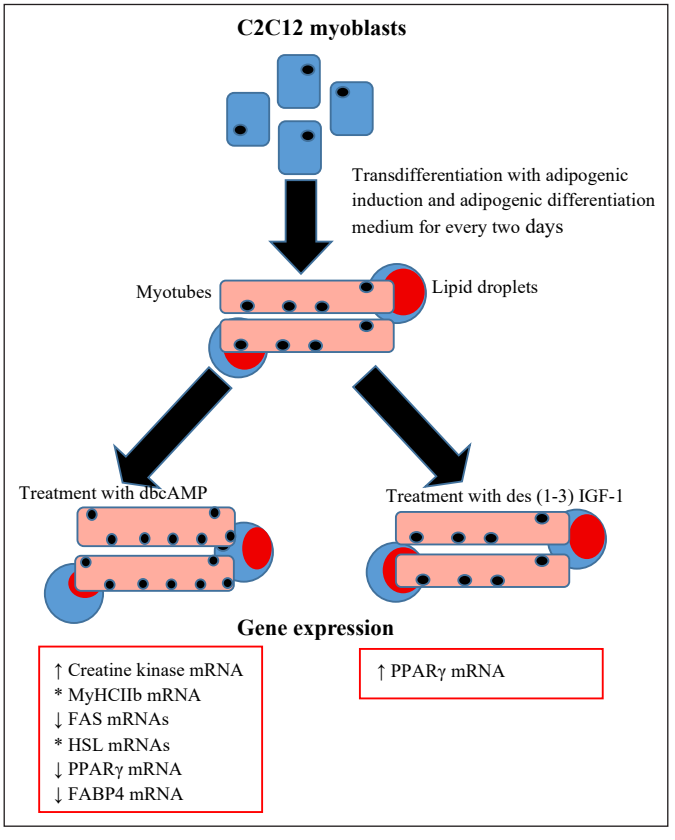


Figure 8. Schematic summary of the myogenic-related, lipogenic-related and adipogenic-related mRNA expressions upon treatment with dbcAMP and des (1-3) IGF-1 in the transdifferentiated C2C12 myoblasts
Note. The arrows adjacent to text indicate the changes in the mRNA expression; (↑) indicates an increase, (↓) indicates a decrease, and (*) indicates a trend. Other mRNA expressions were affected by a time-dependent effect

treatment increased CK mRNA expression, suggesting potential stimulation of the transcriptional pathway associated with cell differentiation. This effect was comparable to the BA treatment (clenbuterol) on neonatal rat muscle cell lines, which stimulated fusion and formation of myotubes, indicated by increased CK activity (McMillan et al., 1992). This observation implied that dbcAMP, along with other agents capable of stimulating cAMP production, such as BA, may contribute to the transcriptional regulation associated with myogenesis, supporting the role of cAMP-dependent signalling in mediating skeletal muscle hypertrophic response in vivo (Johnson et al., 2014; Lynch & Ryall, 2008).

Myf5, MyoD, Myogenin and MRF4 are several myogenic markers involved in controlling the myogenic development and regulation from progenitor cell commitment to myogenesis through to mononucleated myoblasts fusion into multinucleated myotubes, thus categorised as myogenic regulatory factors (MRFs) (Sabourin & Rudnicki, 2000; Yan et al., 2013). In this study, all MRFs mRNA expressions (except MRF4, which was not measured in this study) were increased at day 1, and reduced after 3 days of treatments, suggesting the early transcriptional response associated with myoblast differentiation to form myotubes, regardless the treatments, while adipogenic transcriptional pathway at later stage of the treatments, would supress the myogenic gene expression (Goffette et al., 2024), which are in line are with the observed creatine kinase mRNA expression which is consistent with the transcriptional changes associated with myotube differentiation as previously described.

Alterations in myofibrillar markers were also evident with dbcAMP treatment compared to other groups. Specifically, dbcAMP tended to increase MyHCIIb expression. This might be attributed to MyHCIIb being the predominant and widely distributed isoform in rodent skeletal muscle, which is also highly responsive towards experimental treatment (Harrison et al., 2011). This result also suggests that dbcAMP tended to induce the transcriptional response of fibre transition towards fast fibre characteristics, which is associated with myotube hypertrophy (Brearley et al., 2021; Brown et al., 2012; Hemmings et al., 2015). Meanwhile, MyHCI, MyHCIIa, and MyHCIIx mRNA expressions were reduced after 3 days of treatment, probably associated with the concurrent reduction in MRFs mRNA expressions, as well as the hypertrophic and contractility of the myotubes after prolonged treatment (Brown et al., 2012; Cerny & Bandman, 1986; Torgan & Daniels, 2001).

Meanwhile, in the aspect of lipid metabolism, the treatment with dbcAMP or des (1-3) IGF-1 led to a notable alteration in several lipogenic and lipolytic gene expressions, accompanied by the reduction in the lipid droplet deposition in all the treatment groups. The suppression of FAS by dbcAMP indicated reduced transcriptional activity associated with lipogenesis. In addition, the HSL expression showed a tendency to decline under dbcAMP treatment, suggesting a potential role of dbcAMP in reducing lipid turnover in the cells. Since BA action (mimics the action of dbcAMP) and mediated through cAMP-dependent protein kinase, has been reported to suppress lipogenesis of fat tissue in porcine (Peterla

& Scanes, 1990), suggesting the reduction of lipid turnover might also be associated with dbcAMP-mediated suppression of lipogenesis. This was also further supported by the marked decline of FAS mRNA expression after 3 days of treatment, whereas ATGL remained unaffected and HSL was only minimally affected, suggesting reduced lipogenic capacity after prolonged treatment.

Adipogenesis has been demonstrated to be regulated by des (1-3) IGF-1, which increased the expression of the key adipogenic gene, PPARγ. It has been reported that IGF-1 is the mediator in the GH response, thus suggesting that des (1-3) IGF-1 is one of its variants (Ballard et al., 1996). GH has been demonstrated to increase PPARγ expression in the 3T3-L1 preadipocytes via the JAK/STAT pathway (Kawai et al., 2007). In addition, the activation of PPARγ was induced by Akt signalling, which was apparently activated by IGF-1 during adipocyte differentiation as the activation was blocked by an Akt/PI3K inhibitor and knockdown of Akt1 (Kim et al., 2010). The induction of PPARγ would indicate a transcriptional response associated with adipogenic differentiation, which may be involved in the transdifferentiation of adipocyte-like cells from myoblasts and is associated with the adipocyte-like phenotype observed during C2C12 transdifferentiation. However, this effect did not align with the reduction in lipid droplets deposition, thus, it was unclear as PPARγ was reported to regulate the perilipin-1 gene (Plin1) expression, which is the essential in lipid droplets formation (Arimura et al., 2004; Dalen et al., 2004), suggesting the lipid droplets formation might be due to reduced post-translational activity of PPARγ, which was not measured in this study. This inconsistency between increased PPARγ mRNA expression and reduction in lipid droplets probably highlights the complexity of adipogenic regulation, as the mRNA abundance alone cannot reflect its protein abundance or post-translational activity PPARγ, which could be due to the altered post-transcriptional regulation, varying translational control efficiency, protein stability or degradation (Greenbaum et al., 2003). Meanwhile, according to Hu et al. (1995), ectopic expression of PPARγ did not induce the formation of lipid droplets, unless the effect was synergised with C/EBPα. The expression of C/EBPα mRNA was not significantly affected in this study, suggesting that increased PPARγ mRNA expression may not be accompanied by the transcription of C/EBPα, which may partly explain the limited accumulation of lipid droplets in the treated cells. The number of lipid droplets in des (1-3) IGF-1-treated cells was not as high as in the control cells. This may be due to deposition of lipid being more associated with the lipogenic gene, such as FAS and/or FABP4, whilst the effect of PPARγ is primarily associated with adipogenic differentiation, and these genes play slightly different roles in lipid metabolism.

In general, the adipogenic media induced the expression of FABP4 in all groups. Since FABP4 is categorised as a specific adipocyte marker due to its specific expression in adipose tissue, its downregulation following dbcAMP treatment suggested a reduction in the transcriptional program associated with adipogenesis. Jurie et al. (2007) reported

that FABP4 mRNA expression, in vivo, is closely linked to the intramuscular fat (IMF) formation, and this suggests the role of dbcAMP may influence molecular pathways associated with lipid accumulation, which was associated with a reduction in lipid droplet formation, and potentially in the mechanism of reduced IMF deposition. Another study by Okuda et al. (1989) demonstrated that p-aminophenol, a compound structurally similar to dbcAMP, promoted lipolysis in rat adipocytes, suggesting dbcAMP may modulate adipogenic and lipid metabolic pathways through cAMP-associated signalling. However, the present mRNA data in this study do not establish whether these transcriptional changes translate into increased lipolytic activity. As noted earlier, dbcAMP markedly reduced lipid turnover, and the suppression of FABP4 expression supports the hypothesis that dbcAMP, and by extension BA, enhance cAMP signalling, and may be involved in lipid accumulation and IMF deposition, although confirmation at the protein and functional levels is required.

CONCLUSION

The role of BA was demonstrated by the effect of dbcAMP in vitro, used as a cAMP analogue to modulate the mRNA expression related to myogenesis and adipogenesis in the transdifferentiated C2C12 model. The increase in myogenic mRNA expressions, including CK and MyHCIIb, suggests that cAMP-associated signalling contributes to the regulation of myogenic differentiation and fast-fibre characteristics. Meanwhile, des (1-3) IGF-1 was used to investigate the potential effects associated with GH involved in promoting the expression of genes associated with adipogenesis. Both agents appeared to modulate the expression of genes related to lipid metabolism by reducing lipogenesis and influencing lipid turnover by the changes in lipid droplet deposition, which suggested that both treatments may influence the balance between myogenic and adipogenic metabolic processes. Meanwhile, the transdifferentiation of a C2C12 mixed-cell-type (adipogenic and myogenic) culture model could be used as a tool to assess the potential effects of factors that alter muscle metabolism, thereby indicating their potential effects in muscles in vivo. However, the present study primarily determined mRNA expression, which does not establish corresponding changes in posttranslational regulation, protein abundance, and functional activity, which require further validation of those parameters. This could potentially apply in the identification of treatments that may allow the development of lean and healthy meat or understanding the mechanisms for reducing fat deposition in muscles in animals for healthy food production.

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