Whole egg consumption increases gene expression within the glutathione pathway in the liver of Zucker Diabetic Fatty rats

Joe L. Webb  
*Iowa State University*

Amanda E. Bries  
*Iowa State University*, aebries@iastate.edu

Brooke Vogel  
*Iowa State University*

Claudia Carrillo  
*Iowa State University*, claudiac@iastate.edu

Lily Harvison  
*Iowa State University*, lilyh@iastate.edu

*See next page for additional authors*

Follow this and additional works at: [https://lib.dr.iastate.edu/fshn_hs_pubs](https://lib.dr.iastate.edu/fshn_hs_pubs)  
*Part of the* [Dietetics and Clinical Nutrition Commons](https://lib.dr.iastate.edu/fshn_hs_pubs), [Endocrinology, Diabetes, and Metabolism Commons](https://lib.dr.iastate.edu/fshn_hs_pubs), [Exercise Science Commons](https://lib.dr.iastate.edu/fshn_hs_pubs), [Food Chemistry Commons](https://lib.dr.iastate.edu/fshn_hs_pubs), [Human and Clinical Nutrition Commons](https://lib.dr.iastate.edu/fshn_hs_pubs), and the [Molecular, Genetic, and Biochemical Nutrition Commons](https://lib.dr.iastate.edu/fshn_hs_pubs)  

The complete bibliographic information for this item can be found at [https://lib.dr.iastate.edu/fshn_hs_pubs/38](https://lib.dr.iastate.edu/fshn_hs_pubs/38). For information on how to cite this item, please visit [http://lib.dr.iastate.edu/howtocite.html](http://lib.dr.iastate.edu/howtocite.html).
Whole egg consumption increases gene expression within the glutathione pathway in the liver of Zucker Diabetic Fatty rats

Abstract
Nutrigenomic evidence supports the idea that Type 2 Diabetes Mellitus (T2DM) arises due to the interactions between the transcriptome, individual genetic profiles, lifestyle, and diet. Since eggs are a nutrient dense food containing bioactive ingredients that modify gene expression, our goal was to examine the role of whole egg consumption on the transcriptome during T2DM. We analyzed whether whole egg consumption in Zucker Diabetic Fatty (ZDF) rats alters microRNA and mRNA expression across the adipose, liver, kidney, and prefrontal cortex tissue. Male ZDF (fa/fa) rats (n = 12) and their lean controls (fa/+) (n = 12) were obtained at 6 wk of age. Rats had ad libitum access to water and were randomly assigned to a modified semi-purified AIN93G casein-based diet or a whole egg-based diet, both providing 20% protein (w/w). TotalRNA libraries were prepared using QuantSeq 3’ mRNA-Seq and Lexogen smallRNA library prep kits and were further sequenced on an Illumina HighSeq3000. Differential gene expression was conducted using DESeq2 in R and Benjamini-Hochberg adjusted P-values controlling for false discovery rate at 5%. We identified 9 microRNAs and 583 genes that were differentially expressed in response to 8 wk of consuming whole egg-based diets. Kyto Encyclopedia of Genes and Genomes/Gene ontology pathway analyses demonstrated that 12 genes in the glutathione metabolism pathway were upregulated in the liver and kidney of ZDF rats fed whole egg. Whole egg consumption primarily altered glutathione pathways such as conjugation, methylation, glucuronidation, and detoxification of reactive oxygen species. These pathways are often negatively affected during T2DM, therefore this data provides unique insight into the nutrigenomic response of dietary whole egg consumption during the progression of T2DM.

Disciplines
Dietetics and Clinical Nutrition | Endocrinology, Diabetes, and Metabolism | Exercise Science | Food Chemistry | Human and Clinical Nutrition | Molecular, Genetic, and Biochemical Nutrition

Comments
Webb JL, Bries AE, Vogel B, Carrillo C, Harvison L, Day TA, et al. (2020) Whole egg consumption increases gene expression within the glutathione pathway in the liver of Zucker Diabetic Fatty rats. PLoS ONE 15(11): e0240885. doi:10.1371/journal.pone.0240885.

Creative Commons License
This work is licensed under a Creative Commons Attribution-Noncommercial 4.0 License

Authors
Joe L. Webb, Amanda E. Bries, Brooke Vogel, Claudia Carrillo, Lily Harvison, Timothy A. Day, Michael J. Kimber, Rudy J. Valentine, Matthew J. Rowling, Stephanie Clark, Elizabeth M. McNiell, and Kevin L. Schalinske

This article is available at Iowa State University Digital Repository: https://lib.dr.iastate.edu/fshn_hs_pubs/38
Whole egg consumption increases gene expression within the glutathione pathway in the liver of Zucker Diabetic Fatty rats

Joe L. Webb1,2*, Amanda E. Bries1,2*, Brooke Vogel1, Claudia Carrillo1, Lily Harvison1, Timothy A. Day3, Michael J. Kimber1,2, Rudy J. Valentine4, Matthew J. Rowling1,2, Stephanie Clark1, Elizabeth M. McNeill1,2,5, Kevin L. Schalinske1,2*

1 Department of Food Science and Human Nutrition, Iowa State University, Ames, IA, United States of America, 2 Interdepartmental Graduate Program in Nutritional Sciences, Iowa State University, Ames, IA, United States of America, 3 Department of Biomedical Sciences, Iowa State University College of Veterinary Medicine, Ames, IA, United States of America, 4 Department of Kinesiology, Iowa State University, Ames, IA, United States of America, 5 Genetics and Genomics Graduate Program, Iowa State University, Ames, IA, United States of America.

* These authors contributed equally to this work.
+ kschalina@iastate.edu

Abstract

Nutrigenomic evidence supports the idea that Type 2 Diabetes Mellitus (T2DM) arises due to the interactions between the transcriptome, individual genetic profiles, lifestyle, and diet. Since eggs are a nutrient dense food containing bioactive ingredients that modify gene expression, our goal was to examine the role of whole egg consumption on the transcriptome during T2DM. We analyzed whether whole egg consumption in Zucker Diabetic Fatty (ZDF) rats alters microRNA and mRNA expression across the adipose, liver, kidney, and prefrontal cortex tissue. Male ZDF (fa/fa) rats (n = 12) and their lean controls (fa/+), (n = 12) were obtained at 6 wk of age. Rats had ad libitum access to water and were randomly assigned to a modified semi-purified AIN93G casein-based diet or a whole egg-based diet, both providing 20% protein (w/w). TotalRNA libraries were prepared using QuantSeq 3’ mRNA-Seq and Lexogen smallRNA library prep kits and were further sequenced on an Illumina HighSeq3000. Differential gene expression was conducted using DESeq2 in R and Benjamini-Hochberg adjusted P-values controlling for false discovery rate at 5%. We identified 9 microRNAs and 583 genes that were differentially expressed in response to 8 wk of consuming whole egg. Kyto Encyclopedia of Genes and Genomes/Gene ontology pathway analyses demonstrated that 12 genes in the glutathione metabolism pathway were upregulated in the liver and kidney of ZDF rats fed whole egg. Whole egg consumption primarily altered glutathione pathways such as conjugation, methylation, glucuronidation, and detoxification of reactive oxygen species. These pathways are often negatively affected during T2DM, therefore this data provides unique insight into the nutrigenomic response of dietary whole egg consumption during the progression of T2DM.
Introduction

Type 2 Diabetes Mellitus (T2DM) is an insulin independent metabolic disease characterized by chronic hyperglycemia and concomitant insulin resistance and it is estimated that greater than 415 million adults worldwide have T2DM [1]. Oxidative stress is a potential key mediator in the pathogenesis of T2DM and may underlie the progressive development of hyperglycemia and insulin resistance [2]. More specifically, reports demonstrate that glutathione (a major intracellular antioxidant) enzymes are diminished in the liver and brain of T2DM animal models [3]. Sekhar and colleagues examined the ability of patients with uncontrolled and controlled T2DM to synthesize glutathione via measuring isotopically labelled glycine [4]. They reported that patients with uncontrolled T2DM were severely deficient in the ability to maintain glutathione metabolism in cardiac tissue [5], which may be, in part, due to hyperglycemia decreasing L-cysteine concentrations [6] and the reduced flux of methionine to cysteine [7]. Because of the deleterious effects of hyperglycemia on organ function, it is important to consider the global transcriptomic effects of T2DM. Similar to humans, the Zucker Diabetic Fatty (ZDF) rat model of T2DM also displays increased oxidative stress [8], whereby endogenous protective antioxidants like glutathione are similarly downregulated in ZDF rats [9]. The gene expression profiles in animal models of T2DM, such as the ZDF rat, is consistent with gene expression profiles of humans with T2DM [8], making this a suitable model to explore the global gene expression effects of diet in the ZDF rat.

Dietary treatments with bioactive foods such as cocoa or Shenyuan granules [9, 10] in ZDF rats have been shown to reduce oxidative stress or attenuate renal injury in the presence of T2DM-related nephropathy [11]. Consumption of eggs as a bioactive food during T2DM in humans remains controversial [12–15], but eggs have been shown to display antioxidative properties, which may be beneficial during the progression of T2DM [16]. Additionally, our laboratory has consistently reported that long-term whole egg (WE) consumption improves metabolic parameters during T2DM such as the maintenance of circulating vitamin D concentrations, decreased weight gain, and nephroprotection via reduced proteinuria in male ZDF rats [17, 18]. These are important findings, as vitamin D deficiency, increased adiposity, and kidney failure have collectively been suggested to exacerbate oxidative stress during T2DM [19].

While the literature surrounding the effects of dietary WE on insulin resistance during T2DM is inconclusive in both rodent [20] and human population studies [21], there are no studies to date examining the molecular mechanisms underlying how WE consumption affects the transcriptome across multiple tissues. Longitudinal, prospective, and comprehensive meta-analyses have been performed to assess the independent risk factors of increased dietary egg consumption on chronic diseases [22, 23]. Because of the highly controversial science of whole egg consumption on increased cardiovascular disease in patients with T2DM, it is important to examine the possible underlying molecular targets and drivers of whole egg consumption on disease. Ultimately, analyzing the transcriptomic impact of egg consumption would provide us with a better understanding of the nutrigenomic actions that dietary egg consumption contributes to T2DM, and bridge the gap in our understanding of how whole eggs may affect the physiological progression of T2DM. Therefore, the objective of this study was to determine the influence of WE consumption on gene and microRNA expression profiles in a ZDF rat model of progressive T2DM.

We examined the transcriptomes from the adipose, liver, kidney, and prefrontal cortex (PFC) tissues to determine how WE consumption alters gene expression and examined whether these changes correspond to altered microRNA expression profiles in T2DM.

Results and discussion

Whole eggs have predominantly been criticized for their associated risk of developing chronic diseases [24], yet the benefits of WE consumption have also been reported [13]. For instance,
several groups have suggested that WE provide antioxidant properties [13, 25], either through antioxidant peptides in the egg yolk [11] or other reactive oxygen species-reducing nutrients [26]. Other studies examining the role of quail egg consumption in rat models of T2DM have demonstrated upregulation of glutathione metabolism in alloxan-induced T2DM in Wistar rats [25] and improved oxidative stress profiles in streptozotocin-injected rats [27]. Raza and colleagues [27] identified that in diabetic rat liver glutathione content and glutathione S-transferase (GST) activity were decreased 65% while also observing that brain glutathione and GST activity were increased two-fold as a result of a T2DM phenotype.

**Total RNASeq differential expression**

When comparing the WE versus casein (CAS) in ZDF rats and their lean controls, differential expression analyses of the mRNAseq data resulted in 583 differentially expressed genes (DEGs) across four tissues in both genotypes (Table 1). S1 Table contains the results from DESeq2 with the results for each gene across all four tissues with data on individual genes. S2 Table contains raw mRNA read counts for each tissue and rat across both genotypes. Among the lean controls, 13 genes were differentially expressed in the adipose tissue, 32 in the liver, and 6 in the kidney. Notably, none of the genes were differentially expressed in the PFC between dietary treatments in the lean rats. In the ZDF rats, dietary WE consumption resulted in 532 total DEGs across all tissues where 50 genes were differentially expressed in adipose tissue, 474 in the liver, 6 in the kidney and 2 genes in the PFC following multiple testing correction using the false discovery rate (FDR) threshold of 5%. We demonstrated that consuming WE-based diets for 8 wk resulted in significant alterations in oxidative stress pathways, as well as glutathione metabolism pathways. While there were tissue-specific changes in gene expression, glutathione metabolism was altered in the kidney and liver among ZDF rats, and in the kidney of lean controls were significantly upregulated. Overall, these data highlight how consumption of WE-based diets can provide beneficial effects through modifying gene expression of oxidative reduction targets.

We previously demonstrated that WE consumption for 8 wk is effective at improving serum vitamin D status and providing nephroprotective benefits [17, 18]; however, despite our gene expression findings in this study we still have yet to elucidate the mechanism underlying how WE consumption leads to decreased weight gain [28, 29]. We also identified that ZDF rats fed WE upregulated 11 genes involved in glutathione metabolism in the liver and kidney. In the PFC, WE consumption had differing effects whereby in the lean PFC, WE consumption did not change the transcriptome, whereas in the ZDF rats WE consumption strongly downregulated the expression of 2, AY172581 exon transcripts. These exon transcripts have yet to be characterized and future proteomic studies may reveal their biological importance. Across both genotypes, the most significantly altered genes were involved in the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways of: glutathione metabolism, metabolic pathways, steroid biosynthesis, and cholesterol metabolism. After controlling for the genetic background differences of our ZDF rats, a combined analysis indicated that 428 unique genes were differentially expressed across these tissues as a product of WE consumption. Moreover, 13 different glutathione metabolism genes were significantly upregulated across the liver and kidney in both genotypes suggesting that increased whole egg consumption, may increase glutathione metabolism independent of T2DM, and attenuate the decreased glutathione metabolism during diabetes.

To visualize the global differences in the transcriptomes based on dietary treatment, we performed principal component analysis (PCA) and generated volcano plots for genes that exhibited ≥1.5-fold change, respectively. Fig 1 displays the samples in a three-dimensional principal
| Genotype | Tissue     | Ensembl_ID (ENSRNO) | Symbol | Gene Name                           | L2FC | P-value |
|----------|------------|---------------------|--------|------------------------------------|------|---------|
| ZDF      | Adipose    | G00000011039        | Gch1   | GTP cyclohydrolase 1               | -2.71 | 2.1E-05 |
|          | Downregulated |                  |        |                                    |      |         |
|          |            | G000000040108      | RGD1565355 | similar to fatty acid translocase/CD36 | -2.65 | 2.7E-07 |
|          |            | G00000011024       | Zdhc20 | zinc finger DHHC-type palmitoyltransferase 20 | -2.49 | 1.2E-04 |
|          |            | G00000006946       | Arhgap9 | Rho GTPase activating protein 9 | -2.49 | 2.6E-06 |
|          |            | G0000006715        | Ccr1   | C-C motif chemokine receptor 1     | -2.39 | 7.4E-06 |
|          |            | G00000032546       | Dot1l  | DOT1 like histone lysine methyltransferase | -2.12 | 1.9E-06 |
|          |            | G00000034230       | Fcrl1  | Fc receptor-like 1                 | -2.09 | 6.9E-05 |
|          |            | G00000019283       | P2ry2  | purinergic receptor P2Y2           | -2.07 | 1.2E-04 |
|          |            | G00000022975       | Nfam1  | NFAT activating protein with ITAM motif 1 | -1.92 | 3.1E-04 |
|          |            | G00000013917       | Igsf10 | immunoglobulin superfamily, member 10 | -1.91 | 2.4E-05 |
|          |            | G00000049115       | Ccr5   | C-C motif chemokine receptor 5     | -1.89 | 4.3E-07 |
|          |            | G00000015895       | B4galnt6 | beta-1,4-galactosyltransferase 6 | -1.84 | 5.1E-05 |
|          |            | G00000020479       | Pik3c2a | phosphatidylinositol-4-phosphate 3-kinase, catalytic subunit type 2 alpha | -1.83 | 2.2E-04 |
|          |            | G00000061379       | C7     | complement C7                      | -1.82 | 1.8E-04 |
|          |            | G00000011927       | Sdc3   | syndecan 3                         | -1.82 | 2.3E-04 |
|          |            | G00000026644       | Glipr1 | GLI pathogenesis-related 1         | -1.77 | 6.4E-05 |
|          |            | G00000011946       | Ptn    | pleiotrophin                       | -1.74 | 4.8E-05 |
|          |            | G00000013922       | Dok2   | docking protein 2                  | -1.61 | 2.7E-04 |
|          |            | G00000013526       | Rasf4  | Ras association domain family member 4 | -1.60 | 3.1E-06 |
|          |            | G00000001989       | Alcam  | activated leukocyte cell adhesion molecule | -1.57 | 1.5E-05 |
|          |            | G00000016643       | Lpcat2 | lysophosphatidylcholine acyltransferase 2 | -1.52 | 2.9E-04 |
|          |            | G00000003835       | Slc43a2 | solute carrier family 43 member 2 | -1.52 | 1.6E-04 |
|          |            | G00000009077       | Lipa   | lipase A, lysosomal acid type       | -1.51 | 3.8E-05 |
|          |            | G000000099347      | Arhgap25 | Rho GTPase activating protein 25   | -1.49 | 2.1E-04 |
|          |            | G00000000257       | Smpd3  | sphingomyelin phosphodiesterase 3  | -1.47 | 2.8E-04 |
|          |            | G000000012616      | Ppt1   | palmitoyl-protein thioesterase 1   | -1.41 | 2.7E-04 |
|          |            | G000000099331      | Hck    | HCK proto-oncogene, Src family tyrosine kinase | -1.30 | 4.6E-05 |
|          |            | G000000010183      | Gask1b | golgi associated kinase 1B          | -1.26 | 2.7E-04 |
|          |            | G000000017022      | Cerk   | ceramide kinase                    | -1.25 | 3.2E-04 |
|          |            | G000000088465      | Tmem176b | transmembrane protein 176B         | -1.23 | 2.4E-04 |
|          |            | G000000010208      | Timp1  | TIMP metallopeptidase inhibitor 1  | -1.20 | 1.8E-04 |
|          |            | G000000015072      | Ptgr1  | prostaiglandin reductase 1         | 1.15  | 2.2E-04 |
|          |            | G000000010389      | Ndr2   | NDRG family member 2               | 1.30  | 2.0E-04 |
|          |            | G000000037446      | Pxmp2  | peroxisomal membrane protein 2     | 1.30  | 2.3E-04 |
|          |            | G00000002896       | Prdx6  | peroxiredoxin 6                   | 1.37  | 3.1E-04 |
|          |            | G000000019328      | Phgdh  | phosphoglycerate dehydrogenase     | 1.45  | 3.4E-04 |
|          |            | G000000021316      | Tmem98 | transmembrane protein 98           | 1.48  | 2.1E-04 |
|          |            | G000000046858      | MGC109340 | similar to Microsomal signal peptidase 23 kDa subunit (SPase 22 kDa subunit) (SPC22/23) | 1.52  | 9.7E-05 |
|          |            | G000000017012      | Coq7   | coenzyme Q7, hydroxylase           | 1.56  | 4.5E-05 |
|          |            | G000000021524      | Marp   | melanocortin 2 receptor accessory protein | 1.69  | 1.6E-04 |
|          |            | G000000017226      | Slc2a4 | solute carrier family 2 member 4  | 1.87  | 1.8E-04 |
|          |            | G00000002579       | Parm1  | prostate androgen-regulated mucin-like protein 1 | 1.89  | 7.0E-06 |

(Continued)
Table 1. (Continued)

| Lean Adipose Downregulated | Ensembl_ID (ENSRNO) | Gene Symbol | Gene Name | L2FC | P-value |
|-----------------------------|---------------------|-------------|-----------|------|---------|
| G000000016700              | Tcf21               | transcription factor 21 | -2.82 | 3.2E-06|
| Lean Adipose Upregulated   | Ensembl_ID (ENSRNO) | Gene Symbol | Gene Name | L2FC | P-value |
| G00000013733               | Ppp4r1              | protein phosphatase 4, regulatory subunit 1 | 2.68 | 2.4E-05|
| Lean Kidney Downregulated  | Ensembl_ID (ENSRNO) | Gene Symbol | Gene Name | L2FC | P-value |
| G00000033932               | AY172581.22−201     | tyrosine 3-monooxygenase/tryptophan S-monooxygenase activation protein, eta | -5.21 | 1.1E-05|
| Lean Kidney Upregulated    | Ensembl_ID (ENSRNO) | Gene Symbol | Gene Name | L2FC | P-value |
| None                       | None                | None        | None      | None | None    |
| ZDF Kidney Downregulated   | Ensembl_ID (ENSRNO) | Gene Symbol | Gene Name | L2FC | P-value |
| G000000020204              | Srp19               | signal recognition particle 19 | -1.72 | 3.3E-05|
| ZDF Kidney Upregulated     | Ensembl_ID (ENSRNO) | Gene Symbol | Gene Name | L2FC | P-value |
| G00000018237               | Gstp1               | glutathione S-transferase pi 1 | 2.04 | 5.2E-07|
| G00000018940               | Cnt1                | solute carrier family 28 member 1 | 1.65 | 7.8E-05|

(Continued)
Table 1. (Continued)

| Lean Kidney Downregulated | Ensembl_ID (ENSRNO) | Gene Symbol | Gene Name | L2FC | P-value |
|---------------------------|---------------------|-------------|-----------|------|---------|
| G00000020151              | Cdh1                | cadherin 1  | -1.08     | 1.4E-05 |
| G00000013062              | Cyp24a1             | cytochrome P450, family 24, subfamily a, polypeptide 1 | -1.30 | 4.3E-06 |
| G00000012956              | Tgm2                | transglutaminase 2 | -1.34 | 3.5E-05 |
| G00000004019              | Phlda1              | pleckstrin homology-like domain, family A, member 1 | -2.30 | 2.7E-08 |

| Lean Kidney Upregulated | Ensembl_ID (ENSRNO) | Gene Symbol | Gene Name | L2FC | P-value |
|-------------------------|---------------------|-------------|-----------|------|---------|
| G00000029726             | Gstm1               | glutathione S-transferase mu 1 | 1.40 | 1.5E-05 |
| G00000053811             | Arg2                | arginase 2 | 1.51 | 3.5E-05 |
| G00000000576             | Anapc16             | anaphase promoting complex subunit 16 | 2.11 | 3.3E-05 |

| ZDF Liver Downregulated | Ensembl_ID (ENSRNO) | Gene Symbol | Gene Name | L2FC | P-value |
|-------------------------|---------------------|-------------|-----------|------|---------|
| G00000014320             | Inhba               | inhibin subunit beta A | -4.77 | 3.2E-12 |
| G00000007923             | Cgref1              | cell growth regulator with EF hand domain 1 | -3.74 | 4.4E-09 |
| G00000004307             | Tor3a               | torin family 3 | -3.55 | 6.1E-18 |
| G00000034190             | Ighm                | immunoglobulin heavy constant mu | -3.38 | 1.4E-16 |
| G00000003802             | Pttg1               | PTTG1 regulator of sister chromatid separation | -3.35 | 1.2E-05 |
| G00000007060             | Plin2               | perilipin 2 | -3.31 | 6.8E-28 |
| G00000045549             | Fasn                | fatty acid synthase | -3.31 | 1.5E-10 |
| G000000022556             | Cxcl10              | C-X-C motif chemokine ligand 10 | -3.30 | 4.4E-04 |
| G00000009019             | Slc6a6              | solute carrier family 6 member 6 | -3.09 | 6.0E-11 |
| G000000025691             | Pla2g7              | phospholipase A2 group VII | -3.03 | 4.5E-06 |
| G000000020035             | Cyp17a1             | cytochrome P450 | -3.00 | 2.1E-09 |
| G00000004080             | Fads1               | fatty acid desaturase 1 | -3.35 | 1.2E-05 |
| G00000000658             | Acacb               | acetyl-CoA carboxylase beta | -3.31 | 4.0E-18 |
| G000000030154             | Cyp4a2              | cytochrome P450 | -2.88 | 5.0E-04 |
| G000000021802             | Isl15               | ISG15 ubiquitin-like modifier | -2.72 | 2.4E-03 |
| G000000010152             | Slc25a30            | solute carrier family 25 | -2.58 | 1.8E-09 |
| G00000001963             | Mx2                 | MX dynamin like GTPase 2 | -2.56 | 2.1E-03 |
| G000000040151             | Sdr16c6             | short chain dehydrogenase/reductase family 16C | -2.55 | 6.3E-04 |
| G000000017914             | Cavin3              | caveola associated protein 3 | -3.25 | 1.4E-14 |
| G00000006889             | Insig1              | insulin induced gene 1 | -2.50 | 3.4E-13 |
| G00000006204             | Slc30a3             | solute carrier family 30 member 3 | -2.47 | 3.0E-07 |
| G000000016353             | Nim1k               | NIM1 serine/threonine protein kinase | -2.40 | 3.6E-08 |
| G000000016011             | Plekhd1             | pleckstrin homology and RhoGEF domain containing G1 | -2.40 | 7.8E-05 |
| G000000028137             | Mkig7               | marker of proliferation Ki-67 | -2.38 | 1.5E-04 |
| G000000014476             | Evl                 | Enah/Vasp-like | -2.37 | 3.4E-04 |
| G000000008022             | Apaf1               | apoptotic peptidase activating factor 1 | -2.36 | 7.1E-05 |
| G000000053891             | Phf11               | PHD finger protein 11 | -2.34 | 6.4E-08 |
| G000000010819             | Hspa4l              | heat shock protein family A (Hsp70) member 4 like | -2.32 | 6.9E-06 |
| G000000021150             | Pch3                | phospholipase C beta 3 | -2.31 | 3.1E-05 |
| G000000014141             | Serpine1            | serpin family E member 1 | -2.27 | 1.2E-04 |
| G000000016924             | Acly                | ATP citrate lyase | -2.25 | 5.5E-17 |
| G000000045560             | Gvin1               | GTPase | -2.25 | 2.1E-06 |
| G000000020503             | Cbln3               | cerebellin 3 precursor | -2.22 | 1.4E-06 |
| G000000052444             | Samd9               | sterile alpha motif domain containing 9 | -2.22 | 3.3E-04 |
| G00000005209              | Sprd1               | sprouty-related | -2.21 | 1.7E-05 |
| G00000010888             | Ankrd33b            | ankyrin repeat domain 33B | -2.20 | 2.1E-06 |

(Continued)
| Gene ID   | Description                              | Log2 Ratio | P-Value   |
|----------|------------------------------------------|------------|-----------|
| G000000047218 | Clic5 chloride intracellular channel 5 | -2.20      | 1.7E-03   |
| G00000009481  | Ddhd1 DDHD domain containing 1           | -2.19      | 2.0E-04   |
| G00000022242  | Cxcl9 C-X-C motif chemokine ligand 9     | -2.16      | 1.3E-05   |
| G0000008807   | Rp1                                      | -2.08      | 4.7E-05   |
| G0000014426   | Lox lysyl oxidase                        | -2.07      | 1.9E-03   |
| G0000015498   | Il17rb interleukin 17 receptor B         | -2.07      | 2.3E-04   |
| G0000015965   | Smad4 SMAD family member 4               | -2.07      | 8.7E-04   |
| G0000017512   | Aldh3b1 aldehyde dehydrogenase 3 family  | -2.05      | 1.0E-04   |
| G0000057092   | Sfln4 schlafen family member 4           | -2.05      | 6.2E-06   |
| G0000012685   | Adck1 aarF domain containing kinase 1    | -2.04      | 1.8E-03   |
| G0000011268   | Chd5 chromodomain helicase DNA binding protein 5 | -2.02 | 2.3E-03   |
| G0000032374   | Paq9 progesterin and adipocyte receptor family member 9 | -2.01 | 3.3E-14   |
| G0000020272   | Elap1 endosome-lysosome associated apoptosis and autophagy regulator 1 | -1.97 | 1.0E-04   |
| G0000061118   | LOC102551095 uncharacterized LOC102551095 | -1.96 | 9.6E-05   |
| G0000061527   | Gck glucokinase                           | -1.93      | 4.4E-07   |
| G0000053460   | Acot3 acyl-CoA thioesterase 3            | -1.91      | 1.4E-04   |
| G0000055043   | Cpeb2 cytoplasmic polyadenylation element binding protein 2 | -1.91 | 2.0E-03   |
| G0000017332   | Dapk2 death-associated protein kinase 2  | -1.87      | 3.8E-04   |
| G0000034013   | Acaca acetyl-CoA carboxylase alpha        | -1.86      | 4.5E-05   |
| G0000017611   | Tnp1 transition protein 1                | -1.86      | 2.0E-03   |
| G0000012603   | Sestd1 SEC14 and spectrin domain containing 1 | -1.85 | 1.2E-03   |
| G0000025558   | Palm2 paralentin 2                        | -1.84      | 5.7E-06   |
| G0000018461   | Pdgfrb platelet derived growth factor receptor beta | -1.82 | 1.0E-03   |
| G0000016123   | Rnf144b ring finger protein 144B          | -1.80      | 5.5E-17   |
| G0000013111   | Metl3 methyltransferase-like 3            | -1.78      | 6.7E-04   |
| G0000045679   | Apoa1 apolipoprotein A1                   | -1.78      | 1.1E-11   |
| G000001926    | Cldn1 claudin 1                           | -1.78      | 1.8E-06   |
| G000005600    | Nrra2 nuclear receptor subfamily 4        | -1.77      | 4.2E-04   |
| G0000012148   | Trio trio Rho guanine nucleotide exchange factor | -1.76 | 7.0E-04   |
| G0000004626   | Scl3a2 solute carrier family 34 member 2 | -1.76      | 8.7E-05   |
| G0000009360   | Sh3bp1 SH3-domain binding protein 1       | -1.74      | 2.2E-03   |
| G0000010890   | Bmp1 bone morphogenetic protein 1         | -1.71      | 1.8E-07   |
| G0000011820   | Acp3 acid phosphatase 3                   | -1.69      | 1.1E-04   |
| G0000007591   | Scl45a3 solute carrier family 45          | -1.68      | 8.9E-05   |
| G0000006170   | Bach2 BTB domain and CNC homolog 2        | -1.68      | 1.2E-03   |
| G00000028895   | Rtp4 receptor (chemosensory) transporter protein 4 | -1.66 | 5.3E-05   |
| G0000002773   | Rgs4 regulator of G-protein signaling 4   | -1.65      | 5.0E-04   |
| G0000007234   | Cyp51 cytochrome P450                     | -1.64      | 1.2E-09   |
| G0000020918   | Ccdn1 cyclin D1                           | -1.64      | 7.0E-09   |
| G00000028941   | Zbed3 zinc finger                        | -1.63      | 8.4E-06   |
| G0000012681   | Lgals9 galectin 9                         | -1.63      | 2.8E-13   |
| G0000001640   | Tomm70 translocase of outer mitochondrial membrane 70 | -1.63 | 2.6E-03   |
| G0000009117   | Otub2 OTU deubiquitinase                 | -1.62      | 1.9E-04   |
| G0000005726   | Pclo piccolo (presynaptic cytomatrix protein) | -1.62 | 6.2E-04   |
| G00000051171  | G6pc glucose-6-phosphatase              | -1.61      | 1.6E-04   |
| G0000016552   | Hmgcs1 3-hydroxy-3-methylglutaryl-CoA synthase 1 | -1.60 | 4.4E-15   |
| G0000004577   | Fer2 fasciculation and elongation protein zeta 2 | -1.60 | 1.9E-04   |
| G0000000547   | Tspyl4 TSPY-like 4                       | -1.59      | 5.0E-04   |

(Continued)
| Gene ID     | Symbol  | Description                                      | Z-score | P-value          |
|------------|---------|--------------------------------------------------|---------|------------------|
| Abhd2      |         | abhydrolase domain containing 2                 | -1.59   | 1.9E-07          |
| Tgif1      |         | TGFB-induced factor homeobox 1                  | -1.56   | 1.1E-03          |
| Irf1       |         | interferon regulatory factor 1                  | -1.54   | 2.4E-06          |
| Trib3      |         | tribbles pseudokinase 3                         | -1.54   | 8.5E-06          |
| Mitd1      |         | microtubule interacting and trafficking domain containing 1 | -1.52 | 1.9E-03          |
| Dgkd       |         | diacylglycerol kinase                            | -1.52   | 1.2E-05          |
| Dhc7       |         | 7-dehydrocholesterol reductase                  | -1.51   | 1.1E-05          |
| Gpd2       |         | glycerol-3-phosphate dehydrogenase 2            | -1.51   | 5.5E-04          |
| Adora1     |         | adenosine A1 receptor                           | -1.50   | 2.3E-06          |
| Abhd1      |         | abhydrolase domain containing 1                 | -1.50   | 2.1E-07          |
| Dapk1      |         | death associated protein kinase 1               | -1.48   | 5.3E-04          |
| Wdc21      |         | WAP four-disulfide core domain 21               | -1.45   | 1.6E-04          |
| Pcsk9      |         | proprotein convertase subtilisin/kexin type 9   | -1.44   | 4.7E-07          |
| Trim47     |         | tripartite motif-containing 47                  | -1.44   | 1.3E-04          |
| Map4k4     |         | mitogen-activated protein kinase kinase kinase 4 | -1.43   | 1.1E-05          |
| Plekha1    |         | pleckstrin homology domain containing B1        | -1.43   | 8.9E-05          |
| Tmcc3      |         | transmembrane and coiled-coil domain family 3   | -1.43   | 2.6E-05          |
| Ptd1       |         | pentatripeptide repeat domain 1                 | -1.43   | 1.0E-03          |
| Elov12     |         | ELOVL fatty acid elongase 2                     | -1.43   | 2.8E-11          |
| Gbp11I     |         | GC-rich promoter binding protein 1-like 1        | -1.41   | 2.3E-04          |
| Agpat3     |         | 1-acetylglcerol-3-phosphate O-acyltransferase 3 | -1.41   | 3.8E-09          |
| Sh3g3      |         | SH3 domain containing GRB2 like 3               | -1.40   | 4.1E-05          |
| Mmp15      |         | matrix metalloproteinase 15                     | -1.40   | 1.9E-03          |
| Agxt       |         | alanine—glyoxylate and serine—pyruvate aminotransferase | -1.39   | 7.5E-11          |
| Bg4        |         | BCL2-associated athanogene 4                    | -1.38   | 1.2E-03          |
| Dcaf1      |         | DDB1 and CUL4 associated factor 1               | -1.38   | 2.1E-03          |
| Dnajc18    |         | DnaJ heat shock protein family (Hsp40) member C18 | -1.37   | 2.4E-04          |
| S100a10    |         | S100 calcium binding protein A10                | -1.35   | 2.2E-04          |
| Ifih1      |         | interferon induced with helicase C domain 1     | -1.35   | 7.5E-04          |
| Pde8a      |         | phosphodiesterase 8A                            | -1.34   | 1.5E-03          |
| Prnp       |         | prion protein                                   | -1.33   | 1.7E-03          |
| Apoa4      |         | apolipoprotein A4                               | -1.33   | 2.6E-10          |
| Abcb4      |         | ATP binding cassette subfamily B member 4       | -1.33   | 4.1E-10          |
| Cyp2c7     |         | cytochrome P450                                 | -1.32   | 1.0E-08          |
| Lpl        |         | lipoprotein lipase                              | -1.31   | 5.3E-05          |
| AABR07062570 |         | AABR07062570                                   | -1.30   | 2.5E-03          |
| Tmem135    |         | transmembrane protein 135                      | -1.29   | 4.1E-05          |
| Egr1       |         | early growth response 1                         | -1.29   | 1.1E-05          |
| Aabr07024870 |        | Aabr07024870                                   | -1.29   | 1.4E-03          |
| Znfx1      |         | zinc finger                                     | -1.28   | 5.5E-04          |
| Tipal      |         | alpha tocopherol transfer protein like          | -1.27   | 1.7E-03          |
| Lys2       |         | lysozyme 2                                      | -1.26   | 2.7E-05          |
| Polg       |         | DNA polymerase gamma                            | -1.26   | 2.9E-04          |
| Aldh1H2    |         | aldehyde dehydrogenase 1 family                | -1.26   | 3.5E-04          |
| Fabp4      |         | fatty acid binding protein 4                    | -1.25   | 2.5E-03          |
| Mab21I3    |         | mab-21 like 3                                   | -1.25   | 1.0E-04          |
| Psmb9      |         | proteasome 20S subunit beta 9                   | -1.25   | 7.4E-04          |

(Continued)
| Gene ID | Description | Log2 Fold Change | P-value |
|--------|-------------|-----------------|---------|
| G00000015124 | Gpam | glycerol-3-phosphate acyltransferase | -1.25 | 1.4E-03 |
| G00000020871 | Ltbp4 | latent transforming growth factor beta binding protein 4 | -1.22 | 2.1E-03 |
| G00000016516 | Mbp | myelin basic protein | -1.22 | 1.2E-04 |
| G00000007324 | Plxna2 | plexin A2 | -1.22 | 2.4E-07 |
| G00000018211 | Adipoq | adiponectin | -1.21 | 2.9E-03 |
| G00000020573 | Efna1 | ephrin A1 | -1.19 | 1.7E-04 |
| G00000046061 | Meis1 | Meis homeobox 1 | -1.19 | 8.3E-04 |
| G00000016471 | Ets2 | ETS proto-oncogene 2 | -1.17 | 9.1E-09 |
| G00000059043 | Itch | itchy E3 ubiquitin protein ligase | -1.17 | 1.4E-03 |
| G00000067871 | Dhcr24 | 24-dehydrocholesterol reductase | -1.16 | 1.9E-12 |
| G00000151211 | N4bp1 | Nedd4 binding protein 1 | -1.15 | 3.0E-04 |
| G00000042771 | Apol3 | apolipoprotein L | -1.14 | 7.6E-08 |
| G00000023664 | Lepr | leptin receptor | -1.12 | 2.5E-03 |
| G00000004511 | RT1-Ba | RT1 class II | -1.12 | 1.1E-03 |
| G00000127821 | Cemip2 | cell migration inducing hyaluronidase 2 | -1.11 | 1.0E-05 |
| G00000147663 | Galt | galactose-1-phosphate uridylyltransferase | -1.11 | 1.7E-05 |
| G00000147181 | Acsl3 | acyl-CoA synthetase long-chain family member 3 | -1.11 | 1.4E-03 |
| G00000174281 | Map1b | microtubule-associated protein 1B | -1.10 | 1.7E-03 |
| G00000181571 | Trim21 | tripartite motif-containing 21 | -1.09 | 2.0E-03 |
| G00000014261 | Prkripl1 | PRK antibody-related protein 1 | -1.09 | 2.2E-03 |
| G000000284481 | Elov1 | ELOVL fatty acid elongase 1 | -1.08 | 2.2E-03 |
| G00000056951 | Mgp | matrix Gla protein | -1.07 | 1.2E-03 |
| G00000175581 | Tubb2a | tubulin | -1.07 | 7.2E-04 |
| G00000128761 | Sk6a13 | solute carrier family 6 member 13 | -1.07 | 7.1E-05 |
| G00000189601 | Syne1 | spectrin repeat containing nuclear envelope protein 1 | -1.07 | 7.3E-05 |
| G00000179931 | Abch10 | ATP binding cassette subfamily B member 10 | -1.05 | 8.6E-05 |
| G00000075451 | Angpt4 | angiopoietin-4 | -1.04 | 4.1E-07 |
| G00000079901 | Adipor2 | adiponectin receptor 2 | -1.04 | 1.1E-04 |
| G000000201341 | Upt1 | UPF1 | -1.03 | 5.1E-04 |
| G000000274341 | Pitm2 | fat storage-inducing transmembrane protein 2 | -1.02 | 1.7E-05 |
| G00000483151 | Eif2ak2 | eukaryotic translation initiation factor 2-alpha kinase 2 | -1.02 | 6.7E-05 |
| G00000056421 | Frs2 | fibroblast growth factor receptor substrate 2 | -1.02 | 7.7E-04 |
| G00000146041 | Sigmar1 | sigma non-opioid intracellular receptor 1 | -1.01 | 4.9E-09 |
| G00000021751 | Clock | clock circadian regulator | -1.01 | 2.4E-04 |
| G000000427851 | Sesn2 | sestrin 2 | -1.00 | 1.7E-05 |
| G000000234631 | Parp9 | poly (ADP-ribose) polymerase family | -0.99 | 3.5E-04 |
| G000000433771 | Fdps | farnesyl diphosphate synthase | -0.99 | 1.6E-03 |
| G00000005931 | Rev3l | REV3 like | -0.98 | 2.0E-03 |
| G00000192831 | P2ry2 | purinergic receptor P2Y2 | -0.98 | 1.0E-03 |
| G000000240611 | Rarb | retinoic acid receptor | -0.98 | 2.7E-03 |
| G00000172201 | Tcirg1 | T-cell immune regulator 1 | -0.98 | 3.3E-04 |
| G000000210321 | Sphk2 | sphingosine kinase 2 | -0.97 | 2.3E-05 |
| G00000015851 | Nrip1 | nuclear receptor interacting protein 1 | -0.97 | 1.1E-03 |
| G000000388821 | Cep350 | centrosomal protein 350 | -0.96 | 5.2E-04 |
| G00000052921 | Trip11 | thyroid hormone receptor interactor 11 | -0.96 | 2.4E-06 |
| G000000468891 | Dbi | diazepam binding inhibitor | -0.96 | 2.3E-05 |
| G00000066641 | Tpsd2 | tyrosylprotein sulfotransferase 2 | -0.95 | 2.1E-03 |
| G00000049001 | Crem | cAMP responsive element modulator | -0.95 | 2.0E-03 |
Table 1. (Continued)

| Accession | Gene Symbol | Description | Fold Change | P-Value |
|-----------|-------------|-------------|-------------|---------|
| G00000024115 | C6 | complement C6 | -0.93 | 2.9E-04 |
| G00000030225 | Clpx | caseinolytic mitochondrial matrix peptidase chaperone subunit X | -0.92 | 3.8E-05 |
| G00000038012 | Commd6 | COMM domain containing 6 | -0.91 | 1.8E-04 |
| G00000073002 | Fbn1 | fibrillin 1 | -0.91 | 1.4E-03 |
| G00000018420 | Slc2a7 | solute carrier family 22 member 7 | -0.90 | 4.1E-04 |
| G00000026265 | Dedi | Dedi homolog | -0.89 | 1.6E-06 |
| G0000007728 | Gsdmd | gasdermin D | -0.89 | 2.3E-03 |
| G00000026942 | RGD131195 | similar to KIAA2026 protein | -0.88 | 2.9E-05 |
| G00000030466 | Hspa8 | heat shock protein family A (Hsp70) member 8 | -0.87 | 3.0E-04 |
| G00000019372 | Pc | pyruvate carboxylase | -0.87 | 8.5E-06 |
| G00000000177 | Plpp2 | phospholipid phosphatase 2 | -0.87 | 9.6E-04 |
| G00000056703 | Atrx | ATRX | -0.86 | 2.0E-04 |
| G00000016219 | Vnn1 | vanin 1 | -0.86 | 1.5E-04 |
| G00000014338 | Slc25a25 | solute carrier family 25 member 25 | -0.86 | 6.6E-04 |
| G0000013393 | Sorbs2 | sorbin and SH3 domain containing 2 | -0.84 | 9.9E-06 |
| G00000016692 | Hsd12 | hydroxysteroid dehydrogenase like 2 | -0.83 | 1.3E-04 |
| G00000024145 | Trim65 | tripartite motif-containing 65 | -0.83 | 5.9E-05 |
| G00000010947 | Mmp14 | matrix metallopeptidase 14 | -0.83 | 1.7E-03 |
| G00000018584 | Ptma | prothymosin alpha | -0.83 | 1.5E-05 |
| G0000008274 | Xpc | XPC complex subunit | -0.83 | 3.6E-04 |
| G00000112616 | Tct14 | tetrafunctional repeat domain 14 | -0.83 | 2.7E-03 |
| G00000047386 | Smg1 | SMG1 | -0.82 | 2.7E-03 |
| G00000074000 | Sreb2 | sterol regulatory element binding transcription factor 2 | -0.82 | 4.0E-04 |
| G00000028800 | Gsap | gamma-secretase activating protein | -0.82 | 2.1E-03 |
| G00000007700 | Inhbc | inhibin subunit beta C | -0.81 | 5.3E-04 |
| G00000013178 | Cmip | c-Maf-inducing protein | -0.81 | 6.6E-04 |
| G00000002394 | Tyma | thymidine phosphorylase | -0.80 | 5.0E-04 |
| G00000031709 | Ppflb1 | PPFIA binding protein 1 | -0.79 | 6.6E-04 |
| G00000030200 | Slc25a47 | solute carrier family 25 | -0.79 | 1.5E-03 |
| G00000010947 | Gta | gamma-secretase activating protein | -0.78 | 1.6E-03 |
| G000000010497 | RGD1305807 | hypothetical LOC298077 | -0.77 | 1.7E-05 |
| G000000010814 | Tmprss6 | transmembrane serine protease 6 | -0.75 | 3.7E-04 |
| G00000004709 | Foxn3 | forkhead box N3 | -0.73 | 2.2E-04 |
| G00000007681 | Vdr | vitamin D receptor | -0.72 | 2.1E-03 |
| G00000033593 | Osbp9 | oxysterol binding protein-like 9 | -0.72 | 7.9E-04 |
| G00000002212 | Hsd17b13 | hydroxysteroid (17-beta) dehydrogenase 13 | -0.70 | 5.2E-06 |
| G000000053550 | Itga1 | integrin subunit alpha 1 | -0.68 | 1.6E-03 |
| G000000030700 | COX3 | cytochrome c oxidase subunit 3 | -0.67 | 2.8E-04 |
| G000000020425 | Stim1 | stromal interaction molecule 1 | -0.66 | 1.0E-03 |
| G00000057814 | Nsdh | NAD(P) dependent steroid dehydrogenase-like | -0.66 | 2.0E-05 |
| G00000056371 | Pik3ca | phosphatidylinositol-4- | -0.66 | 1.5E-03 |
| G000000016266 | Mphosphi10 | M-phase phosphoprotein 10 | -0.65 | 2.2E-03 |
| G000000015441 | Il4r | interleukin 4 receptor | -0.65 | 1.8E-03 |
| G0000009102 | Fermt2 | fermitin family member 2 | -0.62 | 2.2E-03 |
| G00000040518 | Rabep1 | rabaptin | -0.62 | 1.8E-03 |
| G00000020151 | Cdh1 | cadherin 1 | -0.60 | 2.4E-03 |
| G00000013135 | Ptpn12 | protein tyrosine phosphatase | -0.58 | 2.7E-04 |
| G00000057623 | Copb1 | COPI coat complex subunit beta 1 | -0.53 | 4.7E-04 |

(Continued)
| ZDF Liver Upregulated | Ensembl_ID (ENSRNO) | Gene Symbol | Gene Name | L2FC | P-value |
|-----------------------|---------------------|-------------|-----------|-------|---------|
| G00000003119          | Gc                  | GC          | peroxiredoxin like 2A | 0.45  | 1.1E-03 |
| G00000000610          | Cisd1               | CDGSH iron sulfur domain 1 | 0.46  | 7.7E-04 |
| G00000019629          | Lamp1               | lysosomal-associated membrane protein 1 | 0.50  | 8.8E-05 |
| G00000000701          | Jscu                | iron-sulfur cluster assembly enzyme | 0.51  | 4.0E-05 |
| G00000037850          | Mtarc2              | mitochondrial amidoxime reducing component 2 | 0.51  | 6.0E-04 |
| G00000019048          | Sod2                | superoxide dismutase 2 | 0.54  | 2.8E-04 |
| G00000007967          | Sdhb                | succinate dehydrogenase complex iron sulfur subunit B | 0.54  | 1.8E-03 |
| G00000013928          | Dsp                 | desmplakin | 0.54  | 1.5E-03 |
| G00000016794          | Phyhd1              | phytanoyl-CoA dioxygenase domain containing 1 | 0.55  | 2.0E-03 |
| G00000019626          | Slc27a5             | solute carrier family 27 member 5 | 0.55  | 8.8E-05 |
| G00000028368          | Etnk2               | ethanolamine kinase 2 | 0.55  | 1.4E-03 |
| G00000011535          | Gcsdh               | glycine cleavage system protein H | 0.56  | 9.9E-04 |
| G00000008921          | Dynll2              | dynein light chain LC8-type 2 | 0.56  | 1.3E-03 |
| G00000003494          | Gsta4               | glutathione S-transferase alpha 4 | 0.56  | 1.1E-03 |
| G00000018604          | Tufm                | Tu translation elongation factor | 0.59  | 2.2E-03 |
| G00000017672          | Akr1c14             | aldo-keto reductase family 1 | 0.59  | 3.1E-04 |
| G00000020994          | Slc25a39            | solute carrier family 25 | 0.59  | 7.3E-04 |
| G000000047708          | Gsta1              | glutathione S-transferase zeta 1 | 0.59  | 1.1E-04 |
| G00000013704          | Cps1                | carbamoyl-phosphate synthase 1 | 0.60  | 5.4E-04 |
| G00000043404          | Uroc1               | uracanate hydratase 1 | 0.60  | 1.6E-05 |
| G00000007395          | Baat                | bile acid-CoA:amino acid N-acyltransferase | 0.60  | 5.3E-04 |
| G000000017577          | Bphl                | biphenyl hydrolase like | 0.60  | 6.8E-04 |
| G00000007069          | Adhfe1              | alcohol dehydrogenase | 0.62  | 4.9E-04 |
| G000000023538          | Aldh5a1             | aldehyde dehydrogenase 5 family | 0.62  | 4.9E-04 |
| G00000006653          | Slc38a4             | solute carrier family 38 | 0.62  | 1.2E-04 |
| G00000001333          | A2gg1               | alpha-2-glycoprotein 1 | 0.62  | 8.6E-06 |
| G000000016339          | Uox                 | urate oxidase | 0.63  | 2.8E-05 |
| G000000061876          | Tas1r2              | taste 1 receptor member 2 | 0.63  | 2.4E-04 |
| G00000006916          | Sardh               | sarcosine dehydrogenase | 0.63  | 8.6E-05 |
| G000000029549          | Eci3                | enoyl-Coenzyme A delta isomerase 3 | 0.63  | 8.9E-04 |
| G000000048723          | Pros1               | protein S | 0.64  | 4.9E-04 |
| G00000008205          | Slco2a1             | solute carrier organic anion transporter family | 0.64  | 2.7E-05 |
| G00000007839          | Slc16a7             | solute carrier family 16 member 7 | 0.64  | 8.2E-04 |
| G000000010389          | Ndrg2               | NDRG family member 2 | 0.65  | 5.7E-04 |
| G000000014165          | Ssr1                | signal sequence receptor subunit 1 | 0.65  | 1.0E-04 |
| G000000029735          | Pid1                | phosphorylserine interaction domain containing 1 | 0.65  | 1.9E-03 |
| G00000003466          | Apon                | apolipoprotein N | 0.65  | 1.1E-03 |
| G000000000518          | Cdo1                | cysteine dioxygenase type 1 | 0.65  | 6.6E-06 |
| G00000008364          | Cat                 | catalase | 0.67  | 1.1E-03 |
| G000000061883          | Aqp9                | aquaporin 9 | 0.68  | 1.3E-03 |

(Continued)
Table 1. (Continued)

| GeneID   | Description                          | Fold Change | q-value    |
|----------|--------------------------------------|-------------|------------|
| G00000021916 | Slc16a12 solute carrier family 16     | 0.68        | 2.5E-03    |
| G00000007743 | Mgst1 microsomal glutathione S-transferase 1 | 0.68        | 1.8E-05    |
| G00000003653 | Fh fumarate hydratase               | 0.68        | 1.6E-03    |
| G00000013223 | Fah fumarylactoacetate hydrolase     | 0.69        | 2.4E-04    |
| G00000014700 | Ttc36 tetratricopeptide repeat domain 36 | 0.69        | 8.4E-05    |
| G00000030862 | Atp6v1h ATPase V1 subunit H         | 0.69        | 4.9E-04    |
| G00000003667 | Ppm1b protein phosphatase           | 0.71        | 4.7E-06    |
| G00000004139 | Ndel1 nudE neurodevelopment protein 1-like 1 | 0.72        | 3.8E-05    |
| G00000007927 | Mettl7b methyltransferase like 7B    | 0.72        | 5.0E-05    |
| G00000004147 | Abca8a ATP-binding cassette         | 0.73        | 1.4E-03    |
| G00000029726 | Gstm1 glutathione S-transferase mu 1 | 0.74        | 1.3E-03    |
| G0000003370 | Otc ornithine carbamoyltransferase   | 0.74        | 6.8E-06    |
| G00000013039 | Add1 adducin 1                      | 0.74        | 4.2E-04    |
| G00000014727 | Fahd1 fumarylactoacetate hydrolase domain containing 1 | 0.75        | 4.2E-04    |
| G00000059463 | Slc39a1 solute carrier family 39 member 1 | 0.76        | 1.6E-03    |
| G00000004302 | Pah phenylalanine hydroxylase       | 0.76        | 3.4E-07    |
| G00000029651 | Rdh16 retinol dehydrogenase 16      | 0.76        | 8.2E-04    |
| G00000028746 | Gsto1 glutathione S-transferase omega 1 | 0.77        | 3.2E-04    |
| G00000018426 | NEWGENE_2134 apolipoprotein C1      | 0.77        | 1.3E-06    |
| G00000001053 | Tmed2 transmembrane p24 trafficking protein 2 | 0.77        | 6.7E-04    |
| G00000016173 | Cyp1a2 cytochrome P450              | 0.77        | 6.7E-04    |
| G00000004089 | Enpp2 ectonucleotide pyrophosphatase/phosphodiesterase 2 | 0.78        | 3.5E-04    |
| G00000042274 | Fbxo31 F-box protein 31             | 0.78        | 2.3E-03    |
| G00000000186 | Tst thiosulfate sulfurtransferase    | 0.78        | 8.6E-05    |
| G000000048812 | Gpx1 glutathione peroxidase 1      | 0.79        | 5.0E-04    |
| G000000047986 | Sult2a1 sulfotransferase family 2A member 1 | 0.79        | 2.5E-03    |
| G00000006345 | Sec61b SEC61 translocon subunit beta | 0.79        | 6.2E-04    |
| G00000009779 | Krt8 keratin 8                      | 0.79        | 2.2E-03    |
| G00000006623 | Cd302 CD302 molecule                | 0.80        | 1.5E-04    |
| G00000005987 | Suox sulfite oxidase                | 0.81        | 1.1E-03    |
| G000000061890 | Ust5r integral membrane transport protein UST5r | 0.81        | 2.3E-04    |
| G000000020879 | Nags N-acetylglutamate synthase     | 0.81        | 3.3E-04    |
| G000000008902 | Pon1 paraoxonase 1                 | 0.82        | 9.7E-07    |
| G00000018904 | Dtymk deoxynucleotide kinase       | 0.82        | 2.1E-03    |
| G000000023116 | Agmo alkylglycerol monoxygenase     | 0.82        | 4.0E-05    |
| G000000047816 | Ccs copper chaperone for superoxide dismutase | 0.84        | 1.3E-04    |
| G000000012142 | Glyat glycine-N-acetyltransferase   | 0.84        | 5.6E-07    |
| G000000021206 | Plaat3 phospholipase A and acyltransferase 3 | 0.84        | 7.5E-04    |
| G000000012962 | Nudt16 nudix hydrolase 16          | 0.85        | 1.9E-04    |
| G000000050315 | Dcxr dicarboxyl and L-xylulose reductase | 0.86        | 2.9E-06    |
| G00000000024 | Hebp1 heme binding protein 1       | 0.86        | 2.7E-04    |
| G00000000386 | Pbd1 phenazine biosynthesis-like protein domain containing 1 | 0.87        | 1.3E-05    |
| G00000007378 | Accox2 acyl-CoA oxidase 2          | 0.87        | 7.0E-05    |
| G00000003307 | Gcdh glutaryl-CoA dehydrogenase    | 0.87        | 2.2E-08    |
| G00000002205 | Ociad1 OCIA domain containing 1    | 0.87        | 1.4E-03    |
| G000000014645 | Aldh7a1 aldehyde dehydrogenase 7 family | 0.88        | 8.2E-08    |
| G00000008638 | Angptl3 angiopoietin-like 3        | 0.88        | 2.9E-09    |
| G00000011351 | Mat1a methionine adenosyltransferase 1A | 0.89        | 3.6E-05    |

(Continued)
| Gene ID | Gene Symbol | Description | Log2 Fold Change | P-Value |
|--------|-------------|-------------|-----------------|---------|
| G00000009421 | Ivd | isovaleryl-CoA dehydrogenase | 0.89 | 1.9E-09 |
| G00000036894 | Cisd3 | CDGSH iron sulfur domain 3 | 0.89 | 4.0E-04 |
| G00000014128 | Ecsit | ECST signaling integrator | 0.90 | 1.6E-03 |
| G00000017619 | Aldh1a1 | aldehyde dehydrogenase 1 family | 0.90 | 3.1E-05 |
| G00000018662 | Amacr | alpha-methylacyl-CoA racemase | 0.90 | 3.9E-07 |
| G00000020000 | Tmem219 | transmembrane protein 219 | 0.90 | 5.2E-04 |
| G0000001957 | Sult1e1 | sulfotransferase family 1E member 1 | 0.90 | 2.8E-06 |
| G00000018680 | Rnase4 | ribonuclease A family member 4 | 0.91 | 1.3E-09 |
| G00000014160 | Tcp1 | t-complex 1 | 0.91 | 2.2E-04 |
| G00000048114 | Echdc3 | enoyl-CoA hydratase domain containing 3 | 0.91 | 2.7E-07 |
| G00000032391 | Creg1 | cellular repressor of E1A-stimulated genes 1 | 0.92 | 1.3E-07 |
| G00000008837 | Ass1 | argininosuccinate synthase 1 | 0.92 | 7.7E-04 |
| G00000018159 | Anxa4 | annexin A4 | 0.92 | 2.3E-04 |
| G00000010993 | Dpm1 | dolichyl-phosphate mannosyltransferase subunit 1 | 0.92 | 9.1E-04 |
| G00000019982 | Eth1 | ETHE1 | 0.92 | 2.4E-05 |
| G00000031717 | Esrp2 | epithelial splicing regulatory protein 2 | 0.93 | 9.8E-07 |
| G00000013409 | Gdm | glutamate cysteine ligase | 0.93 | 3.0E-04 |
| G00000018060 | Fetub | fetuin B | 0.93 | 2.9E-04 |
| G00000017291 | Sord | sorbitol dehydrogenase | 0.94 | 7.2E-09 |
| G00000053362 | Gabarap1 | GABA type A receptor associated protein like 1 | 0.94 | 1.4E-07 |
| G00000021174 | Macrod1 | mono-ADP ribosylhydrolase 1 | 0.95 | 7.1E-05 |
| G00000014268 | Abca2 | ATP binding cassette subfamily A member 2 | 0.95 | 9.8E-04 |
| G00000049771 | Gst1 | glutathione S-transferase theta 1 | 0.96 | 8.4E-05 |
| G00000011226 | Timm8a1 | translocase of inner mitochondrial membrane 8A1 | 0.96 | 4.5E-06 |
| G00000005175 | Sgpp1 | sphingosine-1-phosphate phosphatase 1 | 0.97 | 2.0E-03 |
| G00000049464 | Cyp2c13 | cytochrome P450 | 0.97 | 6.0E-10 |
| G00000002210 | Hsd17b11 | hydroxysteroid (17-beta) dehydrogenase 11 | 0.97 | 4.4E-10 |
| G00000012786 | Pgrmc1 | progestosterone receptor membrane component 1 | 0.99 | 1.2E-07 |
| G00000004327 | Ddc | dops decarboxylase | 0.99 | 4.8E-05 |
| G000000046357 | Adh5 | alcohol dehydrogenase 5 (class III) | 0.99 | 1.2E-11 |
| G00000050409 | Prelid2 | PRELI domain containing 2 | 0.99 | 7.6E-04 |
| G00000004442 | Dgucy | D-glutamate cyclase | 0.99 | 1.6E-03 |
| G00000004876 | Lpin2 | lipin 2 | 1.00 | 3.9E-04 |
| G00000012911 | Erlin1 | ER lipid raft associated 1 | 1.00 | 6.8E-04 |
| G00000053314 | Msrb1 | methionine sulfoxide reductase B1 | 1.00 | 1.1E-07 |
| G00000006619 | Dnacj9 | DnaJ heat shock protein family (Hsp40) member C9 | 1.01 | 6.5E-04 |
| G00000018937 | Gstm7 | glutathione S-transferase | 1.01 | 1.6E-04 |
| G00000027016 | Cobbl1 | cordon-bleu WH2 repeat protein-like 1 | 1.01 | 1.4E-04 |
| G00000046007 | Clbn3 | claudin 3 | 1.02 | 2.8E-04 |
| G00000036009 | Irf1 | iroquois homeobox 1 | 1.02 | 2.0E-03 |
| G00000017777 | Ahcy | adenosylhomocysteinase | 1.02 | 1.5E-05 |
| G00000019180 | Acsl4 | acyl-CoA synthetase long-chain family member 4 | 1.02 | 1.0E-08 |
| G00000022932 | Serh2 | serine hydrolase-like 2 | 1.03 | 1.5E-04 |
| G00000016484 | Gstk1 | glutathione S-transferase kappa 1 | 1.03 | 1.5E-07 |
| G00000003620 | Fmo3 | flavin containing dimethylaminoline monooxygenase 3 | 1.04 | 1.7E-05 |
| G00000032895 | Cyp4f4 | cytochrome P450 | 1.04 | 5.0E-08 |
| G00000032737 | F7 | coagulation factor VII | 1.05 | 2.1E-04 |
| G00000023816 | Aph1a | aph-1 homolog A | 1.05 | 1.6E-03 |

(Continued)
| Gene Symbol | Description                          | Ratio | p-value      |
|------------|-------------------------------------|-------|--------------|
| Cyb5a      | cytochrome b5 type A                | 1.06  | 9.6E-07      |
| Ugp2       | UDP-glucose pyrophosphorylase 2     | 1.06  | 4.1E-08      |
| Cnn3       | calponin 3                          | 1.07  | 5.6E-05      |
| Gsta1      | glutathione S-transferase alpha-1   | 1.07  | 4.2E-10      |
| Mup5       | major urinary protein 5             | 1.07  | 1.5E-04      |
| Pmpca      | peptidase                           | 1.08  | 3.9E-04      |
| Hpd        | 4-hydroxyphenylpyruvate dioxygenase | 1.08  | 7.7E-06      |
| Ripk4      | receptor-interacting serine-threonine kinase 4 | 1.09  | 2.3E-03      |
| Ubd        | ubiquitin D                         | 1.10  | 2.4E-05      |
| Lrtm2      | leucine-rich repeats and transmembrane domains 2 | 1.10  | 1.8E-08      |
| Chchd7     | coiled-helix-coiled-helix domain containing 7 | 1.10  | 4.4E-04      |
| Cyp2c23    | cytochrome P450                      | 1.10  | 4.4E-07      |
| Cyp27a1    | cytochrome P450                      | 1.11  | 1.7E-08      |
| Fam126b    | family with sequence similarity 126 | 1.13  | 4.7E-04      |
| Crym       | crystallin                           | 1.14  | 2.1E-04      |
| Mccx2      | methylcrotonoyl-CoA carboxylase 2   | 1.16  | 1.8E-05      |
| Pdlim1     | PDZ and LIM domain 1                | 1.16  | 7.7E-07      |
| Ca3        | carbonic anhydrase 3                | 1.17  | 4.7E-10      |
| Polg2      | DNA polymerase gamma 2              | 1.17  | 6.2E-04      |
| Rpl13a     | ribosomal protein L13A              | 1.19  | 2.6E-03      |
| Prlbp      | pyridoxal phosphate binding protein | 1.19  | 2.3E-06      |
| Por        | cytochrome P450 oxidoreductase       | 1.19  | 1.2E-09      |
| Ecd        | ecdysoneless cell cycle regulator   | 1.20  | 6.0E-04      |
| Per2       | period circadian regulator 2        | 1.20  | 3.1E-04      |
| Rgn        | regucalcin                           | 1.21  | 8.8E-08      |
| Qdpr       | quinoid dihydropteridine reductase  | 1.21  | 3.4E-09      |
| Ephx1      | epoxide hydrolase 1                 | 1.22  | 7.9E-07      |
| Gch1       | GTP cyclohydrolase 1                | 1.23  | 2.8E-07      |
| Bco2       | beta-carotene oxygenase 2           | 1.24  | 6.3E-07      |
| Hsd11b1    | hydroxysteroid 11-beta dehydrogenase 1 | 1.24  | 4.3E-09    |
| Slc16a10   | solute carrier family 16 member 10  | 1.24  | 1.6E-05      |
| AsrGl1     | asparaginase and isoaspartyl peptidase 1 | 1.25  | 2.7E-03      |
| Mtrr       | 5-methyltetrahydrofolate-homocysteine methyltransferase reductase | 1.26  | 1.6E-03      |
| Bud23      | BUD23                               | 1.27  | 1.7E-03      |
| Prodh1     | proline dehydrogenase 1             | 1.28  | 2.1E-11      |
| Acsn2      | acyl-CoA synthetase medium-chain family member 2 | 1.30  | 3.4E-09      |
| Zfp189     | zinc finger protein 189             | 1.30  | 1.5E-03      |
| Tsku       | tsukushi                             | 1.32  | 7.2E-04      |
| Glyat2     | glycine-N-acetyltransferase-like 2  | 1.33  | 7.3E-07      |
| Sat2       | spermidine/spermine N1-acetyltransferase family member 2 | 1.33  | 6.9E-05      |
| Rup2       | urinary protein 2                   | 1.34  | 7.8E-04      |
| Cyp2c22    | cytochrome P450                      | 1.35  | 9.3E-08      |
| Ppp1r3c    | protein phosphatase 1               | 1.36  | 4.1E-11      |
| Pbx1       | PBX homeobox 1                      | 1.36  | 1.4E-03      |
| Snx8       | sorting nexin 8                     | 1.37  | 2.0E-04      |
| Rnd2       | Rho family GTPase 2                 | 1.37  | 6.0E-05      |
| G00000051227 |                             | 1.38  | 6.2E-04      |

(Continued)
Table 1. (Continued)

| Gene ID | Gene Symbol | Description | Fold Change | p-value |
|---------|-------------|-------------|-------------|---------|
| G00000052810 | Cyp2c11 | cytochrome P450 | 1.39 | 2.0E-11 |
| G00000012436 | Adh6 | alcohol dehydrogenase 6 (class V) | 1.41 | 4.4E-15 |
| G00000015936 | Gng5 | G protein subunit gamma 5 | 1.41 | 2.6E-03 |
| G00000018413 | Per3 | period circadian regulator 3 | 1.42 | 1.6E-04 |
| G00000016967 | Hfe | homeostatic iron regulator | 1.42 | 2.9E-07 |
| G0000001376 | Mettl7a | methyltransferase like 7A | 1.43 | 3.1E-04 |
| G00000056940 | Cited2 | Cbp/p300-interacting transactivator | 1.44 | 1.5E-11 |
| G00000015002 | Abhd15 | abhydrolase domain containing 15 | 1.44 | 1.5E-04 |
| G00000032959 | Adh7 | alcohol dehydrogenase 7 (class IV) | 1.45 | 7.6E-09 |
| G00000050232 | LOC680406 | similar to Urinary protein 2 precursor (RUP-2) | 1.46 | 1.5E-09 |
| G00000020700 | Rnaseh2c | ribonuclease H2 | 1.48 | 7.7E-04 |
| G00000011635 | Ces2e | carboxylesterase 2E | 1.49 | 2.9E-08 |
| G00000015354 | Aox1 | aldehyde oxidase 1 | 1.54 | 2.6E-12 |
| G00000061450 | Homer2 | homer scaffold protein 2 | 1.54 | 2.5E-05 |
| G00000009629 | Car2 | carbonic anhydrase 2 | 1.55 | 2.9E-05 |
| G00000042111 | Sult1c2a | sulfotransferase family | 1.55 | 2.3E-03 |
| G00000057072 | Slc12a3 | solute carrier family 12 member 3 | 1.55 | 3.1E-04 |
| G00000040099 | Xpupеп2 | X-prolyl aminopeptidase 2 | 1.57 | 1.1E-08 |
| G00000013313 | Ncch1 | neutral cholesterol ester hydrolyase 1 | 1.57 | 8.8E-07 |
| G00000015438 | LOC501233 | LRRG70080 | 1.58 | 1.2E-13 |
| G00000015076 | Cyp26b1 | cytochrome P450 | 1.62 | 6.3E-04 |
| G00000016456 | Il33 | interleukin 33 | 1.65 | 4.2E-18 |
| G00000017166 | Tfrc | transferrin receptor | 1.67 | 1.1E-04 |
| G00000011718 | C1r1 | complement C1r subcomponent like | 1.68 | 1.2E-06 |
| G00000013949 | Idh2 | isocitrate dehydrogenase (NADP(+)) 2 | 1.68 | 2.3E-16 |
| G00000018740 | Ugt1a6 | UDP glucuronosyltransferase family 1 member A6 | 1.69 | 6.1E-14 |
| G00000016807 | Oat | ornithine aminotransferase | 1.72 | 1.2E-05 |
| G00000025418 | Armc9 | armadillo repeat containing 9 | 1.74 | 4.5E-04 |
| G00000023778 | Gcnt2 | glucosaminyl (N-acetyl) transferase 2 (II blood group) | 1.77 | 6.8E-05 |
| G00000056596 | Alas1 | 5'-aminolevulinate synthase 1 | 1.80 | 2.3E-15 |
| G00000046664 | Cyp3a9 | cytochrome P450 | 1.82 | 5.3E-04 |
| G00000032360 | Nr1i3 | nuclear receptor subfamily 1 | 1.84 | 6.4E-05 |
| G00000011158 | Abcg1 | ATP binding cassette subfamily G member 1 | 1.86 | 3.0E-05 |
| G00000020250 | Pcgf6 | polycomb group ring finger 6 | 1.88 | 9.2E-04 |
| G00000006420 | Rbm38 | RNA binding motif protein 38 | 1.89 | 2.0E-04 |
| G00000012458 | Cyp2c1 | cytochrome P450 | 1.91 | 1.3E-19 |
| G00000022258 | Tmem150c | transmembrane protein 150C | 1.94 | 9.3E-05 |
| G00000013982 | Hsd17b2 | hydroxysteroid (17-beta) dehydrogenase 2 | 1.94 | 5.1E-04 |
| G00000021027 | Dbp | D-box binding PAR bZIP transcription factor | 1.94 | 3.2E-05 |
| G00000044337 | Map2k6 | mitogen-activated protein kinase kinase 6 | 2.07 | 1.1E-08 |
| G00000032246 | Acsm3 | acyl-CoA synthetase medium-chain family member 3 | 2.19 | 2.3E-16 |
| G00000014490 | Bdh2 | 3-hydroxybutyrate dehydrogenase 2 | 2.21 | 2.5E-14 |
| G00000036687 | Alyref | Aly/REF export factor | 2.23 | 8.9E-04 |
| G00000015519 | Ces1d | carboxylesterase 1D | 2.24 | 6.9E-32 |
| G0000009598 | Ncaph2 | non-SMC condensin II complex | 2.41 | 3.8E-05 |
| G00000043131 | LOC100360095 | urinary protein 1-like | 2.43 | 4.9E-19 |
| G00000034191 | Fmo1 | flavin containing dimethyline monooxygenase 1 | 2.46 | 2.8E-25 |
| G00000005985 | Kcnma1 | potassium calcium-activated channel subfamily M alpha 1 | 2.82 | 1.6E-04 |
Table 1. (Continued)

| Lean Liver Downregulated Ensembl_ID (ENSRNO) | Gene Symbol | Gene Name | L2FC | P-value |
|---------------------------------------------|-------------|-----------|------|---------|
| G00000029668                               | Wdfc21      | WAP four-disulfide core domain 21 | -2.72 | 1.3E-04 |
| G00000020480                               | Fads1       | fatty acid desaturase 1 | -2.53 | 4.0E-08 |
| G0000006889                                | Insig1      | insulin induced gene 1 | -2.32 | 3.2E-05 |
| G00000057557                               | Prlr        | prolactin receptor | -2.27 | 2.3E-04 |
| G00000055909                               | Apoa4       | apolipoprotein A4 | -1.93 | 2.3E-04 |
| G00000030151                               | Cyp4a2      | cytochrome P450 | -1.75 | 2.2E-08 |
| G00000019776                               | Sh3gl3      | SH3 domain containing GRB2 like 3 | -1.62 | 8.9E-05 |
| G00000046889                               | Dbi         | diazepam binding inhibitor | -1.61 | 1.1E-05 |
| G00000014702                               | Elov2       | ELOVL fatty acid elongase 2 | -1.60 | 1.8E-05 |
| G00000032297                               | Msno1       | methylsterol monoxygenase 1 | -1.56 | 3.6E-05 |
| G00000072341                               | Cyp51       | cytochrome P450 | -1.56 | 4.9E-05 |
| G00000020989                               | Tm7sf2      | transmembrane 7 superfamily member 2 | -1.45 | 6.6E-05 |

| Lean Liver Upregulated Ensembl_ID (ENSRNO) | Gene Symbol | Gene Name | L2FC | P-value |
|-------------------------------------------|-------------|-----------|------|---------|
| G0000001376                                | Mettl7a     | methyltransferase like 7A | 1.16 | 1.7E-04 |
| G00000048114                               | Echdc3      | enoyl CoA hydratase domain containing 3 | 1.17 | 1.2E-04 |
| G00000023116                               | Agmo        | alkylglycerol monoxygenase | 1.21 | 1.7E-04 |
| G00000002643                               | Ugdh        | UDP-glucose 6-dehydrogenase | 1.29 | 7.6E-05 |
| G00000015354                               | Aox1        | aldehyde oxidase 1 | 1.33 | 1.2E-04 |
| G00000004089                               | Enpp2       | ectonucleotide pyrophosphatase/phosphodiesterase 2 | 1.34 | 9.6E-05 |
| G00000034191                               | Fmo1        | flavin containing dimethylenine monoxygenase 1 | 1.37 | 1.7E-04 |
| G00000013291                               | Cyp2c23     | cytochrome P450 | 1.47 | 1.1E-04 |
| G00000003809                               | Sat1        | spermidine/spermine N1-acetyl transferase 1 | 1.58 | 3.9E-05 |
| G00000018740                               | Ugt1a6      | UDP glucuronosyltransferase family 1 member A6 | 1.80 | 2.6E-05 |
| G00000015519                               | Ces1d       | carboxylesterase 1D | 1.94 | 5.6E-10 |
| G00000033570                               | Arhgap8     | Rho GTPase activating protein 8 | 2.03 | 1.5E-04 |
| G00000051912                               | Acnat2      | acyl-coenzyme A amino acid N-acyltransferase 2 | 2.05 | 1.1E-04 |
| G00000001158                               | Abcg1       | ATP binding cassette subfamily G member 1 | 2.21 | 1.1E-04 |
| G00000001388                               | Sds         | serine dehydratase | 2.29 | 8.0E-06 |
| G000000047613                               | AABR07048463.1 | AABR07048463.1 | 2.34 | 1.4E-06 |
| G00000013552                               | Scd         | stearoyl-CoA desaturase | 2.36 | 4.8E-06 |
| G00000001242                               | Gst3        | glutathione S-transferase | 2.93 | 1.4E-09 |
| G00000021924                               | Cyp2c22     | cytochrome P450 | 3.22 | 5.2E-15 |
| G00000009488                               | Cyp7a1      | cytochrome P450 family 7 subfamily A member 1 | 3.36 | 1.5E-09 |

1 All genes were analyzed using DESeq2 for differential analysis.
2 Abbreviations used: ZDF, Zucker Diabetic Fatty; L2FC, log2 fold change; PFC, prefrontal cortex.
3 Benjamini–Hochberg adjusted P-values controlling for false discovery rate at 5%, where P < 0.05 was considered significant.

https://doi.org/10.1371/journal.pone.0240885.t001
component space, whereby samples are colored in red or black to distinguish either WE or CAS, respectively. In the mRNA samples, rats on the same dietary treatment (i.e. black or red) clustered together, while animals belonging to different dietary treatments separated, indicating distinctly different patterns across global mRNA expression. These results were further visualized using volcano plots for each tissue as presented in Fig 2. These volcano plots demonstrate the degree to which genes were upregulated or downregulated across each tissue. For instance, volcano plots indicate a relatively equal number of upregulated and downregulated genes in the lean PFC following WE consumption, whereas WE consumption primarily resulted in downregulated gene expression in the ZDF PFC.

During T2DM, reports indicate that genes within the oxidative stress-related pathways upregulated [26]. Evans et al. suggested that oxidative stress was driven by the hyperglycemic environment concomitant with increased concentrations of free fatty acids in the plasma [26]. Corbett et al. [30] reported that protective antioxidant genes such as glutathione peroxidase are downregulated during T2DM, and both glutathione s-transferases (GSTs) and glutathione-dependent enzymes are important in the regulation of pathophysiological alterations in numerous chronic diseases, especially T2DM [30]. Previous work has shown that dietary intervention with direct glutathione supplementation was protective against diabetic nephropathy in an insulin dependent streptozotocin-induced T1DM model [27]. This current study provides new transcriptomic evidence supporting our previous report demonstrating that WE consumption protects against diabetic nephropathy, where WE consumption leads to altered gene expression in the kidney. In this study, we noted that the strongest alterations in glutathione metabolism were in the liver, potentially because hepatic glutathione is produced at much higher concentrations (10 mM), whereas intracellular glutathione concentrations are approximately 1–2 mM [31]. This body of previous work is important in relation to our findings that
several GSTs and glutathione-dependent enzymes are significantly altered during WE consumption in lean controls and during diabetes in the kidneys and livers across both genotypes. Future mechanistic studies identifying the beneficial impact of these two enzymes in chronic diseases like T2DM are warranted.

Outside of the glutathione pathways, we also observed that there were significant differences in early growth response-1 (Egr-1) gene expression following WE consumption. Egr-1 has been implicated in the onset of insulin-resistance, as previous studies in insulin-resistant T2DM mice identified that loss of function in Egr-1 restores insulin sensitivity via increased phosphorylation of the insulin receptor substrate-1 tyrosine kinase [32]. Notably, we observed a 30% decrease in hepatic Egr-1 expression in the ZDF rats fed WE. This is an interesting finding as research by Garnett et al. [33] determined that exposing beta cells to hyperglycemic conditions resulted in a temporal and dose-dependent increase in Egr-1 transcription and translation. Furthermore, Egr-1 null mice are known for their inability of displaying diabetic and obese phenotypes [34] owing to their increased energy expenditure. These data suggest that consumption of WE may lead to altered Egr-1 expression which may play a key role in regulating energy expenditure.

We also demonstrated that WE consumption resulted in tissue-specific alterations in gene expression and that there were distinct transcriptomic differences between genotypes. WE consumption did not influence gene expression in the PFC of lean animals, while 2 genes were significantly altered in the ZDF PFC. There were more stark differences when comparing the liver tissues between the two genotypes, where more than 400 genes were altered in ZDF livers that were not altered in the liver of lean controls. It has been shown that T2DM impacts a variety of tissues [1] but previous studies have provided very little evidence of how T2DM alters the nutrigenomic responses to foods in specific tissues. It is still unknown which specific egg components lead to phenotypic differences in gene expression and future studies should focus on identifying the specific egg constituents that mediate these gene expression differences.

https://doi.org/10.1371/journal.pone.0240885.g002

Fig 2. Volcano plots. Genes upregulated (green) or downregulated (red) by WE consumption, correspond to a 1.5 decrease or increase in log fold changes. Each panel corresponds to a tissue in a given genotype: A) lean adipose; B) lean PFC; C) lean kidney; D) lean liver; E) ZDF adipose; F) ZDF PFC; G) ZDF kidney; and H) ZDF liver.
These collective findings are likely mediated through the alteration of several genes; therefore, we aimed to further examine microRNA changes involved in the underlying progression of T2DM during WE consumption.

**MicroRNA sequencing differential expression**

We examined if endogenously expressed microRNA profiles in the adipose, liver, kidney, and prefrontal cortex tissues would be altered following 8 wk consumption of dietary WE. Differential expression analyses of the ZDF microRNA data resulted in 1 differentially expressed microRNA in the adipose tissue, none in the liver, none in the kidney and 2 in the PFC that surpassed multiple testing correction. Among the lean rats, there were 2 marginally differentially expressed microRNAs in the adipose tissue, 4 in the liver, none in the kidney and none in the PFC that survived multiple testing correction. Table 2 presents the differentially expressed microRNAs in the adipose, liver, kidney, and PFC tissues across both genotypes. S3 Table contains results from DESeq2 with the results for each microRNA across all four tissues and raw microRNA read counts are contained in S4 Table.

Based on the microRNA sequencing analysis, 9 microRNAs were differentially expressed following multiple testing correction. Several of these microRNAs have been previously correlated with gestational diabetes or show to be altered in the plasma of individuals with diabetes. Very few studies to date have examined the tissue-specific changes of endogenous microRNA expression in response to dietary patterns and this is the first study to demonstrate that endogenous microRNA expression in the liver, adipose, and PFC can be altered following 8 wks of WE consumption. Future studies should focus on identifying if similar foods such as quail eggs alter microRNA expression in these tissues and determine the smallest effective dosage of egg required to recapitulate these changes in microRNAs.

**Mapping between microRNAs and target genes**

Next, we sought to determine if these significantly altered microRNAs were responsible for the tissue-specific differential expression of their predicted target genes. MicroRNA mapping analyses of the differentially expressed microRNAs and their target genes demonstrates that in each of the tissues with differentially expressed microRNAs, key target genes of these microRNAs were altered. For instance, in the lean liver microRNA-181a-3p was upregulated and two of its

| Genotype | Tissue               | MicroRNA     | L2FC  | Non adjusted p-value | P-value$^3$ |
|----------|----------------------|--------------|-------|----------------------|-------------|
| ZDF      | Adipose Downregulated| rno-miR-221-3p| -1.60 | 9.53E-05             | 0.007       |
| ZDF      | PFC Upregulated      | rno-miR-29a-3p| 0.59  | 0.0001               | 0.022       |
| ZDF      | PFC Upregulated      | rno-miR-151-5p| 0.89  | 0.0005               | 0.036       |
| Lean     | Adipose Downregulated| rno-miR-125a-5p| -1.48 | 0.0022               | 0.069       |
| Lean     | Adipose Downregulated| rno-miR-125b-5p| -1.78 | 0.0029               | 0.069       |
| Lean     | Liver Upregulated    | rno-miR-9a-5p | 1.89  | 9.08E-05             | 0.0063      |
| Lean     | Liver Upregulated    | rno-miR-181a-5p| 1.10  | 0.0007               | 0.024       |
| Lean     | Liver Upregulated    | rno-miR-10b-5p| 1.37  | 0.0011               | 0.024       |
| Lean     | Liver Downregulated  | rno-miR-192-5p| -0.57 | 0.0013               | 0.024       |

$^1$All miRNAs were analyzed using DESeq2 for differential analysis.

$^2$Abbreviations used: ZDF, Zucker Diabetic Fatty; WE, whole egg; CAS, casein; L2FC, log2 fold change; and PFC, prefrontal cortex.

$^3$Benjamini-Hochberg adjusted P-values controlling for false discovery rate at 5%, where P< 0.05 was considered significant.

https://doi.org/10.1371/journal.pone.0240885.1002
mRNA target genes were differentially expressed, Cytochrome P450 Family 7 Subfamily A Member 1 (Cyp7a1) and stearoyl-CoA desaturase (Scd). Similarly, in the lean adipose, microRNA-125b-5p was downregulated while its target gene phosphoglycolate phosphatase (Pgp) was upregulated. The microRNAs in the PFC and kidney tissue did not map to any differentially expressed genes. Table 3 summarizes the mapping between microRNAs and their gene targets.

While examining the relationship between significantly altered microRNAs and their target genes, we identified that in the livers of lean rats fed WE, the upregulated microRNA-181a-5p affected target genes involved in steroid hormone biosynthesis such as Cyp7a1 and Scd. Notably, only Cyp7a1 was upregulated in the liver of ZDF rats fed the WE-based diet while both Scd and Cyp7a1 were upregulated in the livers of lean control rats. In rodent models of diabetes, liver expression of Cyp7a1 has been shown to be decreased and thought to play a key role in regulating whole body energy homeostasis [35]. Similarly, transgenic mice overexpressing Cyp7a1 were shown to become resistant to weight gain and fatty liver disease [35]. Experiments examining the role of Scd in rat hepatocytes has demonstrated that Scd expression regulating hepatic insulin resistance during diabetes [36], but very few studies have determined the expression of Scd genes in the context of dietary consumption. Based on the data, WE consumption more strongly upregulated hepatic expression of Cyp7a1 in ZDF animals than in the lean controls and this might suggest that WE consumption can prevent or reverse the loss of hepatic Cyp7a1 expression due to diabetes.

In our lean rats, we also identified that microRNA-125b-5p was downregulated in adipose tissue where its gene target Pgp was strongly upregulated. Pgp is known to hydrolyze glycerol-3-phosphate into glycerol, and overexpression experiments in rodents showed that upregulation of Pgp leads to a reduction in body weight gain and improves hepatic glucose regulation [37]. Additionally, we observed the upregulation of liver microRNA-9a-5p, which has been correlated with gestational diabetes in humans [38]. While the gene targets of microRNA-9a-5p were not differentially expressed in the liver, future studies should look into whether endogenous microRNA expression fluctuates in response to consuming other eggs, such as quail eggs, or egg yolk alone.

**KEGG and GO functional enrichment analysis**

To further examine the molecular function of the identified DEGs, KEGG pathway analysis indicated that the most prevalent pathways influenced by dietary WE across multiple tissues in
the ZDF rats were: glutathione metabolism; oxidation-reduction; metabolism of xenobiotics; steroid hormone biosynthesis; and fatty acid synthesis pathways. In the livers of lean control rats, the most significantly expressed pathways included metabolic pathways and retinol metabolism. All the differentially expressed genes that map to KEGG and gene ontology (GO) pathways analyses are presented in S5 Table.

To further investigate the specific genes involved in the glutathione metabolism pathways, genes were categorized into the corresponding reactions identified by Reactome.org in Fig 3. Glutathione metabolism functions in antioxidant defense, signal transduction, cytokine production, and other cellular processes such as detoxification. The role of GST, GSTK, GSTO dimers, and GPX1 which function in glutathione conjugation, glucuronidation, methylation, and detoxification of reactive oxygen species, respectively, are detailed within Fig 3. These reactions within glutathione metabolism are essential for recycling of glutathione disulfide or the conjugation of GSH that can be utilized in redox reactions.

KEGG pathway analysis highlighted that in addition to an upregulation of glutathione metabolism pathways, several of the same gene products mediate metabolism of xenobiotics, a pathway upregulated in our rats fed WE-based diets. Xenobiotic metabolism has previously been shown to be downregulated during insulin dependent T1DM [27], where in this study these pathways were upregulated in response to feeding WE-based diets. These observed effects appear to be tissue specific, as these alterations were the most prominent in ZDF liver, whereas one gene, glutathione s-transferase p (Gstp1), was differentially upregulated in the kidney of ZDF rats while glutathione s-transferase mu 1 (Gstm1) was upregulated in the lean kidney. These findings support the previous observation that WE consumption affects obese phenotypes differently than a lean phenotype [17], in part, due to the different transcriptomic

![Detoxification of Reactive Oxygen Species](image1)

![Methylation](image2)

![Glutathione Conjugation](image3)

![Glucuronidation](image4)

Fig 3. Differentially expressed genes involved in glutathione metabolism. This figure was adapted from D’Eustachio, P., and Jassal, B. from the Reactome [39]. Glutathione metabolism reactions can be categorized into glutathione conjugation, glucuronidation, methylation, or detoxification of reactive oxygen species. All genes are listed within each reaction category followed by their corresponding log2Fold change in parentheses for each given tissue. Abbreviations used: ZDF, Zucker Diabetic fatty rat; GSSG, glutathione disulfide; GSH, glutathione; AS3MT, arsenite 3-methyltransferase; AdoMet, S-adenosyl methionine; AdoHcy, S-adenosyl homocysteine; CDNB, 1-chloro-2, 4-dinitrobenzene; DNPSG, S-(2,4-dinitrophenyl)glutathione; glu, glutamate; cys, cysteine; gly, glycine; gGluCys, gamma-glutamyl-L-cysteine; GST, glutathione s-transferase; and GPX, glutathione peroxidase.

https://doi.org/10.1371/journal.pone.0240885.g003
responses to dietary WE. We previously hypothesized that these differences in response to WE consumption were not due to satiety, because there was increased food intake in the WE group [18]; the present study identifies a potential molecular response to egg partially explaining these previous findings. Other suggested mechanisms that might explain differences between obese and lean genotypes include thermogenesis [40], altered methylation patterns [41], intestinal microbiome alterations [42], and changes in energy expenditure [43]. While there have been numerous studies highlighting differences in the microbiota between obese and lean phenotypes in rats [42] and humans [40], one recent study examining WE consumption concluded that it did not influence the intestinal microbiome in postmenopausal women [44]. Taken together, these observations support the idea that phenotypic alterations during T2DM may depend strongly on obesity status and energy expenditures on a molecular level, potentially in response to changes in the transcriptome.

qPCR analyses

Finally, we examined the relationship between our qPCR data for several genes to validate the results from the Quantseq analysis. Confirmatory analysis with qPCR demonstrated that across the genes selected, the qPCR data highly correlates with the mRNA Quantseq results \( R^2 = 0.72; \) S1 Fig indicating strong similarities between these two methods.

Strengths and limitations

The strengths and limitations of this study should be addressed to better understand how these results fit into the larger context of the current literature. It is estimated that in 2019, people in the United States consumed on average, 5.6 eggs per week [45]. The dose of egg used in this study would equate to roughly 14 eggs per day for a human. While our study demonstrated that consuming a large dose of WE may alter gene expression of various metabolic pathways, particularly during T2DM, this quantity of egg would not be a standard dietary practice in humans. We do recognize that our whole egg dosage was high, but the goal was to examine whether there was a transcriptomic response from consuming dietary whole egg in a T2DM model. It is worth noting that our laboratory has previously reported in ZDF rats that even smaller dosages, such as the human equivalent of <2 eggs/day, significantly reduced weight gain in the ZDF rat and therefore may be effective in identifying oxidative stress outcomes from long-term dietary whole egg consumption [18]. After the examination of the transcriptome following our high WE-based diet, it is warranted to examine these specific genes in a follow-up intervention study. Future studies will focus on titrating down the egg dosages to discern the smallest dosage to elicit similar transcriptomic responses to egg consumption that will be more translatable to human consumption patterns. Overall, our findings are significant as we are the first to report that whole hen egg consumption promotes glutathione metabolism expression during T2DM and alters the transcriptome of multiple tissues using next-generation sequencing. Additionally, we provide evidence supporting the idea that egg consumption modifies endogenous microRNA expression in a tissue-specific manner.

In summary, we examined whether feeding WE modifies expression of microRNAs or gene expression profiles across multiple tissues in a diabetic versus a lean rat model. Across all tissues examined with next generation sequencing, we identified that 9 microRNAs were differentially expressed in response to consuming WE. Additionally, we have shown that these microRNAs were related to tissue-specific changes in gene expression, and that 8 wk of consuming diets high in whole egg modified 583 genes across the PFC, kidney, liver, and adipose tissue. KEGG/GO analyses identified that glutathione metabolism was highly upregulated in response to feeding WE and qPCR results validated the sequencing results. These data suggest
that high WE consumption may provide beneficial effects during T2DM by improving glutathione metabolism gene expression across multiple tissues and decreasing gene expression in oxidative stress pathways.

**Materials and methods**

The data discussed in this publication have been deposited in NCBI’s Gene Expression Omnibus [46] and are accessible through GEO Series accession number GSE157491 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE157491). All protocols used within this study have been made publicly available at protocols.io. Protocols have been zipped into one file and can be accessed at dx.doi.org/10.17504/protocols.io.bjgakjse.

**Animal housing and experimental design**

This animal study was approved by the Institutional Animal Care and Use Committee (IACUC) at Iowa State University. All animal care was performed according to Laboratory Animal Resources Guidelines at Iowa State University. Male ZDF (fa/fa) rats (n = 12) and their lean controls (fa/+; n = 12) were obtained at 6-wk of age (Charles River, Wilmington, MA). Rats were dual-caged and acclimated for 72 h in conventional cages in a temperature-controlled room (25˚C) with a 12-h light-dark cycle. Rats were randomly assigned to an experimental diet (Table 4) consisting of either a casein (CAS)-based diet, or a WE-based diet containing dried WE powder (Rose Acre Farms).

Both diets provided 20% protein (w/w) from either vitamin-free CAS or WE powder. To match the diets for total lipid content (18.3%), corn oil was added to the control diet. Both diets were prepared in-house weekly by mixing all ingredients into a powdered form and

| Ingredient (g/kg)     | CAS     | WE     |
|-----------------------|---------|--------|
| Casein                | 200     | -      |
| Whole Egg             | -       | 435    |
| Cornstarch            | 417     | 365    |
| Corn Oil              | 183     | -      |
| Glucose Monohydrate   | 150     | 150    |
| Mineral Mix           | 35      | 35     |
| Vitamin Mix           | 10      | 10     |
| Choline Bitartrate    | 2       | 2      |
| L-methionine          | 3       | 3      |
| Biotin (1%)           | -       | 0.4    |
| Macronutrients (% total kcal)\(^3\) | 17 | 17 |
| Protein               | 48      | 48     |
| Carbohydrate          | 35      | 35     |
| Fat                   | 4,715   | 4,715  |

\(^1\) All ingredients were purchased from Envigo except for dried whole egg (Rose Acre Farms, Guthrie Center, IA), as well as L-methionine and choline bitartrate (Sigma-Aldrich). Abbreviations used: CAS, casein-based diet, WE, whole egg-based diet.

\(^2\) Total protein and lipid content provided by 435 g of dried WE was 46% (200 g) and 42% (183 g), respectively.

\(^3\) To formulate all diets such that protein was provided at 20% (w/w).
administered daily in a standard amount for both lean and ZDF rats. For the remainder of the study, rats were fed ad libitum for 8 wk and at the end of the experimental period, rats were anesthetized with a dissociative agent combination of ketamine:xylaxine (90:10 mg/kg body weight) via an intraperitoneal injection of 1μL/g body weight. Two methods of animal euthanasia were performed according to the American Veterinary Medical Association guidelines for the Euthanasia of Animals: 2020 edition [47]. Cardiac exsanguination of whole blood on the anesthetized rat was performed and serum was subsequently stored at −80˚C for downstream analysis. The second method of exsanguination was the procurement of organs. Following cardiac puncture, tissues were immediately excised, weighed, and snap frozen in liquid nitrogen for storage at −80˚C in RNALater.

RNA extraction and analysis

Tissue samples (20 mg) were rapidly thawed on ice and largeRNA and smallRNA fractions were extracted from the same isolate using the RNA SPLIT Kit (Lexogen) according to the manufacturer’s instructions. Briefly, samples were homogenized in an isolation buffer and phase separated using a phenol/chloroform extraction followed by a spin column-based purification procedure. All samples were aliquoted and stored at −80˚C for downstream analysis. Following extraction, sample concentrations for the largeRNA fraction were analyzed using a Qubit 2.0 fluorometer (Thermo Fisher) using the Qubit™ Broad Range RNA Assay Kit. RNA integrity was assessed using the Bioanalyzer 2100 (Agilent Technologies) and samples with low RNA integrity number (RIN) values <5 were discarded and re-extracted. SmallRNA concentrations were measured using a Qubit 2.0 fluorometer (Thermo Fisher) using the Qubit™ microRNA Assay Kit.

TotalRNA and smallRNA sequencing

Libraries for totalRNA were prepared using an automated protocol according to the manufacturer’s instructions for half reactions on the QuantSeq 3’ mRNA-Seq Library Prep Kit (Lexogen) using a MANTIS® Liquid Handler pipetting robot (Formulatrix). All totalRNA samples were multiplexed together across two lanes on an Illumina High-Seq 3000. SmallRNA Libraries were prepared manually using the SmallRNA-Seq Library Prep Kit (Lexogen). Briefly, 100 ng of enriched smallRNA was used as input and 3’ and 5’ adapters were ligated followed by column purifications. Subsequently, the ligation products were reverse transcribed and double stranded cDNA libraries were generated. Finally, individual sample barcodes for multiplexing were introduced via 17 cycles of PCR. All libraries were assessed on the Bioanalyzer 2100 (Agilent) to examine if adapter dimers formed during PCR. All libraries were further prepared using a bead purification module (Lexogen) and pooled into a single sample at 2 nM (20 μL reaction) for sequencing.

Sequencing quality control and adapter trimming

For both totalRNA and smallRNA samples, the resulting FASTQ files were analyzed using Fast-QC [48] and sequencing adapters were trimmed using on BBDUK [49] with an example of the trimming procedure: bbduk.sh in = reads.fq out = clean.fq maq = 10 ref = /bbmap/resources/adapters.fa. For smallRNA samples, reads were additionally trimmed using the literal flag to remove the Lexogen specific sequence “5’ –TGGAAATTTCGGGTGC CAAG-GAACTCCAGTCAC– 3’” following similar trimming procedures. Briefly, any read segments that matched Illumina Truseq or Nextera adapters, along with reads containing integrity scores <10 were trimmed out.
Alignment and read quantification

For totalRNA, reads were mapped to the Ensembl release 94 of the Rattus Norvegicus RNO_6.0 genome using RNA STAR [50]. TotalRNA read counts were generated during the read alignment using the—genecounts function in STAR. For smallRNA samples, reference fasta files from www.RNACentral.org were downloaded for microRNA, piwiRNA, snRNA, rRNA, rRNA, and tRNA. Indexes were generated using Bowtie [50] and alignment was conducted using the smallrnaseq python tool [51]. Read counts for all reference indices and Iso-miRs were generated using the smallrnaseq python tool.

Data filtering and normalization

Following read count generation, Quantsseq gene expression data was merged into a single data frame for analysis in R (version 3.6.0). Genes were discarded from the analysis if there were <3 samples without a single read for that given gene. TotalRNA data initially generated read counts for 32,883 genes and over 50% of the trimmed reads from each sample mapped to the RNO_6 version 94 genome. Prior to normalization, remaining gene counts across all four tissues contained between 8,700–12,000 genes for analysis. The microRNA data originally generated read counts for over 350 microRNAs and the formal analysis was conducted on 60–150 targets across each tissue. For totalRNA and smallRNA fractions, all samples were normalized using the Trimmed Mean of M values (TMM) method [52]. Briefly, TMM accounts for variable depth between samples by normalizing them according to the weighted trimmed mean of the log expression ratios across all samples prior to analysis.

Differential expression analysis

All differential expression analyses were conducted using R (version 3.6.0). Differential expression was conducted using DESeq2 from Bioconductor. DESeq-DataSetFromMatrix generated p-values and Benjamini-Hochberg [53] adjusted P-values controlling false discovery rate (FDR) at 5%. Significance was determined at adj P<0.05.

Heatmaps, principal component analysis, and volcano plots

Principal Component Analysis (PCA) was used to visualize sample relatedness across treatments and tissues. Subsequent hierarchical clustering grouped samples according to transcriptomic relatedness, while volcano plots were constructed to visualize samples with absolute log-fold changes >1.5. All figures were generated with Matplotlib in Python version 3.2.0rc1.

KEGG/GO pathway analysis

Biological pathways for each DEG were generated using the KEGG pathway analysis and GO analysis conducted via the Database for Annotation, Visualization, and Integrated Discovery (DAVID) v6.7 software tool.

qPCR validation analyses

TotalRNA from each tissue was aliquoted and frozen at -80°C, and 2 μg of total RNA was reverse-transcribed into cDNA using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Catalog # 4368813). cDNA was diluted to 250 ng/μL and qPCR reactions were performed using 250 ng of total cDNA with primers at 300 nM concentration in 10 μL FastStart Sybr Green Master (Roche) according to the manufacturer’s instructions. Briefly, the thermocycling protocol followed a pre-incubation at 95°C for 10 minutes, followed by 45 cycles of 3-Step amplification: 1) denature at 95°C for 20 seconds; 2) anneal and extend at
60°C for 20 seconds; and 3) elongate at 72°C for 20 seconds. All qPCR reactions were conducted in a Roche LightCycler 96 Real-Time PCR System. Primers sequences for qPCR are as follows: Fatty Acid Synthase FWD: GCCGAGTCTATGCCACTATTC, REV: GCTGATACAGA GAACGGATGAG; Indolethylamine N-methyltransferase FWD: CTGGGAAGGAGAGGTTAG AA, REV: CCGGCAACCACGAAGTATAA; Cytochrome P450, family 2, subfamily c, polypeptide 22 FWD: AGAGAGAGAGAGAGAGAGAGAGA, REV: GAGACCCTCTGACATCCTATAAC; 18S Ribosomal Subunit FWD: AAGACGAACCAGAGCGAAAG, REV: TGCGGAACCTACGACGGTATCT; Cytochrome P450, family 51 FWD: CCTTCCAGTGGTGCTCTTATT, REV: CTAAGCCAC-TACCCAAAAGACTATAC. In all qPCR experiments, 18s RNA expression was used to normalize gene expression within each tissue sample that was processed in triplicate. All data were analyzed using the Livak Delta-Delta CT method [54].

MicroRNA bioinformatic analysis
All microRNA fastq files were processed using the smallrnaseq [51] package in python. Smallrnaseq automates standard bioinformatic processes for quantification and analysis of small non-coding RNA species such as microRNA quantification and novel microRNA prediction. Briefly, smallrnaseq uses bowtie to align fastq files to user defined reference sequences and all reference sequences were downloaded from www.RNAcentral.org (version 14). Following alignment to the Rattus Norvegicus genome and reference tRNA, rRNA, microRNA, lncRNA, and snRNA files, novel microRNA predictions are conducted using microRNADeep2. Additionally, differential expression was automated using the DEseq2 package in R.

Supporting information
S1 Fig. qPCR correlation with mRNA sequencing. Log fold change comparisons between qPCR and mRNA sequencing of several genes suggesting strong relationship between these two methods. (TIF)

S1 Table. mRNA raw read counts. Raw microRNA counts used in the analysis for comparing dietary treatment groups. (XLSX)

S2 Table. mRNA DeSEQ2 summary statistics. Summary statistics for the mRNA data from the DeSEQ2 analysis in R. (XLSX)

S3 Table. MicroRNA DeSEQ2 summary statistics. Summary statistics for the microRNA data from the DeSEQ2 analysis in R. (XLSX)

S4 Table. Raw microRNA read counts. Raw microRNA read counts used in the analyses. (XLSX)

S5 Table. KEGG/GO analysis. Gene ontology pathways that were upregulated/downregulated for each set of differentially expressed genes within each tissue using the DAVID database. (XLSX)

Acknowledgments
We would like to thank Dr. Peng Liu, Department of Statistics, for aiding in planning this project, and the ISU DNA facility staff members Kevin Calvalin, Tanya Murtha and Dr. Mike
Baker for their assistance sequencing our samples. Additionally, the authors would like to thank the undergraduate research assistants that helped conduct the experiments and work with the rats.

**Author Contributions**

**Conceptualization:** Joe L. Webb, Amanda E. Bries, Timothy A. Day, Michael J. Kimber, Rudy J. Valentine, Matthew J. Rowling, Stephanie Clark, Elizabeth M. McNeill, Kevin L. Schalinske.

**Data curation:** Joe L. Webb, Amanda E. Bries.

**Formal analysis:** Joe L. Webb, Amanda E. Bries.

**Funding acquisition:** Timothy A. Day, Michael J. Kimber, Rudy J. Valentine, Matthew J. Rowling, Stephanie Clark, Elizabeth M. McNeill, Kevin L. Schalinske.

**Investigation:** Joe L. Webb, Amanda E. Bries, Brooke Vogel, Claudia Carrillo, Lily Harvison.

**Methodology:** Joe L. Webb, Amanda E. Bries, Kevin L. Schalinske.

**Project administration:** Joe L. Webb, Amanda E. Bries, Matthew J. Rowling, Stephanie Clark, Elizabeth M. McNeill, Kevin L. Schalinske.

**Resources:** Kevin L. Schalinske.

**Software:** Joe L. Webb, Amanda E. Bries.

**Validation:** Elizabeth M. McNeill.

**Visualization:** Joe L. Webb, Amanda E. Bries, Elizabeth M. McNeill, Kevin L. Schalinske.

**Writing – original draft:** Joe L. Webb, Amanda E. Bries.

**Writing – review & editing:** Timothy A. Day, Michael J. Kimber, Rudy J. Valentine, Matthew J. Rowling, Stephanie Clark, Elizabeth M. McNeill, Kevin L. Schalinske.

**References**

1. Zheng Y, Ley SH, Hu FB. Global aetiology and epidemiology of type 2 diabetes mellitus and its complications. Nature Reviews Endocrinology. Nature Publishing Group; 2018. pp. 88–98. https://doi.org/10.1038/nrendo.2017.151 PMID: 29219149

2. Folli F, Corradi D, Fanti P, Davalli A, Paez A, Giaccari A, et al. The Role of Oxidative Stress in the Pathogenesis of Type 2 Diabetes Mellitus Micro- and Macrovascular Complications: Avenues for a Mechanistic-Based Therapeutic Approach. Curr Diabetes Rev. 2012; 7: 313–324. https://doi.org/10.2174/157339911797415585 PMID: 21838680

3. Raza H, John A, Howarth FC. Increased Oxidative Stress and Mitochondrial Dysfunction in Zucker Diabetic Rat Liver and Brain. Cell Physiol Biochem. 2015; 35: 1241–1251. https://doi.org/10.1159/000379947 PMID: 25766534

4. Sekhar R V., Mckay S V., Patel SG, Guthikonda AP, Reddy VT, Balasubramanyan A, et al. Glutathione synthesis is diminished in patients with uncontrolled diabetes and restored by dietary supplementation with cysteine and glycine. Diabetes Care. 2011; 34: 162–167. https://doi.org/10.2337/dc10-1006 PMID: 20929994

5. Raza H, John A, Howarth FC. Alterations in glutathione redox metabolism, oxidative stress, and mitochondrial function in the left ventricle of elderly zucker diabetic fatty rat heart. Int J Mol Sci. 2012; 13: 16241–16254. https://doi.org/10.3390/ijms131216241 PMID: 23203193

6. Kanikarla-Marie P, Micinski D, Jain SK. Hyperglycemia (high-glucose) decreases I-cysteine and glutathione levels in cultured monocytes and blood of Zucker diabetic rats. Mol Cell Biochem. 2018; 459: 151–156. https://doi.org/10.1007/s11010-019-03998-z PMID: 31172396

7. Tessari P. Effects of insulin on whole-body and regional amino acid metabolism. Diabetes Metab Rev. 1994; 10: 253–285. https://doi.org/10.1002/dmr.5610100304 PMID: 7835172
8. Patel D, Rooney R, Groom S. Gene Expression Profiles for the Zucker Fatty Rat Versus Zucker Diabetic Fatty Rat are Highly Consistent with Those Observed in Human Patients. Available: www.criver.com

9. Cordero-Herrera I, Martín MÁ, Goya L, Ramos S. Cocoa intake ameliorates hepatic oxidative stress in young Zucker diabetic fatty rats. Food Res Int. 2015; 69: 194–201. https://doi.org/10.1016/j.foodres.2014.12.039

10. Zou XR, Zhan LR, Chen L, Long QH, Yuan J, Wang L, et al. Influence of the klotho/FGF23/egr1 signaling pathway on calciumphosphorus metabolism in diabetic nephropathy and the intervention of she-nyuan granules. J Biol Regul Homeost Agents. 2019; 33: 1695–1702. https://doi.org/10.23812/19-207-A PMID: 31989808

11. Your M, Howell N. Antioxidant and ACE inhibitory bioactive peptides purified from egg yolk proteins. Int J Mol Sci. 2015; 16: 29161–29178. https://doi.org/10.3390/ijms161226155 PMID: 26690134

12. Fuller NR, Caterson ID, Sainsbury A, Denyer G, Fong M, Gerofi J, et al. The effect of a high-egg diet on cardiovascular risk factors in people with type 2 diabetes: the Diabetes and Egg (DIABEGG) study—a 3-mo randomized controlled trial. Am J Clin Nutr. 2015; 101: 705–713. https://doi.org/10.3945/ajcn.114.096925 PMID: 25833969

13. Fuller NR, Sainsbury A, Caterson ID, Markovi TP. Egg consumption and human cardio-metabolic health in people with and without diabetes. Nutrients. MDPI AG; 2015. pp. 7399–7420. https://doi.org/10.3390/nut7095344 PMID: 26404366

14. Djoussé L, Khawaja OA, Gazziano JM. Egg consumption and risk of type 2 diabetes: a meta-analysis of prospective studies.1. Am J Clin Nutr. 2016; 103: 474–480. https://doi.org/10.3945/ajcn.115.119933 PMID: 26790305

15. Virtanen JK, Mursu J, Tuomainen T-P, Virtanen HE. Voutilainen S. Egg consumption and risk of incident type 2 diabetes in men: the Kuopio Ischaemic Heart Disease Risk Factor Study. Am J Clin Nutr. 2015; 101: 1086–1096. https://doi.org/10.3945/ajcn.114.104109 PMID: 25823339

16. García-Rímon M, González C, Urangá JA, López-Miranda V, López-Fandiño R, Miguel M. Pepsin Egg White Hydrolysate Ameliorates Obesity-Related Oxidative Stress, Inflammation and Steatosis in Zucker Fatty Rats. Peterson J, editor. PLoS One. 2016; 11: e0151193. https://doi.org/10.1371/journal.pone.0151193 PMID: 26985993

17. Saande CJ, Jones SK, Rowling MJ, Schalinske KL. Whole Egg Consumption Exerts a Nephroprotective Effect in an Acute Rodent Model of Type 1 Diabetes. J Agric Food Chem. 2018; 66: 866–870. https://doi.org/10.1021/acs.jafc.7b04774 PMID: 29345464

18. Saande CJ, Webb JL, Curry PE, Rowling MJ, Schalinske KL. Dietary Whole Egg Reduces Body Weight Gain in a Dose-Dependent Manner in Zucker Diabetic Fatty Rats. J Nutr. 2018; 149: 1766–1775. https://doi.org/10.1093/jn/nxz143 PMID: 31254347

19. Dhas Y, Mishra N, Banerjee J. Vitamin D Deficiency and Oxidative Stress in Type 2 Diabetic Population of India. Cardiovasc Hematol Agents Med Chem. 2017; 14: 82–89. https://doi.org/10.2174/187152571466160426150233 PMID: 27114101

20. Saande CJ, Steffes MA, Webb JL, Valentine RJ, Rowling MJ, Schalinske KL. Whole Egg Consumption Impairs Insulin Sensitivity in a Rat Model of Obesity and Type 2 Diabetes. Curr Dev Nutr. 2019;3. 7: 121. https://doi.org/10.1093/cdn/nzz099 PMID: 32258994

21. Pouratfshar S, Akhavan NS, George KS, Foley EM, Johnson SA, Keshavarz B, et al. Egg consumption may improve factors associated with glycemic control and insulin sensitivity in adults with pre- and type II diabetes. Food Funct. 2018; 9: 4469–4479. https://doi.org/10.1039/c8fo00194d PMID: 30073224

22. Dehghan M, Mente A, Rangarajan S, Mohan V, Lear S, Swaminathan S, et al. Association of egg intake with blood lipids, cardiovascular disease, and mortality in 177,000 people in 50 countries. Am J Clin Nutr. 2020; 111: 795–803. https://doi.org/10.1093/ajcn/nqz348 PMID: 31965140

23. Geiker NRW, Lytken Larsen M, Dyerberg J, Stender S, Astrup A. Egg consumption, cardiovascular diseases and type 2 diabetes. European Journal of Clinical Nutrition. Nature Publishing Group; 2018. pp. 44–56. https://doi.org/10.1038/ejcn.2017.153 PMID: 28952608

24. Tran NL, Barraj L, Heilman J, Scrallford C. Egg consumption and cardiovascular disease among diabetic individuals: a systematic review of the literature. Diabetes, Metab Syndr Obes Targets Ther. 2014; 7: 121. https://doi.org/10.2147/DMSO.S98668 PMID: 24711708

25. Aba P, Igwebuke D, Onah J. Effects of Various Concentrations of Quail Egg Solution on Glycemia and Antioxidant Parameters of Alloxan-induced Diabetic Rats. J Adv Med Pharm Sci. 2016; 5: 1–7. https://doi.org/10.9734/jamps/2016/22723

26. Evans JL, Goldfine ID, Maddux BA, Grodsky GM. Are oxidative stress—Activated signaling pathways mediators of insulin resistance and β-cell dysfunction? Diabetes. American Diabetes Association; 2003. pp. 1–8. https://doi.org/10.2337/diabetes.52.1.1 PMID: 12502486
27. Raza H, Ahmed I, John A, Sharma AK. Modulation of xenobiotic metabolism and oxidative stress in chronic streptozotocin-induced diabetic rats fed with Momordica charantia extract. J Biochem Mol Toxicol. 2000; 14: 131–139. https://doi.org/10.1002/(sici)1099-0461(2000)14:3<131::aid-jbt2>3.0.co;2-q PMID: 10711628

28. Quigley JD. Effects of Spray-Dried Whole Egg and Biotin in Calf Milk Replacer. J Dairy Sci. American Dairy Science Association; 2002. https://doi.org/10.3168/jds.S0022-0302(02)74068-X PMID: 11860112

29. Chen X, Du Y, Boni GF, Liu X, Kuang J, Geng Z. Consuming egg yolk decreases body weight and increases serum HDL and brain expression of TrkB in male SD rats. J Sci Food Agric. 2019; 99: 3879–3885. https://doi.org/10.1002/jsfa.9610 PMID: 30680735

30. Corbett SW, Stern JS, Keeseey RE. Energy expenditure in rats with diet-induced obesity. Am J Clin Nutr. 1986; 44: 173–180. https://doi.org/10.1093/ajcn/44.2.173 PMID: 3728354

31. Montero D, Tachibana C, Rahn Winther J, Appenzeller-Herzog C. Intracellular glutathione pools are heterogeneously concentrated. Redox Biol. 2013; 1: 508–513. https://doi.org/10.1016/j.redox.2013.10.005 PMID: 24251119

32. Shen N, Yu X, Pan FY, Gao X, Xue B, Li CJ. An early response transcription factor, Egr-1, enhances insulin resistance in type 2 diabetes with chronic hyperinsulinism. J Biol Chem. 2011; 286: 14508–14515. https://doi.org/10.1074/jbc.M110.190165 PMID: 21321112

33. Garnett KE, Chambers JA, Waddell ID, Boam DSW. Differential gene expression between Zucker Fatty rats and Zucker Diabetic Fatty rats: A potential role for the immediate-early gene Egr-1 in regulation of beta cell proliferation. J Mol Endocrinol. 2005; 35: 13–25. https://doi.org/10.1677/jme.1.01792 PMID: 16087718

34. Zhang J, Zhang Y, Sun T, Guo F, Huang S, Chandalia M, et al. Dietary obesity-induced Egr-1 in adipocytes facilitates energy storage via suppression of FOXO2. Sci Rep. 2013; 3: 1–10. https://doi.org/10.1038/srep01476 PMID: 23502673

35. Li Tiangang, Owsley Erika, Matozel Michelle, Hsu Peter, Chiang John Y.L.. Transgenic expression of CYP7A1 in the liver prevents high fat diet-induced obesity and insulin resistance in mice | the FASEB Journal. Pharmacol Ther. 2010 [cited 18 Jun 2020]. Available: https://www.fasebj.org/doi/abs/10.1096/fasebj.24.4_supplement.570.4

36. Gutiérrez-Juárez R, Pocal A, Mulas C, Ono H, Bhanot S, Monia BP, et al. Critical role of stearoyl-CoA desaturase—1 (SCD1) in the onset of diet-induced hepatic insulin resistance. J Clin Invest. 2006; 116: 1686–1695. https://doi.org/10.1172/JCI26991 PMID: 16741579

37. Mugabo Y, Zhao S, Seifried A, Gezzar S, Al-Mass A, Zhang D, et al. Identification of a mammalian glycerol-3-phosphate phosphatase: Role in metabolism and signaling in pancreatic β-cells and hepatocytes. Proc Natl Acad Sci U S A. 2016; 113: E430–E439. https://doi.org/10.1073/pnas.1514375113 PMID: 26755581

38. Zhang M, Zhu X. miR-9-5p plays an important role in gestational diabetes mellitus (GDM) progression by targeting HK-2. Int J Clin Exp Med. 2018. Available: www.ijcem.com/ PMID: 29874342

39. Jassal B, Matthews L, Viteri G, Gong C, Lorente P, Fabregat A, et al. The reactome pathway knowledge base. Nucleic Acids Res. 2020; 48. https://doi.org/10.1093/nar/gkz1031 PMID: 31691815

40. Karst H, Steiniger J, Noack R, Steglich HD. Diet-induced thermogenesis in man: Thermic effects of sinapic acid in chronic streptozotocin-induced diabetic rats fed with Momordica charantia fruit extract. J Biochem Mol Toxicol. 2000; 14: 131–139. https://doi.org/10.1002/(sici)1099-0461(2000)14:3<131::aid-jbt2>3.0.co;2-q PMID: 10711628

41. Kvaløy K, Page CM, Holmen TL. Epigenome-wide methylation differences in a group of lean and obese women–A HUNT Study. Sci Rep. 2018; 8: 1–9. https://doi.org/10.1038/s41598-017-17765-5 PMID: 29311619

42. Chen J, He X, Huang J. Diet Effects in Gut Microbiome and Obesity. J Food Sci. 2014; 79: R442–51. https://doi.org/10.1111/1750-3841.12397 PMID: 24621052

43. Corbett SW, Stern JS, Keeseey RE. Energy expenditure in rats with diet-induced obesity. Am J Clin Nutr. 1986; 44: 173–80. https://doi.org/10.1093/ajcn/44.2.173 PMID: 3728354

44. Zhu C, Sawrey-Kubiec L, Bardagjy AS, Houts H, Tang X, Sacchi R, et al. Whole egg consumption increases plasma choline and betaine without affecting TMAO levels or gut microbiome in overweight postmenopausal women. Nutr Res. 2020 [cited 27 Apr 2020]. https://doi.org/10.1016/j.nutres.2020.04.002 PMID: 32464420

45. M. Shahbandeh, Statistica 2020. Per capita consumption of eggs in the U.S. 2020 | Statista. In: Statistica.com [Internet]. 28 Jan 2020 [cited 7 Aug 2020]. Available: https://www.statista.com/statistics/183678/per-capita-consumption-of-eggs-in-the-us-since-2000/

46. Edgar R, Domrachev M, Lash AE. Gene Expression Omnibus: NCBI gene expression and hybridization array data repository. Nucleic Acids Res. 2002; 30: 207–210. https://doi.org/10.1093/nar/30.1.207 PMID: 11752295
47. Leary S, Johnson CL. AVMA GUIDELINES FOR THE EUTHANASIA OF ANIMALS: 2020 EDITION
AVMA Guidelines for the Euthanasia of Animals: 2020 Edition* Members of the Panel on Euthanasia
AVMA Staff Consultants. 2020.

48. Andrews S. FASTQC. A quality control tool for high throughput sequence data. 2010 [cited 6 Apr 2020].
Available: https://www.bibsonomy.org/person/1f230a919c34360709aa298734d63dca3/author/0

49. Bushnell B, Rood J, Singer E. BBMerge—Accurate paired shotgun read merging via overlap. PLoS One. 2017. https://doi.org/10.1371/journal.pone.0185056 PMID: 29073143

50. Langmead B, Salzberg SL. Fast gapped-read alignment with Bowtie 2. Nat Methods. 2012; 9: 357–359.
https://doi.org/10.1038/nmeth.1923 PMID: 22388266

51. Farrell D. smallrnaseq: short non coding RNA-seq analysis with Python. Bioarxiv. 2017; 110585. https://doi.org/10.1101/110585

52. Robinson MD, Oshlack A. A scaling normalization method for differential expression analysis of RNA-seq data. Genome Biol. 2010; 11: R25. https://doi.org/10.1186/gb-2010-11-3-r25 PMID: 20196867

53. Benjamini, Yoav; Hochberg Y. Controlling the False Discovery Rate—a Practical and Powerful Approach to Multiple Testing. Journal of the Royal Statistical Society Series B-Methodological 1995. pdf. J R Stat Soc Ser B. 1995. https://doi.org/10.2307/2346101

54. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2-ΔΔCT method. Methods. 2001; 25: 402–408. https://doi.org/10.1006/meth.2001.1262 PMID: 11846609