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Effect of dietary restriction and subsequent realimentation on hepatic oxidative phosphorylation in cattle

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ABSTRACT

Compensatory growth (**CG**) is a naturally accelerated growth which occurs upon realimentation, following a prior period of dietary restriction. The process is harnessed worldwide as a management practice to reduce feed costs in beef cattle production. The objective of this study was to assess the potential contribution of hepatic cellular mitochondrial capacity to CG through global hepatic oxidative phosphorylation gene expression analyses as well as functional mitochondrial enzyme activity assays. Holstein–Friesian bulls were separated into two groups: (i) restricted feed allowance for 125 days (Period 1) (**RES**; $n = 30$) followed by *ad-libitum* feeding for 55 days (Period 2) or (ii) *ad-libitum* access to feed throughout (Periods 1 and 2) (**ADLIB**; $n = 30$). At the end of each period, 15 animals from each treatment group were slaughtered and hepatic tissue was collected. Tissue samples were subjected to RNAseq and spectrophotometric analysis for the functional assessment of mitochondria. RES and ADLIB groups grew at 0.6 kg/day and 1.9 kg/day, respectively, during Period 1. During Period 2, the RES group underwent CG growing at 2.5 kg/day, with ADLIB animals gaining 1.4 kg/day. Oxidative phosphorylation genes were differentially expressed in response to both dietary restriction and CG. Spectrophotometric assays indicated that mitochondrial abundance was greater in animals undergoing dietary restriction at the end of Period 1 and subsequently reduced during realimentation ($P < 0.02$). Results indicate that mitochondrial capacity may be enhanced during dietary restriction to more effectively utilize diet-derived nutrients. However, enhanced mitochondrial capacity does not appear to be directly contributing to CG in cattle.
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Implications

Utilization of compensatory growth (CG) facilitates a method to reduce feed costs through a period of improved feed efficiency. However, although widely incorporated into animal production systems, the underlying biology remains to be elucidated fully. In particular, the contribution of enhanced mitochondrial capacity towards the expression of compensatory growth in cattle is yet to be determined. This study provides evidence that mitochondrial capacity may be enhanced during dietary restriction; however, it does not appear to contribute to the enhanced feed efficiency apparent during compensatory growth.

Introduction

As feed can account for up to 75% of the variable costs in beef cattle production systems (Fitzsimons et al., 2017), any means by which these costs may be reduced without compromising overall animal performance would be of benefit to the beef industry. Utilization of the naturally occurring compensatory growth (**CG**) phenomenon provides a method to allow for a reduction in feed input costs (Fitzsimons et al., 2017). Compensatory growth is an accelerated growth which cattle can display upon realimentation following a prior period of dietary restriction (Hornick et al., 2000); this naturally occurring phenomenon has been attributed to a number of factors including enhanced feed efficiency (Fitzsimons et al., 2017).

Mitochondria are cellular organelles responsible for approximately 90% of oxygen consumption as well as the majority of cellular energy production through oxidative phosphorylation (Harper et al., 2002). Oxidative phosphorylation, the final stage in aerobic cell respiration, produces ATP through the transfer of electrons through the electron transport chain on the inner mitochondrial membrane (Bottje et al., 2006). Given the central role of this organelle to cellular energy production, much attention has been focused towards the contribution of mitochondrial functionality to the underlying biology controlling feed efficiency in livestock. Studies conducted to date with cattle have shown that ADP control of oxidative phosphorylation (Lancaster et al., 2014), mitochondrial respiration rate (Kolath et al., 2006) and mitochondrial complex protein concentration (Davis, 2009) are all higher in feed efficient animals compared to inefficient animals. Using a global

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gene expression approach to study CG response in cattle, both (Connor et al., 2010) and (Keogh et al., 2016a) observed an effect of both dietary restriction and subsequent CG on the expression of genes involved in oxidative phosphorylation in hepatic and skeletal muscle tissues, respectively. Indeed, (Connor et al., 2010) suggested that enhanced mitochondrial efficiency in hepatic tissue may be contributing to CG, manifested as greater expression of oxidative phosphorylation genes in cattle undergoing realimentation compared to their non-restricted counterparts. However, in our own work, we did not observe a similar outcome for skeletal muscle tissue, where down-regulation of oxidative phosphorylation genes was evident in the cattle undergoing CG (Keogh et al., 2016a). These varying results may be a consequence of differences in timing of sampling, level and length of the prevailing dietary restriction period, as well as the tissue type under investigation. Although these studies suggest a role for mitochondrial functionality in the expression of CG in cattle at the mRNA level, to date there have been no published studies on the functional role of mitochondria to CG expression in cattle. Therefore, the objective of this study was to first assess alterations to mitochondrial capacity through an assessment of NADH dehydrogenase (Complex I) activity as well as determining mitochondrial abundance through evaluation of citrate synthase activity in hepatic tissue following periods of both dietary restriction and subsequent CG. Activity of Complex I was specifically targeted due to the large effect of both dietary restriction and subsequent realimentation on the expression of genes coding for this protein (Connor et al., 2010; Keogh et al., 2016a), as well as the previous reported role for this enzyme in both cattle and broilers divergent in feed efficiency phenotype (Bottje et al., 2002; Ojano-Dirain et al., 2007; Ramos and Kerley, 2013). Our second objective was to examine transcript abundance of genes governing oxidative phosphorylation in hepatic tissue during feed restriction and realimentation through global gene expression profiling. The liver was chosen as a target tissue for this study as it is a highly metabolically active organ and is clearly both morphologically and biochemically responsive to dietary restriction and subsequent CG (Connor et al., 2010; Keogh et al., 2015; Keogh et al., 2016b). Moreover, our study was focused towards the first 55 days of realimentation-induced CG as the greatest proportion of accelerated growth and feed efficiency occur during this time (Hornick et al., 2000).

Material and methods

Animal model

The experiments described in this study were conducted in association with a larger study designed to examine the effect of restricted growth and subsequent realimentation in Holstein–Friesian bulls (Keogh et al., 2015). Sixty Holstein–Friesian bulls with a mean (SEM) age of 479 (15) days and BW 370 (35) kg were blocked into one of two groups: (i) restricted feed allowance for 125 days (RES; $n = 30$) followed by *ad-libitum* access to feed for 55 days or (ii) *ad-libitum* access to feed throughout (ADLIB; $n = 30$). The first 125 days were denoted as Period 1 and the subsequent 55 days, Period 2. During Period 1, RES animals were managed to achieve a target mean daily growth rate of 0.6 kg/day, whereas ADLIB animals were expected to gain in excess of 1.5 kg/day. Feed allowances for these target growth rates were based on NRC guidelines (National Research Council (NRC), 2000). Diets were fed individually, with the proportion of feed required based on each animal's own individual BW. All animals were offered a total mixed ration diet, consisting of 70% concentrate and 30% grass silage on a DM basis. Throughout the study, animals were weighed every 2 weeks during Period 1 and every week during Period 2. At the end of each period, 15 animals from each treatment group were slaughtered in an EU licensed abattoir (Euro Farm Foods, Duleek, Co. Meath, Ireland).

Hepatic tissue collection

Hepatic tissue was sampled from all animals within 30 min of slaughter, and all samples were collected from the same location within the right lobe of the liver. All surgical instruments used for tissue collection were sterilized and treated with RNA Zap prior to use (Ambion, Applera Ireland, Dublin, Ireland). Samples were washed thoroughly with sterile Dulbecco's phosphate-buffered saline and immediately snap-frozen in liquid nitrogen before subsequent storage at −80 °C.

Functional mitochondrial assays

Liver tissue samples were homogenised as outlined by (Spinazzi et al., 2012) with slight modifications. Homogenization buffer (pH 7.4) was prepared using 20 mM Tris, 40 mM KCl and 2 mM EGTA (Sigma Aldrich Ireland Ltd., Wicklow, Ireland) diluted in distilled water. Fresh sucrose (250 mM; Sigma Aldrich Ireland Ltd., Wicklow, Ireland) was added to the homogenization buffer on each day of use. From 50 mg of liver tissue, visible fat and connective tissue were removed and remaining liver tissue was then dissected into small fragments. Tissue was diluted at a ratio of 1:20 in the ice-cold sucrose (250 mM) homogenization buffer. Tissue samples were homogenized using a clean glass conical tissue grinder on ice. The homogenate was then centrifuged at 600 *g* for 10 min at 4 °C. The resulting supernatant was subsequently kept on ice and used on the same day. The concentration of protein in homogenates was determined using the Pierce BCA Protein Assay Kit (FisherScientific, Ireland).

The activity of citrate synthase was assayed by coupling the rate-limiting reaction catalyzed by citrate synthase (Eq. (1)) to the irreversible chemical reaction (Eq. (2)):

$$\text{Acetyl}-\text{CoA} + \text{oxalacetate} + H_2O = \text{citrate} + \text{CoA}-\text{SH} \quad (1)$$

$$\text{CoA}-\text{SH} + 5,5'-\text{dithiobis}-(2-\text{nitrobenzoic acid } (\textbf{DTNB})) = 5-\text{thio}-2-\text{nitrobenzoic acid } (\textbf{TNB}) + \text{CoA}-\text{S}-\text{S}-\text{TNB} \quad (2)$$

The product of this reaction, TNB, displays a greater absorption at 412 nm with the absorbance increasing linearly with time. This increase in absorption was measured for 4 min, and rate of reaction subsequently calculated. The enzymatic activity of citrate synthase was calculated as nmol/min/mg homogenate protein using a mathematical equation which incorporated the extinction coefficient for thionitrobenzene, which is 13.6 mM/cm (Spinazzi et al., 2012).

The activity of complex I of the electron transport chain in the liver samples was assessed by UV spectrophotometry as described by Spinazzi et al. (Spinazzi et al., 2012). All assays were performed in duplicate at 37 °C in 2 ml cuvettes. Complex I activity was calculated as nmol/min/mg homogenate protein and expressed in units normalized to citrate synthase activity (marker of mitochondrial abundance within the tissue/cell). Complex I activity was measured as a decrease in absorbance at 340 nm following the oxidation of reduced NADH (Sigma Aldrich Ireland Ltd., Wicklow, Ireland). Tissue homogenate (40 μg) containing mitochondria was incubated at 37 °C for 2 min in 700 μl of reaction medium (50 mM Tris-Hydrogen Chloride (Sigma Aldrich Ireland Ltd., Wicklow, Ireland) with 3 mg/ml bovine serum albumin (Fisher Scientific, Ireland), pH 8.0; 300 μM potassium cyanide (Sigma Aldrich Ireland Ltd., Wicklow, Ireland) and 100 μM NADH). Ubiquinone (60 μM; Coenzyme Q1) was then added to initiate the reaction. Absorbance was monitored for 3 min both before and after the addition of 10 μM rotenone (Sigma Aldrich Ireland Ltd., Wicklow, Ireland). The difference in the decrease in absorption due to NADH oxidation was measured in the absence and presence of rotenone, and the rotenone-sensitive activity of Complex I was subsequently quantified using an extinction coefficient of 6.22 mM/cm (Spinazzi et al., 2012).

Statistical analysis of functional mitochondrial assays

Complex 1 and citrate synthase data were checked for normality using the UNIVARIATE procedure of SAS (SAS Inst. Inc., Cary, NC). Where necessary, data were transformed using the TransReg procedure, by raising to the power of λ. Data were analysed using mixed models methodology (PROC MIXED, SAS). Treatment and period as well as their interaction were included as the main effects, with block included as a random effect in the statistical model. Where no interactions were observed, the data were re-analyzed for main effects only. The Tukey critical difference test was performed to determine the existence of statistical differences between treatment LSmean values. The choice of residual covariance structure was based on the magnitude of the Akaike Information Criterion (lowest is best), and compound symmetry was chosen as the covariance structure.

Oxidative phosphorylation gene expression

Methodology employed to isolate total RNA, preparation of cDNA libraries and subsequent sequencing and bioinformatics analyses from liver tissue samples used in this study has previously been described in Keogh et al. (Keogh et al., 2016b) are is only briefly outlined here. Total RNA was isolated from approximately 60 mg of frozen liver tissue using the Qiagen RNeasy mini kit (Qiagen). The quantity and quality of the resultant RNA were assessed by measuring the absorbance at 260 nm using a Nanodrop spectrophotometer ND-1 000 (Nanodrop Technologies, DE, USA) and by determining the RNA integrity number using an Agilent Bioanalyser 2100 RNA 6 000 Nano Lab Chip (Agilent Technologies Ireland Ltd., Dublin, Ireland), respectively. cDNA libraries were prepared from 3 μg of total RNA from 10 high-quality RNA samples from each treatment group over each period using the Illumina TruSeq RNA sample prep kit (Illumina, San Diego, CA, USA). RNA-seq libraries were sequenced at 100 bp/sequence single-end read using an Illumina HiSeq 2000 sequencer.

Raw sequence reads were first checked for quality using FASTQC software (version 0.10.0) and then aligned to the bovine reference genome (UMD3.1) using TopHat (v2.0.9). The software package HTSeq (v0.5.4p5) (http://pypi.python.org/pypi/HTSeq) was employed to calculate the abundance of mRNA transcripts for all annotated genes, and the R (v2.14.1) Bioconductor package, EdgeR (v3.4.1), was then applied to identify statistically significant differentially expressed genes (**DEG**s). Differentially expressed genes reported in the present study had a Benjamini–Hochberg corrected *P*-value of 0.1. The following treatment comparisons were tested for DEGs: (i) RES vs ADLIB at the end of Period 1; (ii) RES vs ADLIB at the end of Period 2; (iii) RES Period 2 vs RES Period 1 and (iv) ADLIB Period 2 vs ADLIB Period 1. Pathway analysis of DEGs for each comparison was then undertaken using Ingenuity Pathway Analysis (**IPA**) ((Kramer et al., 2014); Ingenuity Systems, Redwood City, California; http://www.ingenuity.com) to determine if the oxidative phosphorylation pathway was significantly enriched in response to dietary restriction and subsequent CG. Pathways were deemed statistically significantly enriched when there were more DEGs in the pathway than would be expected given the size and gene length distribution.

Results

Animal performance

Differences in average daily gain, feed intake and animal performance are outlined in detail by Keogh et al. (Keogh et al., 2015). As planned during Period 1, RES animals consumed less feed than the ADLIB group (DM-intake: RES: 5.46 kg/day; ADLIB: 12.7 kg/day); however, during realimentation in Period 2, RES animals' dietary intake increased (DM intake: RES: 11.8 kg/day; ADLIB: 12.22 kg/day). RES animals achieved their target growth rate of 0.6 kg/d during Period 1, with ADLIB gaining 1.9 kg/d in the same period. This resulted in BWs of 442 kg and 603 kg for RES and ADLIB, respectively, at the end of Period 1. In Period 2, previously restricted animals (during Period 1) undergoing CG exhibited a live weight gain of 2.5 kg/d, while their contemporaries from the control group (fed *ad-libitum* throughout the trial) grew at 1.4 kg/d during Period 2. At the end of Period 2, BWs were 594 kg and 678 kg for RES and ADLIB, respectively. A CG index provides a method of quantifying the extent of the CG response achieved; in the current study, RES animals displayed a CG index of 48% after only 55 days realimentation. Although full body compensation had not occurred by day 55 of realimentation, the liver had compensated for 95% of the prior growth restriction (liver weight, Period 1: RES: 4.47 kg; ADLIB: 8.52 kg; Period 2: RES: 8.46 kg; ADLIB: 8.71 kg), thus providing a sound biological basis to study the biochemical responses of hepatic tissue during dietary restriction and subsequent CG.

Table 1
Effect of dietary restriction and compensatory growth in cattle on citrate synthase and Complex 1 activities.

	RES[1]		ADLIB[2]			*P*-values[3]		
	Period 1	Period 2	Period 1	Period 2	SEM	T[4]	P[5]	T*P
Citrate synthase[6]	76.35	67.7	55.8693	79.8889	6.96067	ns	ns	*
Complex 1[7]	1.2593	1.269	1.1293	0.8995	0.18115	ns	ns	ns

[1] RES: dietary restricted group.
[2] ADLIB, *ad-libitum* fed group.
[3] *P*-values: * = $P < 0.05$, ns = not significant.
[4] Treatment.
[5] Period.
[6] Citrate synthase (CS) units: nmol/min/mg homogenate protein.
[7] Complex I units: unit//CS.

Mitochondrial assays

Mitochondrial assay results are presented in Table 1. A treatment × period interaction ($P < 0.05$) was evident for citrate synthase activity which was greater in RES at the end of Period 1 compared to ADLIB, subsequently lower in RES at the end of Period 2 compared to ADLIB. There was no effect of dietary restriction nor subsequent realimentation on Complex 1 activity ($P > 0.05$).

Oxidative phosphorylation gene expression

Pathway enrichment results of the oxidative phosphorylation pathway within IPA are presented in Table 2. Eleven genes pertaining to oxidative phosphorylation signalling were differentially expressed (**DE**) between RES and ADLIB animals at the end of Period 1, resulting in the pathway tending towards significance ($P < 0.1$). Differentially expressed genes between RES and ADLIB at the end of Period 1 are presented in Table 3. At the end of Period 2, following 55 days of realimentation and CG, only one oxidative phosphorylation gene (*UCP2*) was DE (fold change of 1.9, up-regulated in RES compared to ADLIB). When analysed within treatment, the oxidative phosphorylation signalling pathway was

Table 2
Pathway enrichment for oxidative phosphorylation pathway following dietary restriction and subsequent compensatory growth in cattle.

Comparison	*P*-value of pathway significance[1]
Period 1, RES[2] v ADLIB[3]	**0.072**
Period 2, RES v ADLIB	ns
RES, Period 2 v Period 1	***
ADLIB, Period 2 v Period 1	ns

[1] *P*-values: *** = $P < 0.001$, ns = not significant.
[2] RES: dietary restricted group.
[3] ADLIB: *ad-libitum* fed group.

Table 3
Oxidative phosphorylation genes differentially expressed in hepatic tissue of cattle following a period of dietary restriction.

Symbol	Entrez Gene Name	Fold change[1]
Complex 1: NADH dehydrogenase		
NDUFAF2	NADH:ubiquinone-oxidoreductase complex assembly factor 2	1.49
NDUFB1	NADH:ubiquinone-oxidoreductase subunit B1	1.272
NDUFB10	NADH:ubiquinone-oxidoreductase subunit B10	1.425
Complex II: Succinate dehydrogenase		
SDHA	Succinate dehydrogenase complex flavoprotein subunit A	−1.533
SDHC	Succinate dehydrogenase complex subunit C	−1.36
Complex III: Q cytochrome *c* reductase		
UQCR11	Ubiquinol-cytochrome-c-reductase, complex III subunit XI	1.266
Complex IV: Cytochrome c oxidase		
COX8A	Cytochrome-c-oxidase subunit 8A	1.31
Complex V: ATPsynthase		
ATP5S	ATP-synthase, H+ transporting, mitochondrial Fo complex subunit s	1.356
Associated proteins		
CYB5A	Cytochrome b5 type A	1.254
SOD2	Superoxide dismutase 2, mitochondrial	−1.411
CPT1A	Carnitine palmitoyltransferase 1A	−1.354

[1] Fold changes are up or down in restricted fed animals compared to *ad-libitum* control animals (Period 1; RES v ADLIB).

significantly enriched in RES animals ($P < 0.001$), with no pathway enrichment identified for the ADLIB group (Table 2). Differentially expressed genes within the RES group across both periods (RES Period 2 v RES Period 1) are presented in Table 4.

Discussion

The CG phenomenon is utilized in production systems worldwide as a means to reduce feed costs (Fitzsimons et al., 2017). Indeed, studies have reported complete compensation following a prior period of reduced growth, evident through a CG index of 100% (Fitzsimons et al., 2017). In the current study, animals compensated for 48% of their prior dietary restriction in just 55 days of realimentation, and although complete body compensation was not achieved during this time, the liver displayed 95% compensation within the same time frame (Keogh et al., 2015). In response to both dietary restriction and subsequent realimentation, the metabolically active organs of the body including the liver and gastrointestinal tract may be largely affected, with these organs representing lower proportional weights (based on BW) after a period of dietary restriction, and subsequently compensating ahead of other tissues upon realimentation (Keogh et al., 2015; Fitzsimons et al., 2017). Such a response is undoubtedly due to the requirement to lower the metabolic rate of such tissues during dietary restriction and also to allow for an increase in metabolic capacity during realimentation. However, additional changes in body composition also occur. For example, in the cattle utilized in the current study, a period of dietary restriction resulted in reduced growth of late maturing body components including muscle and fat, whereas early maturing components including bones were largely unaffected (Keogh et al., 2015). Moreover, during CG muscle deposition was greater in RES compared to ADLIB across both periods, indicating that realimentation and CG may lead to enhanced muscle deposition. A similar result for greater muscle deposition was also reported by Keady (Keady, 2011) in Belgian Blue bulls. Overall though the clear response of hepatic tissue in the animals used in the current study provided a sound biological basis to examine mitochondrial capacity in the liver.

Mitochondria utilize between 80 and 90% of cellular oxygen (Harper et al., 2002); thus, different states of dietary intake or feed efficiency could have substantial effects on mitochondrial ATP production and energy expenditure (Lancaster et al., 2014). Previous studies into feed efficiency in livestock species have provided evidence for a contribution of mitochondrial functionality towards inter-animal variation in feed

Table 4
Oxidative phosphorylation genes differentially expressed in hepatic tissue of cattle during compensatory growth compared to a period of dietary restriction.

Symbol	Entrez Gene Name	Fold change[1]
Complex 1: NADH dehydrogenase		
MT-ND1	NADH dehydrogenase, subunit 1 (complex I)	−1.825
MT-ND3	NADH dehydrogenase, subunit 3 (complex I)	−1.565
MT-ND5	NADH dehydrogenase, subunit 5 (complex I)	−1.793
MT-ND6	NADH dehydrogenase, subunit 6 (complex I)	−2.051
NDUFA1	NADH:ubiquinone-oxidoreductase subunit A1	−1.34
NDUFA2	NADH:ubiquinone-oxidoreductase subunit A2	−1.442
NDUFA3	NADH:ubiquinone-oxidoreductase subunit A3	−1.499
NDUFA4	NDUFA4, mitochondrial-complex-associated	−1.257
NDUFA7	NADH:ubiquinone-oxidoreductase subunit A7	−1.416
NDUFA11	NADH:ubiquinone-oxidoreductase subunit A11	−1.388
NDUFAB1	NADH:ubiquinone-oxidoreductase subunit AB1	−1.394
NDUFAF2	NADH:ubiquinone-oxidoreductase complex assembly factor 2	−1.675
NDUFB1	NADH:ubiquinone-oxidoreductase subunit B1	−1.42
NDUFB3	NADH:ubiquinone-oxidoreductase subunit B3	−1.373
NDUFB4	NADH:ubiquinone-oxidoreductase subunit B4	−1.285
NDUFB5	NADH:ubiquinone-oxidoreductase subunit B5	−1.266
NDUFB6	NADH:ubiquinone-oxidoreductase subunit B6	−1.348
NDUFB7	NADH:ubiquinone-oxidoreductase subunit B7	−1.478
NDUFB8	NADH:ubiquinone-oxidoreductase subunit B8	−1.387
NDUFB9	NADH:ubiquinone-oxidoreductase subunit B9	−1.275
NDUFB10	NADH:ubiquinone-oxidoreductase subunit B10	−1.621
NDUFB11	NADH:ubiquinone-oxidoreductase subunit B11	−1.364
NDUFS4	NADH:ubiquinone-oxidoreductase subunit S4	−1.28
NDUFS8	NADH:ubiquinone-oxidoreductase core subunit S8	−1.347
Complex II: Succinate dehydrogenase		
SDHA	Succinate dehydrogenase complex flavoprotein subunit A	1.468
SDHB	Succinate dehydrogenase complex iron sulfur subunit B	−1.443
Complex III: Q cytochrome *c* reductase		
UQCR10	Ubiquinol-cytochrome-c-reductase, complex III subunit X	−1.275
UQCR11	Ubiquinol-cytochrome-c-reductase, complex III subunit XI	−1.572
UQCRH	Ubiquinol-cytochrome-c-reductase hinge protein	−1.256
UQCRQ	Ubiquinol-cytochrome-c-reductase complex III subunit VII	−1.431
Complex IV: Cytochrome c oxidase		
COX17	COX17, cytochrome-c-oxidase copper chaperone	−1.42
COX4I1	Cytochrome-c-oxidase subunit 4I1	−1.483
COX5B	Cytochrome-c-oxidase subunit 5B	−1.393
COX6B1	Cytochrome-c-oxidase subunit 6B1	−1.482
COX7B	Cytochrome-c-oxidase subunit 7B	−1.34
COX8A	Cytochrome-c-oxidase subunit 8A	−1.452
MT-CO1	Cytochrome-c-oxidase subunit I	−1.756
MT-CO2	Cytochrome-c-oxidase subunit II	−1.896
MT-CO3	Cytochrome-c-oxidase III	−1.573
Complex V: ATPsynthase		
ATP5H	ATP-synthase, H+ transporting, mitochondrial Fo complex subunit D	−1.441
ATP5J	ATP-synthase, H+ transporting, mitochondrial Fo complex subunit F6	−1.324
ATP5J2	ATP-synthase, H+ transporting, mitochondrial Fo complex subunit F2	−1.297
ATP5L	ATP-synthase, H+ transporting, mitochondrial Fo complex subunit G	−1.337
ATP5O	ATP-synthase, H+ transporting, mitochondrial F1 complex, O subunit	−1.265
ATP5S	ATP-synthase, H+ transporting, mitochondrial Fo complex subunit s	−1.74
MT-ATP6	ATP-synthase F0 subunit 6	−1.522
Associated proteins		
MT-CYB	Cytochrome b	−1.543
CYB5A	Cytochrome b5 type A	−1.471

[1] Fold changes are up or down in animals undergoing compensatory growth compared to those undergoing dietary restriction (RES Period 2 vs RES Period 1).

efficiency (Bottje et al., 2006; Kolath et al., 2006). Typically, in these studies, the rate of mitochondrial respiration was increased in feed efficient animals (Kolath et al., 2006). Similarly, in the context of CG, gene expression studies (Connor et al., 2010; Keogh et al., 2016a) have attributed a potential role for mitochondrial electron transport efficiency towards the expression of CG in cattle, in both liver and muscle tissues. However, although these studies suggest an effect of dietary restriction and CG on the expression of genes involved in oxidative phosphorylation, there is a dearth of information in the literature on the relationship between mitochondrial functionality or capacity in response to both dietary restriction and subsequent CG in cattle. Thus again, the objectives of the current study were to investigate: (i) the molecular control of oxidative phosphorylation gene expression and (ii) the biochemical capacity of mitochondria through an examination of Complex 1 and citrate synthase activities under conditions of both feed restriction and subsequent realimentation in liver tissue. Citrate synthase is a vital enzyme in the control of Kreb's cycle, and its kinetic properties have been shown to be tightly correlated with the taxonomic status of mitochondria (Boushel et al., 2007), consequently measuring the activity of this enzyme is accepted as a reliable proxy for mitochondrial abundance (Spinazzi et al., 2012).

Oxidative phosphorylation, the final stage in aerobic cell respiration, produces ATP following shuttling of electrons through the electron transport chain on the inner mitochondrial membrane (Osellame et al., 2012). Electrons are transferred between four enzyme complexes on the inner mitochondrial membrane, with the final step of ATP production occurring at Complex V (Osellame et al., 2012). At the end of Period 1 in the current study, the oxidative phosphorylation biochemical pathway was significantly enriched, with genes from each of the five complexes (Complex I: *NDUFAF2*, *NDUFB1*, *NDUFB10*; Complex II: *SDHA*, *SDHC*; Complex III: *UQCR11*; Complex IV: *COX8A*; Complex V: *ATP5S*) of this signalling pathway DE between RES and ADLIB animals. Additionally, a gene coding for an electron carrier protein, *CYB5A*, was also DE in animals following a period of dietary restriction compared to their non-restricted counterparts. Overall, these genes were up-regulated in animals undergoing dietary restriction compared to *ad-libitum*-fed animals, with the exception of *CPT1A*, *SDHA*, *SDHC* and *SOD2* genes which were down-regulated. Both *SDHA* and *SDHC* encode subunits of Complex II of the electron transport chain (Osellame et al., 2012). *CPT1A* is involved in fatty acid uptake and subsequent beta-oxidation in the mitochondria (Briant et al., 2018), whilst *SOD2* functions in the removal of mitochondrial reactive oxygen species (**ROS**), providing protection against cellular damage (Zorov et al., 2014). A similar outcome was evident in muscle tissue of the same animals used in the current study, where following a period of dietary restriction, all DEGs pertaining to oxidative phosphorylation were up-regulated in RES compared to ADLIB (Keogh et al., 2016a). These transcriptional modifications observed across both muscle and liver tissues suggest a greater capacity for mitochondrial capacity through potential greater complex activity during dietary restriction. Additionally, *COX8A* which was identified as up-regulated in RES animals at the end of dietary restriction in Period 1 also displayed greater expression in rumen papillae of cattle with greater feed efficiency potential (Kong et al., 2016). However, functional mitochondrial assays performed in the current hepatic-based investigation revealed no difference in the enzyme activity for NADH dehydrogenase (Complex I) between treatment groups at the same time. Furthermore, activity of NADH dehydrogenase was also not different following the period of realimentation and CG at the end of Period 2, suggesting that enhanced mitochondrial complex activity or capacity does not directly contribute to the greater growth rate and efficiency typical of CG. However, although mitochondrial NADH dehydrogenase complex activity was not altered at the end of a period of dietary restriction (Period 1), citrate synthase activity was greater in RES animals following a period of dietary restriction compared to their non-restricted counterparts. Overall, these results suggest that mitochondrial abundance may be increased during dietary restriction. This may represent an adaptive response to increase the efficiency of energy derived from nutrient intake given the restricted dietary allowance these animals were offered. These results indicate that mitochondrial capacity may be contributing to greater dietary nutrient utilization through increasing mitochondrial abundance during dietary restriction.

During electron transport in oxidative phosphorylation, ROS may be produced which could be detrimental to cellular function, through the induction of intracellular oxidative stress (Fleury et al., 2002). In the absence of antioxidants, ROS can cause oxidation of cellular molecules including lipids, proteins and DNA in the mitochondrion as well as in other cellular organelles (Fleury et al., 2002). Previous reports on poultry divergent for feed efficiency have highlighted increased ROS production in feed inefficient broilers (Bottje et al., 2006), suggesting that a reduction in ROS production may be associated with enhanced mitochondrial efficiency. In the current study, a possible reduction in ROS production following a period of dietary restriction was established through down-regulation of *SOD2* in RES animals compared to ADLIB animals in our gene expression results. This gene codes for a member of the iron/manganese superoxide dismutase family, and it functions in the clearance of mitochondrial ROS, providing cellular protection (Zorov et al., 2014). Down-regulation of this gene combined with the up-regulation of genes from complexes I, III-V (complex I: *NDUFAF2, NDUFB1, NDUFB10*; Complex III: *UQCR11*; Complex IV: *COX8A*; Complex V: *ATP5S*) suggests enhanced mitochondrial capacity during dietary restriction concomitant with the lack of need for a protein involved in ROS removal. Additionally, although our functional assessment of Complex I activity showed no effect as a consequence of dietary restriction, this does not mean that the other enzyme complexes were not altered during dietary restriction. Consequently, further analyses are warranted in this area.

The results of our previous work using skeletal muscle from the same animals employed in the current study suggested an increase in lipid beta-oxidation following a period of dietary restriction (Keogh et al., 2016a), which may have been necessary to provide additional energy to skeletal muscle tissue following sustained dietary restriction. Up-regulation of beta-oxidation genes as well as genes coding for proteins involved in Complex II of the electron transport chain in that study suggested the requirement for lipid oxidation to meet energy demands in muscle tissue for mitochondrial ATP production. In the current study, we identified down-regulation of three genes in the liver: *CPT1A*, *SDHA* and *SDHC* in animals that had undergone a period of dietary restriction. *SDHA* and *SDHC* code for components of succinate dehydrogenase or Complex II of the electron transport chain, whereas *CPT1A* codes for the liver-specific rate-controlling enzyme of the lipid beta-oxidation pathway. Moreover, Complex II of the electron transport chain represents the point at which Krebs cycle is combined with the electron transport chain. Down-regulation of these genes in the current study may suggest a lessened requirement to derive energy from lipid energy stores specifically within the liver following a sustained period of dietary restriction. Following a period of dietary restriction, the liver has repeatedly been shown to be one of the most responsive tissues, with a reduction in size and weight evident in numerous studies including the animals used for this current study (Keogh et al., 2015; Fitzsimons et al., 2017). It is hypothesized that this reduction in size contributes to an overall reduction in liver metabolic activity, which may be as a consequence of a reduction in feed intake as well as a reduction in energy maintenance requirements. At the end of 125 days dietary restriction in the current study, the liver was reduced in size and may have adapted to be less reliant on lipid-derived sources for energy and may be more metabolically energetically efficient when compared to skeletal muscle tissue at the same time (Keogh et al., 2015).

Following a period of realimentation at the end of Period 2, the oxidative phosphorylation signalling pathway was not significantly enriched between RES and ADLIB animals. When analysed within treatment across dietary phase, the pathway was significantly enriched within the RES group, with genes coding for each complex as well as

electron carrier proteins DE. During realimentation-induced CG, the opposite effect to that during dietary restriction was apparent, where a large number of genes coding for proteins involved in each enzyme complex of the electron transport chain were down-regulated in RES animals at the end of Period 2 compared to RES animals at the end of Period 1. The same effect was evident in skeletal muscle tissue of the same animals used in this study on day 15 of realimentation (Keogh et al., 2016a). However, these results do not agree with those of Connor et al. (Connor et al., 2010) who observed up-regulation of mitochondrial genes during the very early stage of realimentation, with the authors of that study suggesting that such increased transcriptional activity may infer that enhanced mitochondrial function may be contributing to the expression of CG in cattle. However, in the data of Connor et al. (Connor et al., 2010), the initial up-regulation of oxidative phosphorylation genes was subsequently found to be down-regulated or not detectable by day 14 of realimentation and CG in their study, suggesting that the initial up-regulation of oxidative phosphorylation genes in that study may have represented a latent effect of the previous dietary restriction. Evidence for a reduction in mitochondrial capacity during CG is further established through the reduction in citrate synthase activity measured in RES animals at the end of Period 2 compared to ADLIB animals. Overall, these results suggest a potential reduction in mitochondrial capacity in animals undergoing CG. However, this may reflect a need to maintain cellular homeostasis during greater dietary intake which was evident in these animals undergoing CG (Keogh et al., 2015) and ultimately may provide a method to avoid excessive ROS production during greater dietary intake and associated cellular activity. Although we observed the majority of oxidative phosphorylation genes during realimentation to be down-regulated in animals undergoing CG compared to dietary restriction, the *SDHA* gene, which codes for a subunit of Complex II, was in fact up-regulated in these animals during this time. While we do not know why this gene was up-regulated during this time, the large number of down-regulated genes from each enzyme-complex suggests that overall the expression and potential activity of the enzyme complexes may be reduced during realimentation and CG.

Alternatively, the down-regulation of oxidative phosphorylation genes combined with the lower abundance of mitochondria evident through citrate synthase activity at the end of Period 2 may represent an acquired adaptive response to maintain cellular homeostasis. During realimentation, previously diet-restricted animals typically consume a greater amount of feed, compared to their non-restricted counterparts (Keogh et al., 2015); in order to cope with this increase in intake and associated necessary cellular activity and metabolism, an overall increased requirement for cellular homeostasis may be necessary. This effect has also been observed in skeletal muscle, rumen papillae and jejunal epithelium tissues from the same animals used in the current study, where greater expression of genes involved in cellular survival and homeostasis was evident following a period of realimentation (Keogh et al., 2016a; Keogh et al., 2017; Keogh et al., 2018). Indeed, this hypothesis is further established through the identification of the *UCP2* gene as DE at the end of Period 2 between RES and ADLIB groups. As mentioned above, the oxidative phosphorylation pathway was not significantly enriched between RES and ADLIB groups at the end of Period 2; however, we did identify one gene from this pathway to be DE. *UCP2* was up-regulated in RES animals compared to ADLIB animals. Uncoupling proteins (**uncoupling proteins**) are a heterogeneous family of proteins that are involved in a number of cellular functions including control of ATP synthesis and control of ROS production (Echtay, 2007). Genes coding for uncoupling proteins have previously been reported to be up-regulated in feed inefficient broilers (Ojano-Dirain et al., 2007) and cattle (Tizioto et al., 2015) compared to their efficient counterparts. Uncoupling proteins are mitochondrial transporter proteins that create proton leaks across the inner mitochondrial membrane, thus uncoupling oxidative phosphorylation from ATP synthesis. As a result, energy is dissipated in the form of heat. With greater nutrient intake as was apparent in RES animals during Period 2 (Keogh et al., 2015), mitochondrial ATP synthesis may be increased. With this increase, there may be a build-up of ROS and this may be alleviated through the expression of UCP. Thus, greater expression of this gene during CG may contribute to sustaining cellular homeostasis during realimentation.

In conclusion, the results of the current study suggest an increase in mitochondrial abundance and an enhanced nutrient utilization capacity when dietary restriction is imposed in cattle. Additionally, during dietary restriction, there was also evidence for greater mitochondrial homeostasis through a potential reduction in ROS production. However, gene expression and functional mitochondrial results indicate that an enhancement of mitochondrial capacity may not be central to the expression of CG in cattle during realimentation. Instead, the increased dietary intake and rate of metabolism typical of realimentation and CG may lead to a reduction in mitochondrial processes in order to maintain cellular homeostasis and minimize production of ROS. Although this study, for the first time, shows alterations to mitochondrial gene expression and enzyme activity in cattle in relation to dietary restriction and subsequent CG, further investigations are warranted for the measurement of mitochondrial respiration, proton leak and ROS production during states of both dietary restriction and subsequent CG.

Ethics approval

All procedures involving animals were approved by the University College Dublin, Animal Research Ethics Committee and licensed by the Irish Department of Health and Children in accordance with the European Community Directive 86/609/EC.

Data and model availability statement

The RNA-seq data used for this study are publically available in the NCBI Gene Expression Omnibus https://www.ncbi.nlm.nih.gov/geo/ under accession number GSE64285.

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Author contributions

KK, DAK and RPP conceived the study. SMW and DK procured the funding for the study. KK, SMW and CMcK conducted the laboratory analysis. KK conducted the data analysis and prepared the manuscript. All authors contributed to manuscript editing.

Declaration of interest

The authors declare that they have no competing interests.

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