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Mitochondrial abundance and function in skeletal muscle and liver from Simmental beef cattle divergent for residual feed intake

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*Cellular mitochondrial function has been suggested to contribute to variation in feed efficiency (**FE**) among animals. The objective of this study was to determine mitochondrial abundance and activities of various mitochondrial respiratory chain complexes (complex I (**CI**) to complex IV (**CIV**)) in liver and muscle tissue from beef cattle phenotypically divergent for residual feed intake (**RFI**), a measure of FE. Individual DM intake (**DMI**) and growth were measured in purebred Simmental heifers (*n = *24) and bulls (*n = *28) with an initial mean BW (SD) of 372 kg (39.6) and 387 kg (50.6), respectively. All animals were offered concentrates* ad libitum *and 3 kg of grass silage daily, and feed intake was recorded for 70 days. Residuals of the regression of DMI on average daily gain (**ADG**), mid-test BW$^{0.75}$ and backfat (**BF**), using all animals, were used to compute individual RFI coefficients. Animals were ranked within sex, by RFI into high (inefficient; top third of the population), medium (middle third of population) and low (efficient; bottom third of the population) terciles. Statistical analysis was carried out using the MIXED procedure of SAS v 9.3. Overall mean ADG (SD) and daily DMI (SD) for heifers were 1.2 (0.4) and 9.1 (0.5) kg, respectively, and for bulls were 1.8 (0.3) and 9.5 (1.02) kg, respectively. Heifers and bulls ranked as high RFI consumed 10% and 15% more (*P < 0.05*), respectively, than their low RFI counterparts. There was no effect of RFI on mitochondrial abundance in either liver or muscle (*P > 0.05*). An RFI × sex interaction was apparent for CI activity in muscle. High RFI animals had an increased activity (*P < 0.05*) of CIV in liver tissue compared to their low RFI counterparts; however, the relevance of that observation is not clear. Our data provide no clear evidence that cellular mitochondrial function within either skeletal muscle or hepatic tissue has an appreciable contributory role to overall variation in FE among beef cattle.*

Keywords: feed efficiency, gender, oxidative phosphorylation, citrate synthase, bovine

Implications

Selection for low residual intake facilitates improved feed efficiency in beef cattle. However, the cost of identifying low residual feed intake cattle is a limitation to genetic progress in the area. Elucidating the biology underpinning residual feed intake will provide critical information about the potential scientific utility of biomarkers for the trait which will in turn aid in more widespread adoption within beef cattle breeding programmes. While a relationship between mitochondrial abundance/activity and residual feed intake in beef cattle has previously been hypothesised, there is little published information to substantiate this. This study provides evidence highlighting that (i) differing potential for cellular mitochondrial abundance or functionality in muscle and liver is not a major contributor to variation in feed efficiency in beef cattle and (ii) this observation is consistent across gender.

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Introduction

Feed provision is the main cost in beef cattle production systems and consequently there is considerable interest in improved feed efficiency (**FE**) as a means of increasing the economic and environmental sustainability of such enterprises (Fitzsimons *et al.*, 2017; Kenny *et al.*, 2018). Residual feed intake (**RFI**), defined as the difference between observed and expected feed intake requirements for maintenance of BW and growth (Koch *et al.*, 1963; Savietto *et al.*, 2014) is considered a useful index to examine the biological mechanisms associated with variation in FE due to its phenotypical independence from animal performance traits (Berry and Crowley, 2013). In addition, RFI is moderately heritable (Crews *et al.*, 2004; Crowley *et al.*, 2010) which has led to interest in the trait as a candidate for genetic improvement programmes. Research to date has demonstrated that RFI is a complex multifaceted trait (Fitzsimons *et al.*, 2017); thus, like

all key performance traits, a solid understanding of its underlying biological control is necessary to increase both the utility and accuracy of genomic selection-based approaches. While it has been estimated that two-thirds of variation in RFI can be explained by inter-animal variation in the metabolic processes associated with energy expenditure (Herd and Arthur, 2009), there are little data available to substantiate this. Mitochondria account for up to 90% of cellular oxygen consumption and the bulk of adenine tri-phosphate (**ATP**) synthesis (Herd and Arthur, 2009) and thus have an appreciable impact on overall metabolic efficiency in mammals. The electron transport chain comprises four multi-protein complexes (I to IV) which shuttle electrons down the chain creating a proton motive force that drives ATP synthesis (Berg *et al*., 2002). Research indicates differences in activity of the electron transport chain in skeletal muscle (Iqbal *et al*., 2004) and liver (Iqbal *et al*., 2005) of broilers and in the skeletal muscle of lambs (Sharifabadi *et al*., 2012) varying in FE. Furthermore, Kolath *et al.* (2006) demonstrated that efficient cattle have an increased rate of mitochondrial respiration than their inefficient counterparts. Despite this equivocation remains in the published literature regarding the relationship between RFI and mitochondrial abundance and function in beef cattle. The aim of the current study was to investigate whether RFI phenotype has a basis in differential mitochondrial abundance and/or function in two key metabolic organs: liver and muscle, and whether effects were consistent across sex.

Material and methods

Animals and management

A total of 52 purebred Simmental beef cattle (24 heifers and 28 bulls) as previously described by McKenna *et al.* (2018) were used in this study. These animals were derived from a herd previously described by Fitzsimons *et al.* (2014a) in which animals were phenotypically ranked on RFI and characterised for a large number of traits. For the current experiment, the highest ($n = 20$) and lowest ($n = 20$) ranking cows and heifers based on RFI phenotype were bred using artificial insemination and multiple ovulation/embryo recovery technologies, to pedigree AI Simmental sires with estimated breeding values for high or low RFI, respectively (Crowley *et al*., 2010). Frozen-thawed embryos were transferred to crossbred recipients, and the resulting calves were used for the purpose of this study (Šavc *et al*., 2016). Calves were reared by their dams for the first week of life after which they were weaned and subsequently artificially reared on milk replacer and concentrate using an electronic feeding system (Vario; Foster-Tecknik, Engen, Germany) as described by Byrne *et al.* (2017). Following weaning from milk replacer, calves were turned out to pasture and rotationally grazed on perennial ryegrass-dominated swards. At approximately 15 months of age, cattle were housed within pens (heifers and bulls were accommodated separately within the same shed) of between five and seven animals/pen in a slatted floor shed (lying area = 2.82 m^2/animal). Cattle were fed once daily (0800 h) using the Calan gate feeding system (American Calan Inc., Northwood, NH, USA) and were offered concentrate (860 g/kg rolled barley, 60 g/kg soya bean meal, 60 g/kg molasses and 20 g/kg minerals/vitamins) *ad libitum* and 3 kg grass silage to support ruminal function. All animals had continuous access to clean fresh drinking water. Cattle had an acclimatisation period of 14 days to the *ad libitum* dietary regime and test facilities before the experimental recording period commenced, which lasted for 70 days. Mean age (SD) at the start of the RFI measurement period was 413 (24.96) and 422 (23.21) days for heifers and bulls, respectively.

Feed analysis. Concentrate and silage offered was sampled three times weekly and samples were stored at -20°C pending laboratory analysis. Samples of concentrates and silage were subsequently pooled on a weekly basis for DM determination. Concentrate samples were dried in an oven with forced-air circulation at 98°C for 16 h for DM determination and forage samples dried at 40°C for 48 h.

BW and body measurements. Body weight and measurements for this trial have previously been described by McKenna *et al.* (2018). Briefly, animal weights were recorded prior to feeding, at the start and at the end of the trial and on a weekly basis throughout the RFI measurement period. Ultrasonic measurements were taken at the start and end of the RFI measurement period to measure the fat depth at the third lumbar vertebra, the 13th thoracic rib and the rump on the animal's right side.

Computation of traits. Calculation of RFI and other growth traits for heifers and bulls has been previously described by McKenna *et al.* (2018). Briefly, heifers and bulls were considered as separate groups for computation of traits and statistical analysis. Expected DM intake (**DMI**) was computed for each animal using a multiple regression model, regressing DMI on metabolic BW (**MBW**), average daily gain (**ADG**) and mean lumbar backfat (**BF**) change (mm). Some, though not all studies have reported a relationship between ultrasonically measured BF deposition and RFI status in beef cattle and have identified this trait as a small but statistically significant energetic sink. This has been manifested as animals with favourable phenotypes for RFI having leaner carcasses (Nkrumah *et al*., 2007) which can lead to unfavourable implications for carcass quality (Arthur *et al*., 2001; Moraes *et al*., 2017). For this reason, the current study corrected for BF when selecting for RFI to avoid potential negative effects of co-selecting for leaner animals (Hill and Ahola, 2012). Residual feed intake was calculated within sex for each animal as the difference between actual DMI and expected DMI.

The model used to compute expected DMI, as described by McKenna *et al.* (2018), was

$$Y_j = \beta_0 + \tau_i + \beta_1 \text{MBW}_j + \beta_2 \text{ADG}_j + \beta_3 \text{BF}_j + e_j$$

where Y_j is the average DMI for the jth animal, β_0 is the regression intercept, τ_i is the fixed effect of the ith day of birth, β_1 is the partial regression coefficient for MBW, β_2 is the regression coefficient for ADG, β_3 is the regression coefficient for BF and e_j is the random error associated with the jth animal. The coefficient of determination (R^2)for the statistical model produced from this equation accounted for 0.7 ($P < 0.001$) of the variation in DMI and was used to predict DMI for each animal (Supplementary Material S1). Animals were ranked according to RFI phenotypes within sex and then divided into equal terciles of low (efficient), medium and high RFI (inefficient) resulting in eight animals per group in each of the high and low RFI heifer groups, and nine animals per group in each of the high and low RFI bull groups, respectively.

Biopsy sample collection. M. longissimus dorsi biopsies were harvested as described by Kelly *et al.* (2011), and liver tissue was collected by percutaneous punch biopsy as described by McCarthy *et al.* (2009) from animals deemed high and low RFI under local anaesthetic (5 ml s.c. Adrenacaine; Norbrook Laboratories (Ireland) Ltd, Newry, Co. Down, Northern Ireland) at the end of the RFI measurement period. All surgical instruments used for tissue collection were sterilised and treated with 70% ethanol and RNaseZap (Ambion, Applera Ireland, Dublin, Ireland). *M. longissimus dorsi* biopsies were snap frozen in liquid nitrogen directly after collection, and liver biopsies were washed in sterile Dulbecco's phosphate-buffered saline (Fisher Scientific, Dublin, Ireland) and snap frozen in liquid nitrogen. All samples were subsequently stored at −80°C pending further processing.

Tissue homogenate preparation and total protein determination

Tissue homogenates of *M. longissimus dorsi* and liver biopsy samples were prepared as outlined by Spinazzi *et al.* (2012) with slight modifications. Briefly, visible fat and connective tissue were removed from 50 mg of tissue, which was then dissected into small fragments. Tissue was diluted at a ratio of 1 : 20 in ice : cold sucrose (250 mM) homogenisation buffer (pH 7.4) containing tris(hydroxymethyl)aminomethane (**Tris**; Sigma Aldrich Ireland Ltd, Vale Road, Arklow, Wicklow, Ireland), potassium chloride (Sigma Aldrich Ireland Ltd) and ethylenebis(oxyethylenenitrilo)tetraacetic acid (Sigma Aldrich Ireland Ltd) diluted in distilled water. Sucrose (Sigma Aldrich Ireland Ltd) was added on day of use. Tissue was homogenised using a clean glass conical tissue grinder on ice. Homogenate was centrifuged at 600 **g** for 10 min at 4°C and supernatant was kept on ice and used on the same day. Protein concentration of homogenates was determined using the Pierce BCA Protein Assay Kit (Fisher Scientific).

Citrate synthase assay

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

$$\text{Acetyl-CoA} + \text{oxaloacetate} + \text{H}_2\text{O} \rightarrow \text{citrate} + \text{CoA-SH} \quad (1)$$

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

The reaction product 5-thio-2-nitrobenzoic acid has an intense absorption at 412 nm and the absorbance increases linearly with time. This increase in absorption was measured using the Shimadzu UV-2600 spectrophotometer for 4 min and rate of reaction was calculated. The enzymatic activity of citrate synthase was calculated as nmol/min per mg mitochondrial protein using the molar extinction coefficient for citrate synthase, which is 13.6 mM/cm (Spinazzi *et al.*, 2012).

Measurement of mitochondrial electron transport chain complex activities

The activities of electron transport chain complexes were assessed in muscle and liver biopsy samples by UV spectrophotometry as described by Spinazzi *et al.* (2012) with modifications using a UV spectrophotometer. All assays were performed in duplicate at 37°C in 2 ml cuvettes. Activities of all complexes were calculated as nmol/min per mg of protein and expressed in units normalised to citrate synthase activity which is a marker of mitochondrial abundance within a tissue/cell.

Complex I activity. The activity of complex I (**CI**) was assayed as a decrease in absorbance at 340 nm by following the oxidation of reduced NAD (Sigma Aldrich Ireland Ltd). Mitochondria (40 μg of tissue homongenate) were incubated at 37°C for 2 min in 700 μl of reaction medium (50 mM Tris-hydrogen chloride (Sigma Aldrich Ireland Ltd) with 3 mg/ml bovine serum albumin (**BSA**; Fisher Scientific), pH 8.0; 300 μM potassium cyanide (**KCN**; Sigma Aldrich Ireland Ltd) and 100 μM NADH). Sixty micromolar Ubiquinone (coenzyme Q1) was added to initiate the reaction. Absorbance was monitored for 3 min before and after the addition of 10 μM Rotenone (Sigma Aldrich Ireland Ltd). 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 CI was subsequently quantified using an extinction coefficient of 6.22 mM/cm (Spinazzi *et al.*, 2012).

Complex II activity. Complex II (**CII**) activity was determined by following the secondary reduction of 2, 6-dichlorophenolindophenol (**DCPIP**; Sigma Aldrich Ireland Ltd) by decylubiquinol (**DUB**; Sigma Aldrich Ireland Ltd) at 600 nm. Mitochondria (2 μg of protein) were added to a buffer containing 25 mM potassium dihydrogen phosphate (**KH_2PO_4**; Sigma Aldrich Ireland Ltd), 0.10 mM EDTA (Sigma Aldrich Ireland Ltd), BSA (1 mg/ml), 300 μM KCN, 80 μM DCPIP and 20 mM succinate (Sigma Aldrich Ireland Ltd) and incubated for 10 min. Fifty micromolar DUB was added and the reaction was initiated with the addition. Absorbance

was monitored for 3 min before and after the addition of 10 mM malonate (Sigma Aldrich Ireland Ltd) which acts as an inhibitor of the reaction. The difference in the decrease in absorption before and after the addition of malonate represents the complex activity. The extinction coefficient used for this reaction was 19.1 mM/cm (Spinazzi *et al.*, 2012).

Complex III activity. The assay was performed at 550 nm by monitoring the rate of reduction of cytochrome c by ubiquinol-2. Mitochondria (3 μg of protein) were added to medium containing 25 mM KH_2PO_4 (pH 7.8), 0.10 mM EDTA and 75 μM oxidised cytochrome c (Sigma Aldrich Ireland Ltd) and left to incubate for 2 min. The reaction was initiated by the addition of 100 μM decylubiquinol (Sigma Aldrich Ireland Ltd) and followed for 2 min. The non-enzymatic reduction of cytochrome c was measured after the addition of 4 μM antimycin A (Sigma Aldrich Ireland Ltd); the specific activity of complex III (**CIII**) was calculated by subtracting the non-enzymatic rate. An extinction coefficient of 18.5 mM/cm was used (Spinazzi *et al.*, 2012).

Complex IV activity. The activity of complex IV (**CIV**) was measured by following the oxidation of reduced cytochrome c (cytochrome c was reduced with sodium dithionite) as a decrease in absorbance at 550 nm. The reaction medium contained 25 mM KH_2PO_4, 1 mg/ml defatted BSA and mitochondria (25 to 40 μg of protein). The reaction was initiated by adding 60 μM reduced cytochrome c, and the reaction was followed for 60 s. The specific activity was calculated by using 18.5 mM/cm as the extinction coefficient (Spinazzi *et al.*, 2012).

Complex I + III combined activity. In order to measure the flow of electrons between complexes, the combined activity of CI + CIII was measured by monitoring the reduction of oxidised cytochrome c at 550 nm. Mitochondria (25 μg of protein) were added to a medium containing 50 mM KH_2PO_4, 1 mg/ml BSA, 300 μM KCN and 50 μM oxidised cytochrome c and left to incubate, and the baseline for the reaction was recorded for 2 min. The reaction was initiated by the addition of 200 μM NADH. The differences in absorption were measured in the presence and absence of rotenone, and the combined specific activity was recorded as the rotenone sensitive activity. The specific activity was calculated by using 18.5 mM/cm extinction coefficient (Spinazzi *et al.*, 2012).

Complex II + III combined activity. In order to measure the flow of electrons between complexes, the combined activity of CII + CIII was measured by monitoring the reduction of oxidised cytochrome c at 550 nm in the presence of succinate. Mitochondria (3 μg of protein) were added to medium containing 20 mM KH_2PO_4, 1 mg/ml BSA, 300 μM KCN and 10 mM succinate. Samples were pre-incubated with succinate for 10 min to fully activate the enzyme. The reaction was initiated by the addition of 50 μM oxidised cytochrome c. The differences in absorption were measured in the presence and absence of malonate, and the combined specific activity was deemed as the malonate sensitive activity. The specific activity was calculated by using 18.5 mM/cm extinction coefficient (Spinazzi *et al.*, 2012).

Statistical analysis

Normality of data distribution was tested using the UNIVARIATE procedure of SAS 9.1 (SAS Institute Inc., Cary, NC, USA). The LSMEANS statement in PROC MIXED SAS was used to examine the effect of RFI grouping on feed intake, performance traits, body composition, mitochondrial complex activities and mitochondrial abundance. The statistical model used included the fixed effect of RFI group (high *v.* low), sex (heifer *v.* bull) and RFI group × sex interaction. A random sire effect was included in the final model for all traits. Animal age in days was included in the model as a linear covariate. Differences in RFI group were determined by *F* tests using type III sums of squares. The PDIFF option and the Tukey test were applied as appropriate to evaluate pairwise comparisons between RFI group means. The df method used was Kenward Roger and an unstructured covariance structure was chosen as the model included a random term for sire. Effects were considered statistically significant with an adjusted $P < 0.05$ and considered a tendency towards statistical significance with an adjusted $P < 0.10$.

Results

Animal performance and residual feed intake

Animal performance and FE data have been described previously by McKenna *et al.* (2018); however, results pertaining to animals specific to this study have been presented again in Table 1. There were no interactions among the main effects for RFI, DMI, MBW, initial BW, final BW, ADG and BF change ($P > 0.05$). Animals of high and low RFI did not differ ($P > 0.05$) in initial BW, final BW or ADG. There was an effect of sex ($P < 0.05$) on RFI, final BW and ADG. Bulls had greater variation in RFI coefficients, a higher final BW and a greater ADG than heifers. As reported by McKenna *et al.* (2018), heifers and bulls had a mean initial BW (SD) of 372 (39.6) and 387 (50.6) kg, an ADG of 1.2 (0.4) and 1.8 (0.3) kg and DMI of 9.1 (0.5) and 9.5 (1) kg, respectively, RFI averaged 0.00 for both sexes and ranged from −0.4 to 0.6 kg of DM/day for heifers and −0.5 to 0.5 kg DM/day for bulls. Heifers and bulls ranked as high RFI consumed 10% and 15% more than their low RFI counterparts ($P < 0.05$), respectively.

Citrate synthase assay

Results for citrate synthase assays in muscle and liver tissue are presented in Table 2. There was no RFI × sex interaction and no effect ($P > 0.05$) of RFI or sex on citrate synthase activity in muscle or liver tissue.

Mitochondrial electron transport chain complex activities

Results for mitochondrial electron transport chain complex activity assays are presented in Table 2. There was an

Table 1 *Summary of mean phenotypic data of cattle divergent for residual feed intake (RFI)*

	RFI group			Sex			*P*-value		
Trait	High	Low	SD	Bulls	Heifers	SD	RFI	Sex	RFI × sex
No. of animals	17	17	–	18	16	–	–	–	–
DMI (kg/day)	9.70	8.80	0.20	9.47	9.10	0.21	**	ns	ns
RFI (kg DM/day)	0.40	−0.40	0.02	0.03	−0.30	0.01	***	ns	ns
MBW ($kg^{0.75}$)	95.00	95.00	2.50	97.00	93.00	2.51	ns	ns	ns
Initial BW (kg)	382.00	373.00	16.00	382.00	372.00	16.50	ns	ns	ns
Final BW (kg)	488.00	483.00	16.70	509.00	462.00	17.20	ns	**	ns
ADG (kg)	1.50	1.50	0.13	1.80	1.20	0.14	ns	***	ns
Backfat change (mm)	1.30	1.70	0.24	1.30	1.70	0.25	ns	ns	ns

ns = not significant; DMI = DM intake; MBW = mid-test metabolic BW; ADG = Average daily gain.
** $P < 0.01$, *** $P < 0.001$.

Table 2 *Mean activity of citrate synthase and complexes I to IV in muscle and liver of cattle divergent for residual feed intake (RFI)*

	RFI group			Sex			*P*-value		
Enzyme activity	High	Low	SD	Bulls	Heifers	SD	RFI	Sex	RFI × sex
No. of animals	17	17	–	18	16	–	–	–	–
Citrate synthase (CS) (nmol/min per mg)									
Muscle	138.00	137.00	25.90	146.00	129.00	26.60	ns	ns	ns
Liver	61.00	82.00	19.20	80.00	63.00	19.70	ns	ns	ns
Complex activity in muscle (unit/cs)									
CI	0.10	0.10	0.03	0.10	0.20	0.03	ns	**	*
CII	0.40	0.40	0.22	0.20	0.50	0.23	ns	ns	ns
CIII	0.20	0.20	0.04	0.10	0.20	0.05	ns	ns	ns
CIV	0.70	0.40	0.30	0.30	0.80	0.31	ns	ns	ns
Complex activity in liver (unit/cs)									
CI	1.20	0.90	0.30	1.00	1.20	0.31	ns	ns	ns
CII	1.80	1.90	0.55	2.00	1.80	0.56	ns	ns	ns
CIII	0.50	0.40	0.15	0.30	0.50	0.16	ns	ns	ns
CIV	3.20	2.10	0.44	2.40	2.90	0.45	*	ns	ns

nmol/min per mg = specific activity of enzymes; ns = not significant; unit/cs = specific activity normalised to citrate synthase activity; CI to IV = complex I to IV.
* $P < 0.05$, ** $P < 0.01$.

Table 3 *Mean combined activities of complexes I + III and II + III in muscle and liver of cattle divergent for residual feed intake (RFI)*

	RFI group			Sex			*P*-value		
Enzyme activity	High	Low	SD	Bulls	Heifers	SD	RFI	Sex	RFI × sex
No. of animals	17	17	–	18	16	–	–	–	–
C I + III muscle (unit/cs)	0.22	0.15	0.09	0.11	0.26	0.09	ns	ns	ns
C II + III muscle (unit/cs)	0.11	0.09	0.04	0.08	0.11	0.04	ns	ns	ns
C I + III liver (unit/cs)	0.29	0.21	0.07	0.29	0.21	0.08	ns	ns	ns
C II + III liver (unit/cs)	2.21	4.26	2.04	2.64	3.82	1.17	ns	ns	ns

CI to III = Complex I to III; unit/cs = specific activity normalised to citrate synthase activity; ns = not significant.

RFI × sex interaction ($P < 0.05$) for CI activity in muscle tissue, whereby high RFI heifers had higher activity than low RFI heifers ($P = 0.03$) and high RFI bulls tended to have a lower activity than low RFI bulls ($P = 0.07$). Sex affected ($P < 0.01$) CI activity in muscle tissue manifested as heifers having a higher concentration of CI activity than bulls. There was an effect of RFI status ($P < 0.05$) on CIV activity in the liver tissue, whereby high RFI animals had higher activity than low RFI animals.

Combined activities of electron transport chain complexes. Results for the combined activities of CI + CIII and CII + CIII in muscle and liver tissue are presented in Table 3. There was no RFI × sex interaction or effect of sex or RFI status

($P > 0.05$) for activity of CI + CIII or CII + CIII in muscle or liver tissue.

Discussion

The objectives of this study were firstly to examine the effects of RFI status and sex on key performance traits in beef cattle and secondly to examine if mitochondrial abundance and functionality in two metabolically important tissues are affected by either sex or RFI status. The main findings of this study were firstly, that mitochondrial abundance in either *longissimus dorsi* or liver tissue is not affected by RFI status in beef cattle. Secondly, we conclude that while CI activity in muscle and CIV activity in liver are altered in response to RFI status, the overall function of the respiratory chain does not apparently contribute to variation in energetic efficiency among growing beef cattle.

The ADG of the heifers (mean 1.2 kg) in this study was lower than the bulls (mean 1.8 kg) which is not surprising, and it has been widely documented that heifers have a slower growth rate than bulls when managed similarly (McDonald *et al.*, 2002). In addition, the majority of studies comparing growth rate across sexes are confounded with 'system related' effects, and the current study is unique in that it presents results for a contemporary group of genetically similar purebred males and females reared similarly from birth to slaughter. The range in ADG observed was consistent with that of growing beef cattle offered an energy dense diet to appetite (Fitzsimons *et al.*, 2013; Fitzsimons *et al.*, 2014b).

Consistent with our results, previous research has demonstrated that when managed similarly bulls have a higher growth potential than heifers (Bureš and Bartoň, 2012). While BF accretion was numerically greater for heifers compared with bulls, as might be expected, this difference did not reach statistical significance. Additionally, RFI status by design did not affect not only BW change but also BF accretion for either sex, which is consistent with some (Basarab *et al.*, 2003; Nkrumah *et al.*, 2007) but not all studies (Gomes *et al.*, 2012; Fitzsimons *et al.*, 2014b). In accordance with our experimental design, no differences in animal performance were observed between the RFI groups, despite a 10% and 15% difference in DMI within heifers and bulls, respectively. This finding is in agreement with other studies examining the relationship between RFI and other productivity traits where RFI status was not found, either phenotypically (Niemann *et al.*, 2011) or genetically (Crowley *et al.*, 2010), to influence weight or growth rate of beef cattle.

The linear regression model used to compute RFI in the current study accounted for 70% of the variation in DMI. This value is within the range obtained by Fitzsimons *et al.* (2014b) where energy dense diets were offered to cattle of a similar age and genotype. This higher coefficient of determination is not surprising considering animals were offered an energy dense low forage diet. Such diets support high digestibility, low rumen fill and a fast ruminal passage rate compared to more fibrous diets (Forbes, 2007).

There is equivocation in the published literature on the relationship between FE status and measures of body fat content (Fitzsimons *et al.*, 2017). For example, some studies that used the base model (MBW and ADG) to predict DMI have reported either a positive (Arthur *et al.*, 2001; Basarab *et al.*, 2003; Basarab *et al.*, 2007) or no relationship (Gomes *et al.*, 2012; Fitzsimons *et al.*, 2014b) between FE and carcass fatness traits (see review by Kenny *et al.*, 2018). In the current study, we included an adjustment for BF accretion in our DMI prediction model. The conflicting literature regarding the relationship between RFI and subcutaneous BF depth may be due to the variation in the extent and timing of fat deposition in different breeds and also differences in the site selected for ultrasonic measurements. As expected, BF gain was numerically higher for heifers compared with bulls though this bordered ($P = 0.10$) on statistical significance.

Citrate synthase is a vital enzyme in the control of the Krebs cycle and its kinetic properties have been shown to be tightly correlated with the taxonomic status of mitochondria (Else *et al.*, 1988). Measuring the activity of this enzyme is accepted as a reliable proxy for mitochondrial abundance and can be used to normalise mitochondrial functionality, and the use of this assay has been well documented (Spinazzi *et al.*, 2012). A relationship between mitochondrial function and FE has been well documented (Fitzsimons *et al.*, 2017). Therefore, we hypothesised that given the metabolic importance of liver and muscle, and the observations of Bottje *et al.* (2017) who concluded that more efficient broilers had a higher mitochondrial RNA content, an increase in mitochondrial number in these organs could be contributing to variation in RFI. Despite this, we observed no differences in mitochondrial abundance in muscle or liver in animals divergent for RFI, a novel finding in beef cattle. This led us to further speculate that while mitochondrial number is unaffected, mitochondria may be functioning at different efficiencies between the two phenotypes.

As aforementioned, there is a plethora of information relating to mitochondrial function and RFI across a number of species (Fitzsimons *et al.*, 2017). Notwithstanding this, however, there is conflict, however, among published reports in that some authors have observed a higher activity of respiratory chain enzymes in various tissues from more efficient animals (Sharifabadi *et al.*, 2012; Bottje and Kong, 2013) while others have observed a decrease in activities of these enzymes in more efficient animals (Sandelin, 2005). For the majority of these enzyme complexes, we did not see any difference in activity indices for either liver or muscle tissue between RFI classifications, which would indicate that as a whole there is no difference in the function of the electron transport chain between RFI classifications which is consistent with the observations of Kolath *et al.* (2006). Complex I is the initiation point for the electron transport chain and is the largest enzyme in the chain. It has previously been found to be associated with FE at a transcript level in

cattle (Ramos and Kerley, 2013). We did observe an RFI × sex interaction for CI activity in muscle tissue. This observation could indicate that there is a potential relationship between CI and RFI, but it may be a function of sex and warrants further investigation. We observed an effect of RFI on CIV in liver, in which, those animals ranked as high had a higher concentration of this enzyme than their lower RFI contemporaries. This is in agreement with Sandelin *et al.* (2005) who measured protein abundance of the complexes and may be an indication that the mitochondria in the less efficient animals have to 'work harder' to maintain energy homeostasis.

Mitochondrial respiration is the product of the combined action of the different components of the electron transport chain. Firstly, the actions of the complexes, and secondly the electron carriers, coenzyme Q_{10} and cytochrome c, are required to transport electrons from CI and CII to CIII and subsequently the electrons are transported to CIV by cytochrome c. In the individual enzyme complex assays, the electron carriers were included in the assay reaction as electron acceptors or donors (Schwarzer, 2016). However, defects in mitochondrial respiration due to changes in the interaction of individual complexes or limited amounts of endogenous electron carriers may be missed under these reaction conditions. For example, coenzyme Q_{10} deficiency is known to impair mitochondrial function, even in the absence of defects in the mitochondrial complexes (Rooney *et al.*, 2017). With this in mind, it was decided to carry out specific experiments to assess the combined action of two complexes, that is, CI + III activity or CII + III activity (Díaz *et al.*, 2009). We observed no differences in the combined activities of the electron carrier complexes, coenzyme Q_{10} and cytochrome c, between animals divergent for RFI, indicating that there were no differences in electron flow through the mitochondrial respiratory chain between the phenotypes. These observations combined with those of the complex assays provide further evidence that the activity of the electron transport chain is not affected by phenotypic RFI.

In conclusion, our findings highlight that mitochondrial abundance is not related to variation in FE in cattle as measured by RFI. It follows that any changes observed in mitochondrial activity in muscle and liver tissue are as a result of differences in mitochondrial functionality and not due to the presence of more mitochondria. While our work and the work of others highlight a potential shift in energy metabolism between phenotypes, we conclude that it is unlikely that differing potential for cellular mitochondrial functionality is a major contributor to variation in FE in cattle. A logistical limitation of the present study was that frozen tissue was used and, thus, the mitochondria in the respective tissues were no longer respiring. Future investigation should focus on work with fresh biopsy samples. This would allow for measurement of mitochondrial respiration, proton leak and ROS production which may provide further insight into the potential impact of cellular mitochondrial function on variation in the expression of phenotypic RFI.

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Declaration of interest

The authors declare that they have no competing interest.

Ethics statement

All procedures involving animals in this study were conducted under an experimental licence (AE19132/PO11) from the Health Products Regulatory Authority in accordance with the cruelty to Animals Act 1876 and the European Communities (Amendment of Cruelty to Animals Act 1876) Regulation 2002 and 2005.

Software and data repository resources

None of the data were deposited in an official repository.

Supplementary material

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