

University of Nebraska - Lincoln

DigitalCommons@University of Nebraska - Lincoln

---

Roman L. Hruska U.S. Meat Animal Research  
Center

U.S. Department of Agriculture: Agricultural  
Research Service, Lincoln, Nebraska

---

12-1-2021

## Transcriptome profiles of the skeletal muscle of mature cows during feed restriction and realimentation

Hannah C. Cunningham-Hollinger  
*University of Wyoming*

Larry A. Kuehn  
*USDA ARS Roman L. Hruska U.S. Meat Animal Research Center*

Kristi M. Cammack  
*South Dakota State University*

Kristin E. Hales  
*USDA ARS Roman L. Hruska U.S. Meat Animal Research Center*

William T. Oliver  
*USDA ARS Roman L. Hruska U.S. Meat Animal Research Center*

*See next page for additional authors*

Follow this and additional works at: <https://digitalcommons.unl.edu/hruskareports>



Part of the [Beef Science Commons](#), and the [Meat Science Commons](#)

---

Cunningham-Hollinger, Hannah C.; Kuehn, Larry A.; Cammack, Kristi M.; Hales, Kristin E.; Oliver, William T.; Crouse, Matthew S.; Chen, Celine; Freetly, Harvey C.; and Lindholm-Perry, Amanda K., "Transcriptome profiles of the skeletal muscle of mature cows during feed restriction and realimentation" (2021). *Roman L. Hruska U.S. Meat Animal Research Center*. 528.  
<https://digitalcommons.unl.edu/hruskareports/528>

This Article is brought to you for free and open access by the U.S. Department of Agriculture: Agricultural Research Service, Lincoln, Nebraska at DigitalCommons@University of Nebraska - Lincoln. It has been accepted for inclusion in Roman L. Hruska U.S. Meat Animal Research Center by an authorized administrator of DigitalCommons@University of Nebraska - Lincoln.

---

**Authors**


Hannah C. Cunningham-Hollinger, Larry A. Kuehn, Kristi M. Cammack, Kristin E. Hales, William T. Oliver, Matthew S. Crouse, Celine Chen, Harvey C. Freetly, and Amanda K. Lindholm-Perry

RESEARCH NOTE

Open Access



# Transcriptome profiles of the skeletal muscle of mature cows during feed restriction and realimentation

Hannah C. Cunningham-Hollinger<sup>1</sup>, Larry A. Kuehn<sup>3</sup>, Kristi M. Cammack<sup>2</sup>, Kristin E. Hales<sup>3</sup>, William T. Oliver<sup>3</sup>, Matthew S. Crouse<sup>3</sup>, Celine Chen<sup>4</sup>, Harvey C. Freetly<sup>3</sup> and Amanda K. Lindholm-Perry<sup>3\*</sup> 

## Abstract

**Objective:** Realimentation can compensate for weight loss from poor-quality feedstuffs or drought. Mature cows fluctuate in body weight throughout the year due to nutrient availability. The objective of this study was to determine whether cows that differ in weight gain during realimentation also differ in the abundance of transcripts for enzymes associated with energy utilization in skeletal muscle. Mature cows were subjected to feed restriction followed by ad libitum feed. Skeletal muscle transcriptome expression differences during the two feeding periods were determined from cows with greater ( $n = 6$ ) and less ( $n = 6$ ) weight gain during the ad libitum feeding period.

**Results:** A total of 567 differentially expressed genes (408 up- and 159 down-regulated) were identified for the comparison of restriction and ad libitum periods ( $P_{\text{Bonferroni}} < 0.05$ ). These genes were over-represented in lysosome, aminoacyl-tRNA biosynthesis, and glutathione metabolism pathways. Validation of the expression of five of the genes was performed and four were confirmed. These data suggest that realimentation weight gain for all cows is partially controlled by protein turnover, but oxidative stress and cellular signaling pathways are also involved in the muscle tissue. This dataset provides insight into molecular mechanisms utilized by mature cows during realimentation after a period of low abundance feed.

**Keywords:** Skeletal muscle, Microarray, Transcriptome, Body weight gain

## Introduction

Cattle producers are faced with the challenge of increasing production to feed the growing population, and over 2/3 of the budget is the feed to achieve production goals. Compensatory gain (CG) increases production while maintaining or even decreasing inputs and affects an animal's lean tissue and fat deposition. Forage quality fluctuates with season; thus, the mature beef cow can experience periods of nutrient restriction followed by realimentation. This, along with the utilization of CG

for improved feed efficiency, highlights the necessity to understand the mechanisms that allow for certain cattle to respond differently to nutritional shifts.

Previous work investigated these mechanisms in the adipose tissue of mature cows [1] and discovered key metabolic and signaling pathways during CG. Another critical tissue to evaluate for transcriptional response to CG is skeletal muscle. Caton et al. [2] shows that muscle accounts for 21% of total energy; muscle and adipose combined represent 27%. The role of skeletal muscle in energy metabolism substantiates the need to understand the transcriptional response.

Keogh et al. [3] investigated the transcriptome response to CG in the muscle of bulls and found that biological processes associated with feed restriction were lipid

\*Correspondence: [Amanda.lindholm@usda.gov](mailto:Amanda.lindholm@usda.gov)

<sup>3</sup> USDA, ARS, U.S. Meat Animal Research Center, P.O. Box 166, Clay Center, NE 68933, USA

Full list of author information is available at the end of the article



© The Author(s) 2021. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>. The Creative Commons Public Domain Dedication waiver (<http://creativecommons.org/publicdomain/zero/1.0/>) applies to the data made available in this article, unless otherwise stated in a credit line to the data.

metabolism and energy production. During realimentation, these biological processes shifted towards cellular function and organization. It is also evident that cattle differ in their ability to respond and reprogram following feed restriction. Thus, the objective of this study was to determine the transcriptional differences in skeletal muscle of cows with high or low gain during realimentation following nutrient restriction.

### Main text

The samples and phenotypes presented in this paper were collected previously [1]. Crossbred cows ( $n=121$ ) were used in the study. Angus, Hereford, and MARC III composite cows were bred by AI to Angus, Hereford, Simmental, Limousin, Charolais, Gelbvieh and Red Angus bulls. The  $F_1$  bulls from Angus and Hereford dams were mated to  $F_1$  cows from these matings to produce cross progeny. At 5 years, cows were not bred and moved to an individual feed intake facility with Calan Gates (American Calan, Northwood NH). Feed restriction and ad libitum feeding diets are described in [1]. Muscle biopsies were taken at day  $105 \pm 2$  of the feed restriction from the left side and at day  $49 \pm 2$  of the realimentation from the right side. The samples were immediately frozen in liquid nitrogen and stored at  $-80^\circ\text{C}$ .

Cows most divergent in body weight (BW) gain (12 total;  $n=6$  High Gain,  $n=6$  Low Gain) during the realimentation period were selected and skeletal muscle samples were processed. This classification was based on realimentation to identify cows with divergent BW gain during realimentation following nutrient restriction, and represent variation in CG. Cows selected for high gain had higher body weights and feed intakes than cows selected for lower gain ( $P<0.0005$ ). Cows with greater gain displayed average Gain:Feed ratio of 0.12, compared to 0.08 for cows with lesser gain ( $P<0.01$ ).

Total ribonucleic acid (RNA) was isolated from 50–100 mg of tissue with TriPure reagent (Roche, Indianapolis, IN), and quantified using a NanoDrop 8000 spectrophotometer (Thermo Scientific, Wilmington, DE, USA). The 260/280 measurements were  $>1.8$  for all samples. Quality of total RNA was assessed on an Agilent 2100 Bioanalyzer and RNA integrity number (RIN) values were  $\geq 7$  for all samples.

For microarray, 250 ng of total RNA was used with the Bovine 1.1ST array strips on the Affymetrix GeneAtlas System (Santa Clara, CA, USA). Transformed data were analyzed using a repeated measures model where gain class was fitted as a fixed effect and restricted and ad libitum samples as time points. Gain class was tested on the animal error term while time and its interaction with gain class was tested on the residual. Genes were considered differentially expressed when a Bonferroni adjusted

$P$  of  $<0.05$  was obtained. Raw data files were deposited in the NCBI gene expression omnibus (GEO) as series record GSE94777.

A total of 1,007 genes were differentially expressed at a nominal  $P$ -value  $<0.05$  between cows with high and low gain. However, none were significant after Bonferroni correction. After Bonferroni correction, the comparison of feed restriction and realimentation time points produced 567 differentially expressed genes (DEG;  $P_{\text{Bonferroni}}<0.05$ ; Additional file 1: Table S1). Of these, 408 genes were down-regulated and 159 were genes up-regulated during feed restriction. A heat map illustrating the 30 most up-regulated and 30 most down-regulated genes is presented in Additional file 2: Figure S1. The interaction (gain  $\times$  time) analysis produced no DEG after Bonferroni correction. These data reflect a more prominent response, in terms of the numbers of DEG passing correction for multiple testing in skeletal muscle across treatment group (feed restriction versus ad libitum) rather than phenotype differences (high and low gain). Feed restriction and realimentation result in two different physiological states expressed by changes in weight gain and energy utilization [4]. Differentiation between high and low BW gain during realimentation is likely controlled by many genes with small effects, and in combination with small sample size, fewer DEG with large effects may be expected.

Validation using real-time quantitative polymerase chain reaction (RT-qPCR) was performed on five of the DEG (*INHBE*, *GNB3*, *TRHR*, *AP1M1* and *UCN*) from the analysis comparing feed restriction to realimentation (Additional file 3: Table S2). PrimePCR (Bio-Rad, Hercules, CA, USA) arrays for each target gene and *GAPDH* (housekeeping gene) were used. Real-time PCR was performed in triplicate for all samples and genes on a Bio-Rad CFX384 (BioRad) instrument using SsoAdvance SYBR Green master mix (Bio-Rad). The RT-qPCR reaction was performed according to the manufacturer's instructions. Relative transcript abundance of each target gene was calculated using the  $2^{-\Delta\Delta C_t}$  method [5] with the reference gene and a pooled sample. The correlation between microarray data and RT-qPCR 2ddCt values were  $>0.6$  for 4 of the 5 genes tested.

Functional annotation of the 567 DEG identified in the feed restriction and realimentation analysis was performed in the database for annotation, visualization, and integrated discovery (DAVID) v6.8 ([6]; Table 1; Fig. 1). Down-regulated genes were enriched for lysosome ( $P<0.05$ ), with trends for aminoacyl-tRNA biosynthesis, glutathione metabolism, and renin-angiotensin system pathways ( $P<0.1$ ). Up-regulated genes were enriched for phototransduction ( $P<0.05$ ). All genes identified for the lysosome pathway were expressed in higher abundance

**Table 1** Pathways identified by Database for Annotation, Visualization and Integrated Discovery (DAVID) and Ingenuity Pathway Analysis (IPA) containing genes over-represented of cows during feed restriction and ad libitum periods

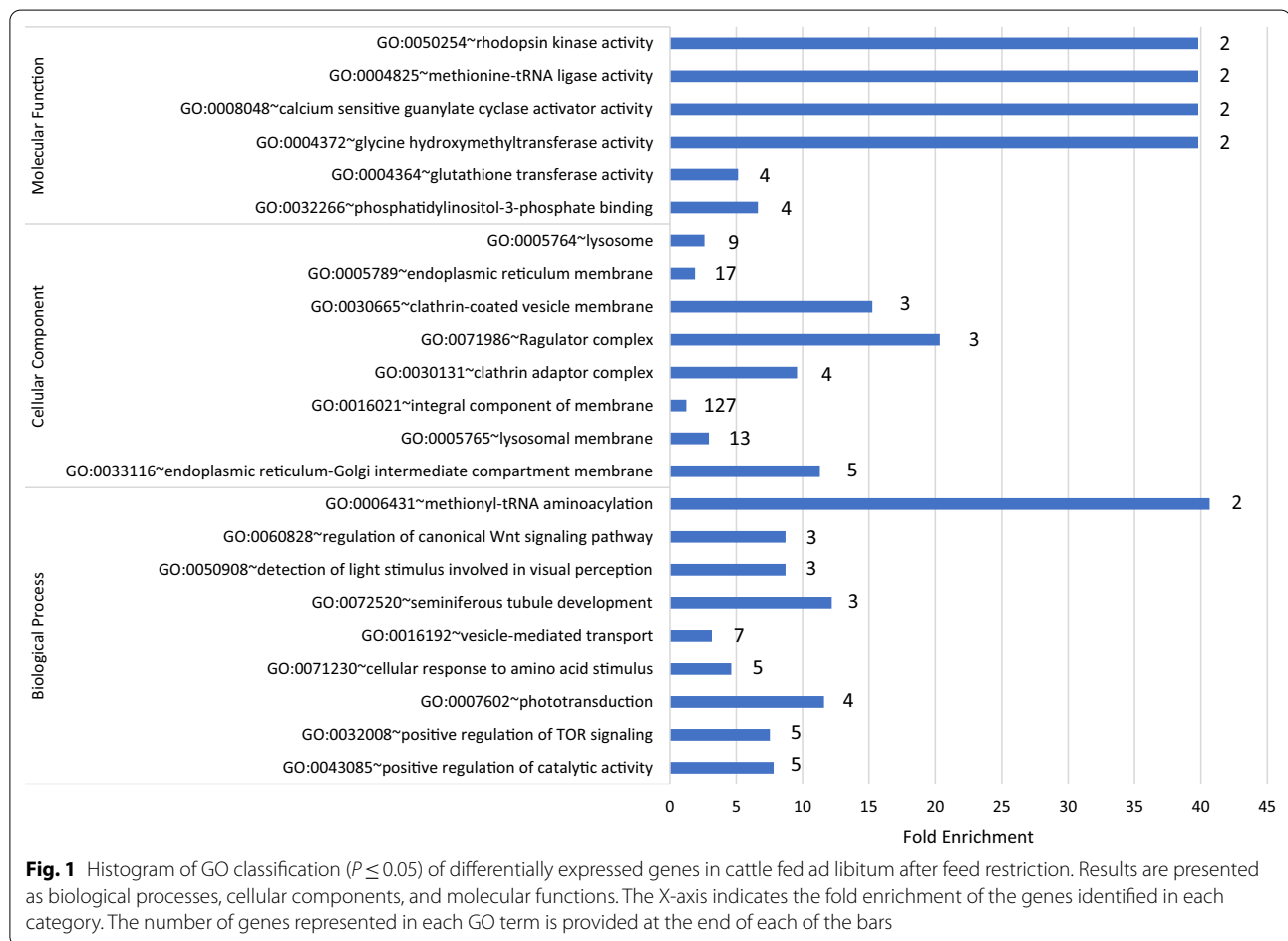
Program	Term <sup>a</sup>	# Genes <sup>b</sup>	P	Genes <sup>c</sup>
DAVID	Lysosome	10	0.0008	<i>CD63, NAGPA, AP1M1, AP1M2, CTSA, ENTPD4, GALC, GGA1, M6PR, MCOLN1</i>
	Aminoacyl-tRNA biosynthesis	4	0.06	<i>MARS, MARS2, SARS2, VARS</i>
	Glutathione metabolism	3	0.09	<i>GSTM2, GSTM3, SRM</i>
	Renin-angiotensin system	3	0.09	<i>ACE, ACE3, CTSA</i>
	Phototransduction	3	0.1	<i>GRK1, CNGB1, RCVRN</i>
	<b>Phototransduction<sup>d</sup></b>	<b>3</b>	<b>0.008</b>	<b><i>GRK7, GUCA1A, GUCA1B</i></b>

<sup>a</sup> Term representing the shared functional annotation or canonical pathway of the differentially expressed genes that were over-represented in the list

<sup>b</sup> Number of DEG identified within the biological process

<sup>c</sup> Official gene symbol of the genes identified as differentially expressed for each term

<sup>d</sup> Term in bold represents up-regulated genes from cows during feed restriction compared to ad libitum periods. Plain text illustrates genes down-regulated during feed restriction compared to ad libitum



during realimentation. Lysosomes are responsible for digesting macromolecules including proteins and organelles [7]. The amino acid products of lysosome digestion are recycled for synthesis of new proteins. The

upregulation of several genes in the lysosome pathway during realimentation suggests an increase in protein turnover. In support of this, roughly 7.5% of the genes identified in this study have a role in protein catalytic

(See figure on next page.)

**Fig. 2** Canonical pathways identified with Ingenuity pathway analysis using the list of 567 differentially expressed genes ( $P_{\text{adjusted}} < 0.05$ ). **A** illustrates the percentage of genes that were up- or down-regulated in each canonical pathway. **B** specifically illustrates the dTMP de novo biosynthesis, folate polyglutamation and folate transformations I pathways and the involvement of differentially expressed genes SHMT1 and SHMT2

activity, indicating a dramatic shift in cellular response to an increase in nutrient availability.

The DEG *MARS*, *MARS2*, and *VARS*, involved in aminoacyl-tRNA biosynthesis were also detected in [3]. The direction of expression was the same for both studies with an increase in transcript abundance of these genes during realimentation. These genes may be of particular interest as biological markers of CG because they have been identified in two different populations and both sexes of beef cattle [3].

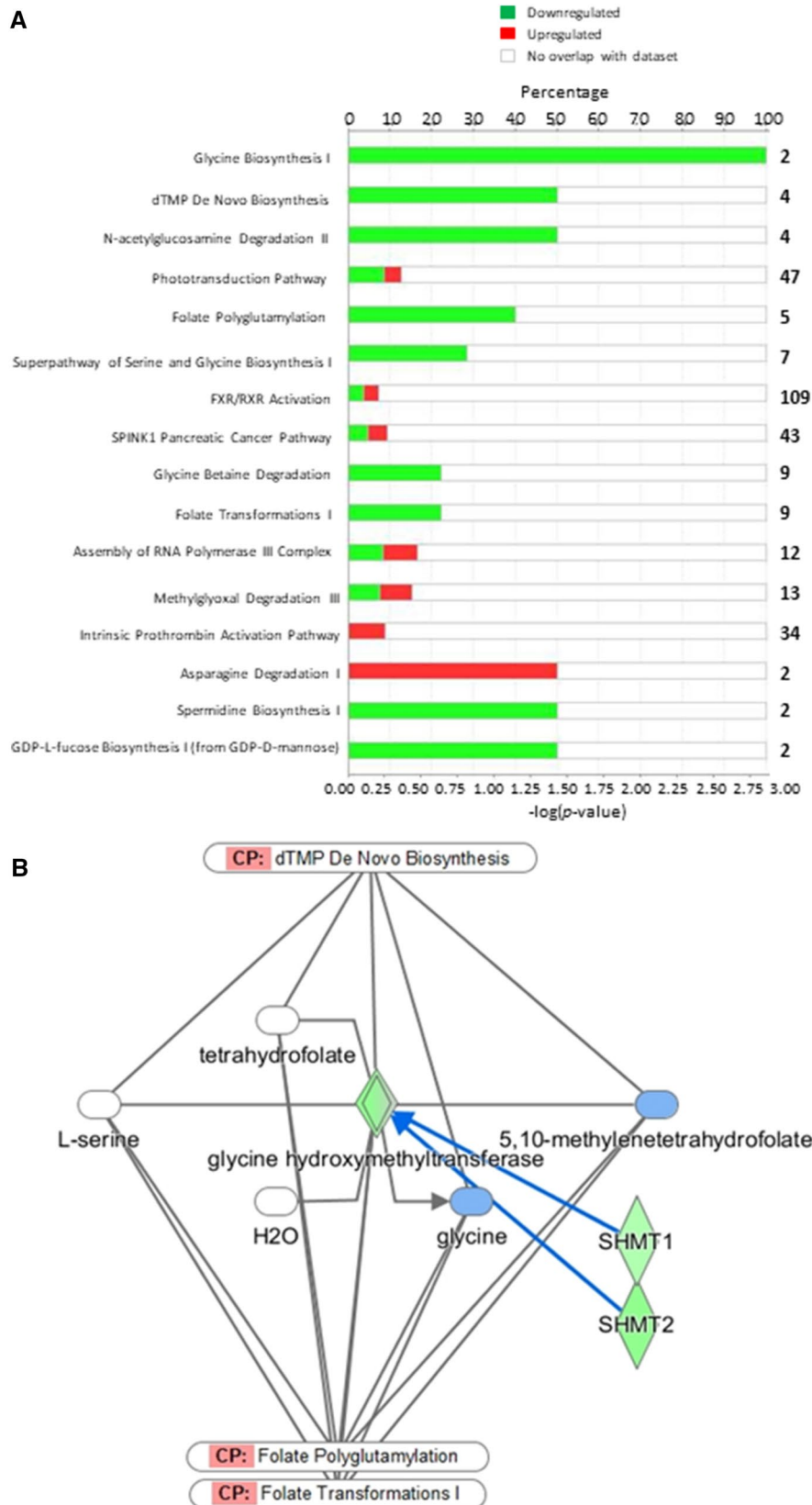
Genes involved in glutathione metabolism (Table 1), an indicator of cellular stress, were upregulated during realimentation. The expression of the glutathione s-transferase (GST) genes, involved in detoxification of oxidative stress products, was higher in mature cows during realimentation. Increased expression of GST genes during CG has been reported in other studies [3, 8, 9]. Studies in mice and humans have shown that increases in caloric intake produce increases in mitochondrial production of hydrogen peroxide in muscle cells [10, 11]. Moreover, mitochondrial dysfunction and oxidative phosphorylation pathways were identified in the adipose tissue of these same cows supporting that the animals are responding to oxidative stress because of increased feed intake.

The list of DEG ( $n=567$ ) between restriction and realimentation was evaluated using the PANTHER Classification System [12, 13] for over-representation in biological processes (Additional file 4: Table S3). Two biological processes were identified: cellular response to organonitrogen compound and response to acid chemical ( $P < 0.05$ ). The genes over-represented were *SHMT1*, *SHMT2*, *LAMTOR1*, *LAMTOR4*, *LOC614531* and *RRAGA*. All were down-regulated in the feed restricted animals, and all annotated genes have functions in amino acid synthesis or cellular responses to amino acid availability. The two *LAMTOR* genes and *RRAGA* encode proteins that are crucial to the activation of the mammalian target of rapamycin complex 1 cascade to promote cell growth in response to cell signals such as nutrient and amino acid levels [14–16]. The list of DEG ( $n=567$ ) between restriction and realimentation was also analyzed with Ingenuity Pathway Analysis (IPA). Two of the canonical pathways also identified by IPA (Fig. 2A) [17], glycine biosynthesis 1 and deoxythymidine monophosphate (dTMP) de novo biosynthesis were identified via

differential expression of *SHMT1* and *SHMT2* (Fig. 2B). These genes encode serine hydroxymethyltransferase enzymes that metabolize tetrahydrofolate to 5,10-methylenetetrahydrofolate and serine to glycine [18]. Synthesis of 5,10-methylenetetrahydrofolate is a divergent point in the folate cycle with three potential outcomes, one of which is synthesis of deoxythymidine monophosphate [19]. Single-carbon biosynthesis via the folate cycle is critical for biosynthesis (proteins, polyamines, nucleotides), amino acid homeostasis, epigenetic maintenance through modification of gene expression via methylation of deoxyribonucleic acid (DNA), RNA, and histones, as well as cellular redox defense through the synthesis of glutathione mentioned previously [19–21].

We have previously evaluated the adipose from these same cows [1] and found overlap with the DEG identified in muscle. A total of 74 DEG were identified in both tissues over the course of feed restriction to realimentation. While these genes did not cluster into functional biology terms, some are of particular interest. For example, *XKR4* has been implicated in gain and feed intake in cattle [22] and *UCN* is involved in mammalian appetite and stress response [23, 24]. Genes involved in transcription or translation were also identified in both tissues (*CUX1*, *ETV1*, *EIF4EBP2*, *FOXJ3*, and *SOX30*). These were all transcribed in higher abundance during realimentation in muscle. Genes with inflammatory functions were identified in the muscle tissue of cows transitioning from restriction to realimentation, including *IL18BP*, *IL18RAP*, *IL34*, *IL36A*, *INHBE*, and *SELE*. Genes *IL34*, *IL36A*, *INHBE* and *SELE* may be of particular importance, as they were detected in the adipose of these cows. These inflammatory response genes differ in function yet are important regulators of cellular response to stresses including inflammation, nutritional stress, and disease [25–31]. The identification of several common genes in both muscle and adipose tissues indicates that they share some of the same responses to CG and may be particularly important as modulators of realimentation.

The nutritional challenges implemented here reflect those experienced by cattle grazing seasonal pastures and native ranges, which represents the majority of the U.S. cow herd. The objective of this discovery study was to investigate differences in the skeletal muscle transcriptome explaining the molecular responses in BW gain in mature cows during realimentation. While some



**Fig. 2** (See legend on previous page.)

pathways associated with protein turnover and energy metabolism were identified, many of the biological processes discovered were modulators of these pathways in some capacity, even if not directly identified as belonging to those biological processes.

In summary, we identified common pathways among cows in the muscle tissue during realimentation, which included protein turnover, tRNA synthesis, and glutathione metabolism. These appear to be pathways that are critical for CG of cows during realimentation and provide insight into the underlying biological mechanisms. This study is the first to evaluate high versus low gaining mature cows for biological differences in the muscle tissue during feed restriction and realimentation. Further studies on larger groups of animals are necessary to better evaluate these genes as potential biological markers for CG.

### Limitations

A limitation of this study was the small sampling size ( $n=12$ ). There were 121 cows in this study and we selected those most divergent in BW gain during realimentation. Obtaining clear differences between animals with high versus low gain phenotype while balancing the cost to perform the microarray experiments were critical factors for our sample size. Future aims include validation of some of the most highly and lowly expressed genes detected in this study in samples from cows biopsied in different years.

### Abbreviations

AI: Artificial insemination; BW: Body weight; CG: Compensatory gain; DAVID: Database for annotation, visualization, and integrated discovery; DEG: Differentially expressed genes; DNA: Deoxyribonucleic acid; GEO: Gene expression omnibus; GO: Gene ontology; GST: Glutathione s-transferase; IPA: Ingenuity pathway analysis; RNA: Ribonucleic acid; RIN: RNA integrity number; RT-qPCR: Real time quantitative polymerase chain reaction.

### Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s13104-021-05757-8>.

**Additional file 1: Table S1.** Statistical analysis data of the expression of genes in the muscle tissue of mature cows for high versus low gain, feed restriction versus ad libitum feed time points, and the interaction between gain and time.

**Additional file 2: Fig. S1.** Heatmap illustrating the changes in expression for the 30 most up-regulated and the 30 most down-regulated genes between the feed restriction diet and ad libitum feed.

**Additional file 3: Table S3.** Comparison of microarray data with RT-qPCR data for five genes identified as differentially expressed in the muscle tissue of cows ( $n = 12$ ) fed ad libitum after feed restriction.

**Additional file 4: Table S4.** PANTHER biological processes identified as over-represented in the muscle tissue of mature cows between feed restriction and ad libitum periods.

### Acknowledgements

The authors would like to thank Chris Haussler and Cindy Felber for their assistance during sample collection, and Linda Flathman for sample processing and laboratory assistance. The authors also appreciate the cattle operations staff for care and handling of the animals. The authors are also grateful to Donna Griess for manuscript preparation and editing.

Mention of trade name proprietary product or specified equipment does not constitute a guarantee or warranty by the USDA and does not imply approval to the exclusion of other products that may be suitable. USDA is an equal opportunity provider and employer.

### Authors' contributions

HCC-H processed tissue samples and analyzed microarray data and drafted the manuscript; LAK performed the analysis for a revision of the manuscript. KMC participated in discussions regarding the project and edited the manuscript; KEH collected samples and edited the manuscript; WTO participated in the writing of the manuscript. MSC participated in the writing of the manuscript. CC performed analyses for a revision of the manuscript. HCF conceived the project and performed animal phenotyping and analyses for animal selection, collected samples, and edited the manuscript; AKL-P conceived and designed experiments, collected samples, participated in discussions regarding data analyses, and drafted the manuscript. All authors made significant contributions editing the manuscript. All authors read and approved the final manuscript.

### Funding

This work received no external funding.

### Availability of data and materials

The datasets generated and/or analysed during the current study are available in the NCBI gene expression omnibus (GEO) as series record GSE94777 at <https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE94777>.

### Declarations

#### Ethics approval and consent to participate

The U.S. Meat Animal Research Center (USMARC) Animal Care and Use Committee reviewed and approved all animal procedures. The procedures for handling cattle complied with the Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching [32]. Informed consent was not applicable for this study.

#### Consent for publication

Not applicable.

#### Competing interests

The authors declare they have no competing interests.

#### Author details

<sup>1</sup>University of Wyoming, Laramie, WY 82071, USA. <sup>2</sup>South Dakota State University, West River Ag Center, Rapid City, SD 57702, USA. <sup>3</sup>USDA, ARS, U.S. Meat Animal Research Center, P.O. Box 166, Clay Center, NE 68933, USA. <sup>4</sup>USDA, ARS, Beltsville Human Nutrition Research Center, Beltsville, MD 20705, USA.

Received: 22 January 2021 Accepted: 23 August 2021

Published online: 16 September 2021

### References

1. Crocker Cunningham H, Cammack KM, Hales KE, Freetly HC, Lindholm-Perry AK. Differential transcript abundance in adipose tissue of mature beef cows during feed restriction and realimentation. *PLoS ONE*. 2018;13:e0194104. <https://doi.org/10.1371/journal.pone.0194104>.
2. Caton JS, Bauer ML, Hidari H. Metabolic components of energy expenditure in growing beef cattle: a review. *Asian-Aus J Anim Sci*. 2000;13:702.
3. Keogh K, Kenny DA, Cormican P, McCabe MS, Kelly AK, Waters SM. Effect of dietary restriction and subsequent re-alimentation on the transcriptional profile of bovine skeletal muscle. *PLoS ONE*. 2016;11:e0149373. <https://doi.org/10.1371/journal.pone.0149373>.



4. Freetly HC, Nienaber JA. Efficiency of energy and nitrogen loss and gain in mature cows. *J Anim Sci*. 1998;76:896–905.
5. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2- $\Delta\Delta$ CT Method. *Methods*. 2001;25:402–8.
6. Huang DW, Sherman BT, Tan Q, Collins JR, Alvord WG, Roayaei J, et al. The DAVID gene functional classification tool: a novel biological module-centric algorithm to functionally analyze large gene lists. *Genome Biol*. 2007;8:R183. <https://doi.org/10.1186/gb-2007-8-9-r183>.
7. Perera RM, Zoncu R. The lysosome as a regulatory hub. *Ann Rev Cell Devel Biol*. 2016;32:223–53.
8. Keogh K, Waters SM, Cormican P, Kelly AK, Kenny DA. Effect of dietary restriction and subsequent re-feeding on the transcriptional profile of bovine jejunal epithelium. *PLoS ONE*. 2018;13(3):e0194445.
9. Connor EE, Kahl S, Elsasser TH, Parker JS, Li RW, Van Tassel CP, et al. Enhanced mitochondrial complex gene function and reduced liver size may mediate improved feed efficiency of beef cattle during compensatory growth. *Funct Integr Genomics*. 2010;10:39–51.
10. Anderson EJ, Lustig ME, Boyle KE, Woodlief TL, Kane DA, Lin C-T, et al. Mitochondrial H<sub>2</sub>O<sub>2</sub> emission and cellular redox state link excess fat intake to insulin resistance in both rodents and humans. *JCI*. 2009;119:573–81.
11. Tan BL, Norhaizan ME, Liew WP. Nutrients and oxidative stress: friend or foe? *Oxid Med Cell Longev*. 2018;2018:9719584. <https://doi.org/10.1155/2018/9719584>.
12. Mi H, Huang X, Muruganujan A, Tang H, Mills C, Kang D, Thomas PD. PANTHER version 11: expanded annotation data from gene ontology and reactome pathways, and data analysis tool enhancements. *Nucl Acids Res*. 2017;45:D183–9.
13. Mi H, Muruganujan A, Casagrande JT, Thomas PD. Large-scale gene function analysis with the PANTHER classification system. *Nat Protoc*. 2013;8:1551–66.
14. Sancak Y, Bar-Peled L, Zoncu R, Markhard AL, Nada S, Sabatini DM. Ragulator-Rag complex targets mTORC1 to the lysosomal surface and is necessary for its activation by amino acids. *Cell*. 2010;141:290–303.
15. Colaco A, Jaattela M. Ragulator – a multifaceted regulator of lysosomal signaling and trafficking. *JBC*. 2017;216:3895.
16. Howell JJ, Manning BD. mTOR couples cellular nutrient sensing to organismal metabolic homeostasis. *Trends Endocrinol Metab*. 2011;22:94–102. <https://doi.org/10.1016/j.tem.2010.12.003>.
17. Kramer A, Green J, Pollard J Jr, Tugendreich S. IPA: causal analysis approaches in ingenuity pathway analysis. *Bioinformatics*. 2014;30:523–30.
18. Appaji RN, Ambili M, Jala VR, Subramanya HS, Savithri HS. Structure-function relationship in serine hydroxymethyltransferase. *Biochem Biophys Acta*. 2003;2003(1647):24–9. [https://doi.org/10.1016/s1570-9639\(03\)00043-8](https://doi.org/10.1016/s1570-9639(03)00043-8).
19. Clare CE, Brassington AH, Kwong WY, Sinclair KD. One-carbon metabolism: Linking nutritional biochemistry to epigenetic programming of long-term development. *Annu Rev Anim Biosci*. 2019;2019(15):263–87. <https://doi.org/10.1146/annurev-animal-020518-115206>.
20. Luccock M. Folic acid: nutritional biochemistry, molecular biology, and role in disease processes. *Mol Genet Metab*. 2000;71:121–38. <https://doi.org/10.1006/mgme.2000.3027>.
21. Ducker GS, Rabinowitz JD. One-carbon metabolism in health and disease. *Cell Metab*. 2017;25:27–42. <https://doi.org/10.1016/j.cmet.2016.08.009>.
22. Lindholm-Perry AK, Kuehn LA, Smith TP, Ferrell CL, Jenkins TG, Freetly HC, Snelling WM. A region on BTA14 that includes the positional candidate genes LYPLA1, XKR4 and TMEM68 is associated with feed intake and growth phenotypes in cattle. *Anim Genet*. 2012;43:216–9.
23. Bradbury MJ, McBurnie MI, Denton DA, Lee KF, Vale WW. Modulation of urocortin-induced hypophagia and weight loss by corticotropin-releasing factor receptor 1 deficiency in mice. *Endocrinology*. 2000;141:2715–24.
24. Wang L, Stengel A, Goebel M, Martinez V, Gourcerol G, Rivier J, Tache Y. Peripheral activation of corticotropin-releasing factor receptor 2 inhibits food intake and alters meal structures in mice. *Peptides*. 2011;32:51–9.
25. Dinarello C. Novel targets for interleukin 18 binding protein. *Ann Rheum Dis*. 2001. <https://doi.org/10.1136/ard.60.90003.iii18>.
26. Grisoni M-L, Proust C, Alanne M, DeSuremain M, Salomaa V, Kuulasmaa K, et al. Lack of association between polymorphisms of the IL18R1 and IL18RAP genes and cardiovascular risk: the MORGAM Project. *BMC Med Genet*. 2009. <https://doi.org/10.1186/1471-2350-10-44>.
27. Masteller EL, Wong BR. Targeting IL-34 in chronic inflammation. *Drug Discovery Today*. 2014;19:1212–6. <https://doi.org/10.1016/j.drudis.2014.05.016>.
28. Conde J, Scotece M, Abella V, Lois A, López V, García-Caballero T, et al. IL-36 $\alpha$ : a novel cytokine involved in the catabolic and inflammatory response in chondrocytes. *Sci Rep*. 2015. <https://doi.org/10.1038/srep16674>.
29. Sugiyama M, Kikuchi A, Misu H, Igawa H, Ashihara M, Kushima Y, et al. Inhibin  $\beta$ E (INHBE) is a possible insulin resistance-associated hepatokine identified by comprehensive gene expression analysis in human liver biopsy samples. *PLoS ONE*. 2018;13: e0194798. <https://doi.org/10.1371/journal.pone.0194798>.
30. Bevilacqua MP, Nelson RM, Mannori G, Cecconi O. Endothelial-leukocyte adhesion molecules in human disease. *Annu Rev Med*. 1994;45:361–78. <https://doi.org/10.1146/annurev.med.45.1.361>.
31. Arvanitis DA, Flouris GA, Spandidos DA. Genomic rearrangements on VCAM1, SELE, APEG1 and AIF1 loci in atherosclerosis. *J Cell Mol Med*. 2005;9:153–9. <https://doi.org/10.1111/j.1582-4934.2005.tb00345.x>.
32. Federation of Animal Science Societies. Guide for the care and use of agricultural animals in research and teaching. Champaign: Federation of Animal Science Societies; 2010.

## Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Ready to submit your research? Choose BMC and benefit from:

- fast, convenient online submission
- thorough peer review by experienced researchers in your field
- rapid publication on acceptance
- support for research data, including large and complex data types
- gold Open Access which fosters wider collaboration and increased citations
- maximum visibility for your research: over 100M website views per year

At BMC, research is always in progress.

Learn more [biomedcentral.com/submissions](https://biomedcentral.com/submissions)

