RESEARCH REPORT
ANIMAL SCIENCE

Title: Determining the biological and metabolic differences between slow and fast growing pigs raised in commercial conditions – **NPB #11-124**

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Industry Summary:
With escalating feed prices, increasing litter sizes, and ever-evolving disease challenges, the difficulties of transitioning pigs during the weaning process is a major priority for pork producers. While many studies have suggested nutritional or environmental interventions to help ease this transition, the root biological causes for differences in growth during the transition period are not well understood. Data from our laboratory shows that poor-transitioning pigs have lower rates of protein and lipid accretion (Jones et al., 2012). In this experiment, we investigated the metabolic basis for poor weaned-pig transition by comparing gene expression in the longissimus dorsi muscle and adipose tissue of pigs selected from the bottom 10th percentile for ADG and better-transitioning contemporaries (60-70th ADG percentile). Poor transitioning pigs had lower expression of genes related to muscle structure and function, higher expression of genes related to muscle protein degradation, and lower expression of genes related to glucose metabolism. Many of the gene expression differences between poor transitioning pigs and their contemporaries indicate that the reduced performance in poor transitioning pigs reared under commercial conditions may be related to low availability of energy and AA in low tADG pigs due to low feed intake.

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Scientific Abstract

Weaning transition is a stressful event in the pig's life. While most pigs recover rapidly from this stressor, a portion of the population lags behind their contemporaries in growth performance. The underlying biological and molecular mechanisms involved in post-weaning differences in growth performance are poorly understood. The objective of this experiment was to determine the metabolic basis for poor weaned-pig transition compared to better-transitioning contemporaries by identifying changes in metabolic pathways that are correlated with, and may control, differences in post-weaning success using transcriptional profiling of muscle and adipose tissue. A total of 1,054 pigs were reared in commercial conditions and weighed at birth, weaning, and 3 wk post-weaning. Transition ADG (tADG) was calculated as the average daily gain between weeks 0 and 3 post-weaning. Nine pigs from each of the lowest 10^{th} percentile (low tADG) and the 60^{th}-70^{th} percentile (high tADG) were harvested at 3 wk post-weaning. We measured body composition and generated transcriptional profiles of longissimus dorsi (LM) and subcutaneous adipose tissue for these animals using RNAseq. Overall, 622 genes were differentially expressed ($q \leq 0.10$, fold change ≥ 1.2) in LM and no differentially expressed transcripts ($q \leq 0.10$) were identified in adipose tissue samples. Pathway analyses of differentially expressed genes in LM indicated a shift to decreased protein synthesis, increased protein degradation, and reduced glucose metabolism in the muscle of low tADG. Some of these responses are likely due to changes in Akt pathway signaling including reduced translation initiation by negative regulators of the mTOR pathway and increased ubiquitin-dependant degradation signaling through FoxO1 and antrogen-1 (Fbx32). Many of the gene expression differences between poor transitioning pigs and their contemporaries may be related to low availability of energy and AA in low tADG pigs. This information provides new insight into the metabolic changes occurring in the post weaning period, helping to lead to new strategies t achieve greater success under commercial conditions.

Introduction

The weaning transition priod is one of the most stressful events in the pig's life and is associated with poor feed intake, reduced growth performance, and impaired intestinal barrier function (Moeser et al., 2007; Wijtten et al., 2011). Improvements in technology related to nutrition, health, and management have been utilized to minimize the adverse effects of weaning stress; however, there is still a tremendous amount of within-population variation in post-weaning growth performance during the transition period. Previous research from our group has shown that poor post-weaning performance is highly correlated with performance and mortality during the wean-to-finish period (Jones, 2012). We have also identified poor feed intake and decreased protein and lipid deposition rates as key factors associated with low ADG during the period immediately post-weaning (Jones et al., 2012). While numerous contributing factors have been identified, many of the underlying biological and molecular mechanisms involved in post-weaning differences in growth performance are poorly understood.

Objective

To determine the metabolic basis for poor weaned-pig transition by identifying changes in metabolic pathways that are correlated with, and may control, differences in post-weaning success.

Materials & Methods

All experimental procedures adhered to the ethical and humane use of animals for research, and were approved by the Iowa State University Institutional Animal Care and Use Committee (#2-11-7095-S).

Animals and Housing

Over a 3.5 d period 1,500 pigs (Danbred 600 × Newsham NC32) were farrowed at a commercial sow farm. Prior to suckling, pigs were weighed individually to obtain birth weights and tagged with a unique identification number. Sow parity and litter size were recorded. All procedures from birth until weaning were

carried out according to normal procedures at the source farm, including cross-fostering among litters to standardize litter size among sows. At 16 or 17 days of age, a total of 1,054 pigs were randomly selected, weaned, and transported to a commercial wean-to-finish barn. Pigs were sorted by sex and randomly allotted to 40 pens with 26 or 27 pigs per pen.

Pigs were weighed individually at 0 and 3 weeks post-weaning. Transition ADG (**tADG**) was calculated as the average daily gain between weeks 0 and 3 post-weaning. One pig from each of the lowest 10^{th} percentile (low tADG) and the 60^{th}-70^{th} percentile (high tADG) for tADG was used to create pairs of pigs derived from litters of the same size and from sows of the same parity. Pigs remained in original pens after initial placement, so a pair of pigs may have been located in different pens. All pigs were fed the same diets in the phase-feeding program utilized by the commercial producer.

Pigs originated from a sow herd that was negative for Porcine Reproductive and Respiratory Syndrome virus (PRRSv). However, a mixed PRRSv and Influenza A virus outbreak was confirmed week 2 post-weaning in the wean-to finishing barn where the study was conducted.

Tissue Collection and Sample Preparation

At 3 weeks post-weaning, 20 pairs of pigs (40 total pigs) were transported approximately 45 min to the Iowa State University Diagnostic Laboratory. Pigs were euthanized via captive-bolt stunning and exsanguination. Samples of longissimus dorsi muscle (**LM**) and backfat were rapidly collected, frozen in liquid nitrogen, and stored at -80°C until RNA isolation. The remainder of each whole carcass, including blood, was stored at -20°C. Carcasses were then ground, homogenized, and subsampled. Sub-samples were then freeze dried, ground through a 1-mm screen and analyzed for percentage DM, ash, crude fat, and N. Briefly, percentage DM and ash were determined according to modified methods 930.15 and 942.05 (AOAC, 2005), respectively, where samples were dried at 105˚C or 600˚C, respectively to a constant weight instead of 2 h. Crude fat was determined by ether extraction without acid hydrolysis according to method 920.39 (AOAC, 2005). Nitrogen content was determined by Kjeldahl according to method 981.13 (AOAC, 2005). Calibration was conduction with a glycine standard (N content 18.7 ± 0.1%). Upon analysis, N content of the glycine standard was 18.7 ± 0.07%. Crude protein was expressed as nitrogen × 6.25. All chemical analyses were carried out in duplicate, and repeated when the intra-duplicate coefficient of variation exceeded 1%. Based on the sub-sample chemical composition, total body composition was calculated for water, protein, lipid, and ash using empty body weight at harvest.

Total RNA was isolated and purified from LM and adipose tissues of a subset of 18 pigs (9 pigs each from the low and high tADG categories) using Ambion MagMAX total RNA isolation kits (Life Technologies, Carlsbad, CA) per manufacturer's instructions. Quality and quantity of RNA were determined by using a Nanodrop ND-1000 (Thermo Scientific, Wilmington, DE) and an Agilent 2100 Bioanalyzer (Agilent Technologies, Inc., Santa Clara, CA). Mean RNA integrity numbers were 8.7 ± 0.60 for LM RNA samples and 7.4 ± 0.61 for adipose tissue RNA samples.

RNA sequencing libraries were prepared using TruSeq RNA Sample Prep Kits (Illumina Inc., San Diego, CA) and samples sequenced by 2 × 100 bps paired-end sequencing on an Illumina HiSeq 2000 instrument.

Transcriptome

Raw sequence data was transferred to the Iowa State University Genome Informatics Facility. The raw read sequences were aligned to the *Sus scrofa* genome build 10.2 (Ensemble release 70) using the Genomic Short-read Nucleotide Alignment Program (GSNAP; Version 2012-07-20) (Wu and Nacu, 2010). Reads that mapped uniquely to the genome were utilized for downstream analysis and differential expression. Raw read counts for each gene were then determined using HTSeq and the current Ensembl gene annotation file.

Quantitative Reverse Transcriptase PCR for Verification of Differentially Expressed Genes

Quantitative Reverse Transcriptase PCR (qRT-PCR) was used to verify differential expression of 32 genes in LM. Total RNA was isolated from LM of all 18 pigs as described above and reverse transcribed to cDNA using SuperScript VILO cDNA synthesis kits (Life Technologies). For the qRT-PCR, DELTAgene

Assays (Fluidigm, San Francisco, CA) were used and specific sequences were obtained after 12 cycles of denaturation at 95°C for 15 seconds and annealing and elongation at 60°C for 4 minutes using 2x Taqman PreAmp Master Mix (Life Technologies). The pre-amplified products were 10-fold diluted and further amplified with the 2x SsoFast EvaGreen Supermix with Low ROX (Biorad; Hercules, CA) in a 48.48 Dynamic Array on a Biomark system (Fluidigm). The relative expression value of each gene was calculated relative to the housekeeping gene TOP2B using the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001).

Statistical analyses

Performance and body composition

Data were analyzed using the PROC MIXED procedure of SAS with tADG (high vs. low) as a fixed effect. Differences between least squares means were separated using the PDIFF option of SAS with results considered significant if $P \leq 0.05$ and tendencies if $P \geq 0.05$ and ≤ 0.10.

Gene expression

Statistical analysis of RNAseq read counts were performed using the QuasiSeq package in R (Lund et al., 2012). The raw read counts were normalized using upper quartile normalization. Normalized read counts were fit as the dependent variable in a generalized linear model, assuming a negative binomial distribution, including the variables tADG (high vs. low), parity (1 vs. 2+) and birth weight (in kg) as fixed effects. This model was compared to a reduced model, including all fixed effects except tADG to test for differences in gene expression as a function of tADG. The QuasiSeq software allows users to account for over-dispersion in count data by modeling the estimated over-dispersion by the total number of read counts in a gene. A spline correction was used to account for some of the effects of over-dispersion. The q-value method was used to correct for multiple testing as a means to control the false discovery rate (Storey and Tibshirani, 2003).

Pathway analysis with gene ontology and Kyoto Encyclopedia Genes and Genomes

Genes in LM that were differentially expressed ($q \leq 0.10$, fold change ≥ 1.2) were divided into genes that were upregulated in high or low tADG pigs. Using the Database for Annotation, Visualization and Integrated Discovery (DAVID) version 6.7, an open-access Web-based functional annotation and clustering program (Dennis et al., 2003), the differentially expressed gene lists were analyzed for overrepresented biological process categories based on Gene Ontology (GO) or Kyoto Encyclopedia Genes and Genomes (KEGG) pathway categories. The *P*-values for overrepresentation were computed by a modified Fisher's exact test, using the transcriptome for LM as background. The GO biological process terms were clustered using functional annotation clustering tool (Dennis et al., 2003), where the enrichment score for each cluster was computed as the negative log of the geometric mean of *P*-values in the cluster. The resulting statistically significant clusters (enrichment score ≥ 1.3) were further curated to keep only GO terms with *P*-values ≤ 0.05.

Quantitative RT-PCR

Data were analyzed using the PROC MIXED procedure of SAS using the same model as the RNAseq data as described above. Differences between least squares means were separated using the PDIFF option of SAS with results considered significant if $P \leq 0.05$ and tendencies if $P \geq 0.05$ and ≤ 0.10.

Results

Growth and Body Composition

High tADG pigs gained 230% more body weight than low tADG pigs (220 vs. 96 g/d, $P < 0.0001$; Table 1) during the 3-wk post-weaning transition period. Birth weight, weaning weight, and ADG from birth to weaning were similar ($P > 0.10$) between high and low tADG pigs. Low tADG pigs were higher in whole body water ($P = 0.01$), tended to have greater ash percentage ($P = 0.06$) and were lower in body lipid content ($P = 0.001$).

Read Alignment and Differential Expression of Genes

For LM, an average of 15.3 million paired-end reads were generated per sample (Table 2). Approximately 1.2 million reads per sample were classified as "no feature", meaning the reads could not be assigned to any feature in the genome, and 1.6 million reads per sample were classified as "ambiguous", meaning the reads were assigned to multiple genomic features and could not be assigned to a specific feature within the genome. For adipose tissue, an average of 16.4 million paired-end reads were generated per sample, with 1.1 and 1.7 million reads per sample classified as "no feature" and "ambiguous", respectively. In total, 82% of LM and 83% of adipose tissue reads were uniquely mapped back to the reference genome after alignment.

RNAseq analyses revealed a total of 622 differentially expressed gene transcripts ($q \leq 0.10$) with a fold change ≥ 1.2 in LM. A total of 317 DE genes transcripts had higher expression in low tADG pigs and 305 DE genes had lower expression in low tADG pigs. No differentially expressed transcripts ($q \leq 0.10$) were identified in adipose tissue samples.

RNAseq Data Validation by qPCR

To verify expression patterns measured by RNAseq in LM, we used qPCR to assay mRNA for 32 genes, which were predicted to be differentially expressed between high and low tADG pigs ($q \leq 0.10$, fold change $\geq$ 1.2) by RNAseq. For all tested genes, expression differences were consistent in direction with the RNAseq results (Supplementary Tables 1 and 2). Statistical significance ($P \leq 0.05$) was confirmed by qPCR 84% of the tested genes in LM and all genes, except insulin-like growth factor 2 (IGF2; $P = 0.70$), had a P-value of < 0.15.

Ontological Pathways Regulated by the Genes Differentially Expressed in LM

To identify biological pathways potentially underlying the effects of tADG on skeletal muscle metabolism and physiology, we functionally annotated the genes that are differentially expressed between high and low tADG pigs using DAVID. Genes with higher expression in low tADG LM produced 3 clusters of biological process terms with enrichment scores ≥ 1.3 (Table 3). The enriched pathways in LM of low tADG pigs included protein catabolic processes, ubiquitin-dependent protein catabolism, and regulation of translational initiation. Genes with lower expression in LM of low tADG pigs were organized into 6 significant annotation clusters (Table 4). The most significant associations were with genes related to muscle contraction, the glycolysis pathway, cytoskeleton organization, muscle development, and blood vessel development.

Analysis of KEGG Pathways using DAVID revealed overrepresentation of the proteasome ($P < 0.001$) in genes with higher expression in low tADG pigs and overrepresentation of glycolysis/gluconeogenesis ($P < 0.0001$), pyruvate metabolism ($P < 0.05$), and the insulin signaling pathway ($P < 0.05$) in genes with lower expression in low tADG pigs.

Discussion

Our results indicate differences in mRNA levels and changes in metabolic pathways in LM between pigs with over a 2-fold difference in ADG during the 3-wk period following weaning. Although alterations in RNA expression do not always correspond to alterations in protein expression or activity, we suggest that systematic changes in the expression of genes that belong to overrepresented GO or KEGG categories imply functional change of pathways represented by these categories.

Adipose tissue transcriptome was not affected by tADG

Subcutaneous adipose tissue is the major site for fatty acid synthesis and storage in the pig. Previous research indicated that poor transitioning pigs had reduced lipid accretion rates (Jones et al., 2012). In the current experiment, the low tADG pigs had less whole body lipid content than the high tADG pigs; however, somewhat surprisingly, there were no statistically significant changes in the adipose tissue transcriptome observed in this study.

Expression of genes involved in protein synthesis and degradation in LM

Previous research indicated that poor transitioning pigs have lower rates of protein accretion than their contemporaries (Jones et al., 2012), and data from the current study indicate that the reduced rate of protein accretion is likely due to changes in both synthesis and degradation of skeletal muscle protein.

High tADG pigs had greater expression of genes that encode muscle structural proteins including actin (alpha 1), tropomyosin 1 (alpha), myomesin 2, and several myosin heavy chain isoforms (including IIA, IIB, IIX, and 13). The major pathway regulating muscle protein synthesis is the mechanistic target of rapamycin (mTOR) pathway. The mTOR pathway enhances global protein synthesis by increasing translation initiation via phosphorylation of both S6 kinase 1 (S6K1) and a repressor protein, eukaryotic initiation factor 4E (eIF4E) - binding protein 1 (4E-BP1). When mTOR signaling is low, the 4E-BP1 protein represses translation initiation by binding to eIF4E, blocking eIF4E-eIF4G complex formation. When 4E-BP1 is phosphorylated, eIF4E is released, which can then bind to eIF4G forming a complex that up-regulate mRNA binding to the 40S ribosomal subunit, thereby increasing translation initiation (Davis et al., 2008).

The mTOR pathway is regulated by a wide variety of cellular signals, including growth factors, nutrients, cellular energy levels, and stress conditions. Feeding increases protein synthesis in skeletal muscle, largely due to the rise in insulin and AA concentrations after feeding (Davis et al., 2002). Insulin activates the activation of phosphatidylinositol 3-kinase (PI3K) and Akt upstream of mTOR, whereas AA directly activate mTOR (Davis et al., 2000). The gene encoding the 4E-BP1 protein had higher expression in the low tADG pigs. Transcriptional control of 4EBP has not been extensively evaluated; however, due to its function as a negative regulator of translational initiation, higher expression may lead to reduced protein synthesis. DNA-damage-inducible transcript 4-like (DDIT4L; also known as REDD2) had higher expression in low tADG pigs. REDD2 is upregulated in various stress-related responses, including DNA damage, hypoxia, and glucocorticoid treatment, and works downstream of Akt to inhibit mTOR functions (Corradetti et al., 2005; Miyazaki and Esser, 2009).

Low tADG pigs had increased expression of genes and pathways involved in muscle protein degradation. Several genes involved in ubiquitin-dependant catabolism, including ubiquitin specific proteases and several components of the 26S proteasome were more highly expressed in low tADG pigs.

Akt signaling is linked to muscle ubiquitin-dependant protein degradation and muscle atrophy via the forkhead-box O (FoxO) transcription factors. In the presence of insulin, the Akt pathway sequesters FoxO transcription factors in the cytoplasm via phosphorylation (Stitt et al., 2004). When insulin signaling is low, Akt is inactive, and therefore, FoxOs are translocated to the nucleus and induce the transcription of target genes that regulate protein degradation (Kamei et al., 2004). In our experiment, low tADG pigs had higher expression of FoxO1 and FbxO32, a key transcriptional target of FoxO1. FbxO32 (also known as atrogin 1 or MAFbx) is the substrate recognition component of a muscle-specific E3 ubiquitin ligase. Atrogin 1 binds to target proteins to induce ubiquitin binding and degradation through the proteasome. Atrogin 1 is a marker of the atrophy process and has been shown to be induced in multiple models of skeletal muscle atrophy resulting from a variety of conditions including muscle inactivity, multiple disease states, and starvation (Schiaffino et al., 2013). Over-expression of FoxO1 in transgenic mice showed markedly reduced muscle mass (Kamei et al., 2004). FoxO knockdown blocks the upregulation of antrogin 1, preventing muscle loss during atrophy (Sandri et al., 2004). FoxO1 was identified as a due to its negative association with several genes with higher expression in high tADG pigs. The expression of both FoxO1 and Fbx32 are linked to increased glucocorticoid signaling.

Genes involved in energy metabolism in LM

Poor transitioning pigs are often associated with low feed intake (Huang et al., 2011; Jones et al., 2012). During periods of low energy intake, skeletal muscle is capable of sparing glucose to ensure continued glucose supply to the brain. One possible way that glucose utilization in muscle may be by decreasing transcription of genes encoding glycolytic enzymes. We found reductions in the mRNA levels for genes involved in glycolysis including glucose-6-phosphate isomerase, phosphoglycerate kinase, phosphoglycerate mutase, and pyruvate kinase in low tADG pigs. The mRNA levels for three key enzymes involved in glycogenolysis, glycogen phosphorylase, phosphorylase kinae, and the glycogen debranching enzyme amylo-alpha-1, 6-glucosidase, 4-

alpha-glucanotransferase were also downregulated in the low tADG pigs. Jagoe et al. (2002) demonstrated decreases in similar glycolytic and glycogenolytic enzymes using a food depravation model in mice.

Literature Cited

AOAC International. 2005. Official Methods of Analysis of AOAC International. 18th ed. Assoc. Off. Anal. Chem., Gaithersburg, MD.

Corradetti, M. N., K. Inoki, and K.-L. Guan. 2005. The Stress-inducted Proteins RTP801 and RTP801L Are Negative Regulators of the Mammalian Target of Rapamycin Pathway. J. Biol. Chem. 280: 9769-9772.

Davis, T. A., M. L. Fiorotto, D. G. Burrin, P. J. Reeds, H. V. Nguyen, P. R. Beckett, R. C. Vann, and P. M. O'Connor. 2002. Stimulation of protein synthesis by both insulin and amino acids is unique to skeletal muscle in neonatal pigs. Am J Physiol Endocrinol Metab. 282: E880-890.

Davis, T. A., H. V. Nguyen, A. Suryawan, J. A. Bush, L. S. Jefferson, and S. R. Kimball. 2000. Developmental changes in the feeding-induced stimulation of translation initiation in muscle of neonatal pigs. Am J Physiol Endocrinol Metab. 279: E1226-1234.

Davis, T. A., A. Suryawan, R. A. Orellana, H. V. Nguyen, and M. L. Fiorotto. 2008. Postnatal ontogeny of skeletal muscle protein synthesis in pigs. J. Anim. Sci. 86: E13-E18.

Dennis, G., Jr., B. T. Sherman, D. A. Hosack, J. Yang, W. Gao, H. C. Lane, and R. A. Lempicki. 2003. DAVID: Database for Annotation, Visualization, and Integrated Discovery. Genome Biol. 4: P3.

Huang, Y., S. Henry, R. Friendship, K. Schwartz, and J. Harding. 2011. Clinical presentation, case definition, and diagnostic guidelines for porcine periweaning failure to thrive syndrome. Journal of Swine Health and Production 19: 340-344.

Jagoe, R. T., S. H. Lecker, M. Gomes, and A. L. Goldberg. 2002. Patterns of gene expression in atrophying skeletal muscles: response to food deprivation. FASEB J. 16: 1697-1712.

Jones, C. K. 2012. Characterizing the fallback pig, Iowa State University, Ames, Iowa.

Jones, C. K., N. K. Gabler, R. G. Main, and J. F. Patience. 2012. Characterizing growth and carcass composition differences in pigs with varying weaning weights and postweaning performance. J. Anim. Sci. 90: 4072-4080.

Kamei, Y., S. Miura, M. Suzuki, Y. Kai, J. Mizukami, T. Taniguchi, K. Mochida, T. Hata, J. Matsuda, H. Aburatani, I. Nishino, and O. Ezaki. 2004. Skeletal muscle FOXO1 (FKHR) transgenic mice have less skeletal muscle mass, down-regulated Type I (slow twitch/red muscle) fiber genes, and impaired glycemic control. J Biol Chem. 279: 41114-41123.

Livak, K. J., and T. D. Schmittgen. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods. 25: 402-408.

Lund, S. P., D. Nettleton, D. J. McCarthy, and G. K. Smyth. 2012. Detecting differential expression in RNA-sequence data using quasi-likelihood with shrunken dispersion estimates. Stat Appl Genet Mol Biol. 11(5). /j/sagmb.2012.2011.issue-2015/1544-6115.1826/1544-6115.1826.xml. doi: 2010.1515/1544-6115.1826.

Miyazaki, M., and K. A. Esser. 2009. REDD2 is enriched in skeletal muscle and inhibits mTOR signaling in response to leucine and stretch. Am J Physiol Cell Physiol. 296: C583-592. doi: 510.1152/ajpcell.00464.02008. Epub 02009 Jan 00467.

Moeser, A. J., C. V. Klok, K. A. Ryan, J. G. Wooten, D. Little, V. L. Cook, and A. T. Blikslager. 2007. Stress signaling pathways activated by weaning mediate intestinal dysfunction in the pig. Am J Physiol Gastrointest Liver Physiol. 292: G173-181.

Sandri, M., C. Sandri, A. Gilbert, C. Skurk, E. Calabria, A. Picard, K. Walsh, S. Schiaffino, S. H. Lecker, and A. L. Goldberg. 2004. Foxo Transcription Factors Induce the Atrophy-Related Ubiquitin Ligase Atrogin-1 and Cause Skeletal Muscle Atrophy. Cell 117: 399-412.

Schiaffino, S., K. A. Dyar, S. Ciciliot, B. Blaauw, and M. Sandri. 2013. Mechanisms regulating skeletal muscle growth and atrophy. FEBS J. 280: 4294-4314.

Stitt, T. N., D. Drujan, B. A. Clarke, F. Panaro, Y. Timofeyva, W. O. Kline, M. Gonzalez, G. D. Yancopoulos, and D. J. Glass. 2004. The IGF-1/PI3K/Akt Pathway Prevents Expression of Muscle Atrophy-Induced Ubiquitin Ligases by Inhibiting FOXO Transcription Factors. Mol. Cell 14: 395-403.

Storey, J. D., and R. Tibshirani. 2003. Statistical significance for genomewide studies. Proc Natl Acad Sci U S A. 100: 9440-9445.

Wijtten, P. J., J. van der Meulen, and M. W. Verstegen. 2011. Intestinal barrier function and absorption in pigs after weaning: a review. Br J Nutr. 105: 967-981.

Wu, T. D., and S. Nacu. 2010. Fast and SNP-tolerant detection of complex variants and splicing in short reads. Bioinformatics. 26: 873-881.

Table 1. Body weight, average daily gain, and body composition of low and high transition average daily gain (tADG) pigs.

	Low tADG	High tADG	SEM	P-value
n =	9	9	-	-
Body weight, kg				
Birth	1.26	1.44	0.10	0.24
Weaning	4.51	5.23	0.35	0.16
3 wk post-weaning	6.23	9.19	0.38	<0.0001
ADG, g/d				
Birth to weaning	197	230	17	0.20
0-3 wk post-weaning	96	220	5	<0.0001
Body composition[1], %				
Water	68.5	66.2	0.5	0.01
Protein	15.9	15.9	0.1	0.99
Lipid	12.6	15.5	0.5	0.001
Ash	2.95	2.39	0.2	0.06

[1]Measured at 3-wk post-weaning

Table 2. Reads from Illumina

	LM		Adipose	
	Mean ± SD	Range	Mean ± SD	Range
Number of reads (in millions)				
Total	15.7 ± 1.01	14.4 – 18.8	16.4 ± 1.46	13.6 – 19.7
No feature	1.2 ± 0.10	1.0 – 1.4	1.1 ± 0.16	0.8 – 1.4
Ambiguous	1.6 ± 0.13	1.3 – 1.9	1.7 ± 0.20	1.1 – 1.9
Uniquely aligned	12.9 ± 0.92	11.8 – 15.2	13.6 ± 1.21	11.3 – 16.5
Aligned, %	92 ± 0.7%	91 – 94%	93 ± 0.7%	92 – 95%
Uniquely mapped, %	82 ± 1.2%	81 – 85%	83 ± 1.1%	82 – 87%

Paired end reads were aligned to the Sus scrofa genome (build 10.2) using GSNAP software.

Table 3. Functional annotation clusters and corresponding GO biological process terms for genes with higher express ($q < 0.10$ and fold-change ≥ 1.2) in logissimus dorsi tissue of low transition average daily gain pigs[1]

ES	GO Biological Process	Count	*P*-value
	Cluster 1		
1.8	GO:0006511~ubiquitin-dependent protein catabolic process	12	6.8E-03
	GO:0030163~protein catabolic process	22	7.2E-03
	GO:0051603~proteolysis involved in cellular protein catabolic process	21	1.1E-02
	GO:0044257~cellular protein catabolic process	21	1.1E-02
	GO:0044265~cellular macromolecule catabolic process	24	1.2E-02
	GO:0019941~modification-dependent protein catabolic process	20	1.3E-02
	GO:0043632~modification-dependent macromolecule catabolic process	20	1.3E-02
	GO:0009057~macromolecule catabolic process	25	1.3E-02
	Cluster 2		
1.3	GO:0006511~ubiquitin-dependent protein catabolic process	12	6.8E-03
	GO:0051436~negative regulation of ubiquitin-protein ligase activity during mitotic cell cycle	6	9.3E-03
	GO:0031145~anaphase-promoting complex-dependent proteasomal ubiquitin-dependent protein catabolic process	6	9.3E-03
	GO:0051352~negative regulation of ligase activity	6	1.0E-02
	GO:0051444~negative regulation of ubiquitin-protein ligase activity	6	1.0E-02
	GO:0032269~negative regulation of cellular protein metabolic process	10	1.0E-02
	GO:0051437~positive regulation of ubiquitin-protein ligase activity during mitotic cell cycle	6	1.1E-02
	GO:0051248~negative regulation of protein metabolic process	10	1.1E-02
	GO:0051443~positive regulation of ubiquitin-protein ligase activity	6	1.2E-02
	GO:0051439~regulation of ubiquitin-protein ligase activity during mitotic cell cycle	6	1.3E-02
	GO:0051351~positive regulation of ligase activity	6	1.5E-02
	GO:0031397~negative regulation of protein ubiquitination	6	1.6E-02
	GO:0032268~regulation of cellular protein metabolic process	17	1.7E-02
	GO:0051438~regulation of ubiquitin-protein ligase activity	6	1.9E-02
	GO:0051340~regulation of ligase activity	6	2.3E-02
	GO:0031398~positive regulation of protein ubiquitination	6	2.6E-02
	GO:0031396~regulation of protein ubiquitination	6	5.0E-02
	Cluster 3		
1.3	GO:0006446~regulation of translational initiation	5	1.1E-02
	GO:0045947~negative regulation of translational initiation	3	3.6E-02
	GO:0010608~posttranscriptional regulation of gene expression	9	4.6E-02

[1]An enrichment score (ES) of 1.3 is equivalent to a nonlog scale value of 0.05. Count refers to the gene members that belong to an annotation term. Fisher Exact *P*-value represents the degree of enrichment of the Gene Ontology (GO) term

Table 4. Functional annotation clusters and corresponding GO biological process terms for genes with lower expression ($q \leq 0.10$ and fold-change ≥ 1.2) in logissimus dorsi tissue of low transition average daily gain pigs[1]

ES	GO Biological Process	Count	*P*-value
	Cluster 1		
5.8	GO:0006936~muscle contraction	15	1.1E-07
	GO:0003012~muscle system process	15	4.2E-07
	GO:0006941~striated muscle contraction	7	1.1E-04
	Cluster 2		
3.2	GO:0006006~glucose metabolic process	15	3.4E-07
	GO:0019318~hexose metabolic process	15	7.2E-06
	GO:0005996~monosaccharide metabolic process	15	3.5E-05
	GO:0006096~glycolysis	7	9.0E-05
	GO:0006090~pyruvate metabolic process	7	1.3E-04
	GO:0044275~cellular carbohydrate catabolic process	8	3.4E-04
	GO:0006007~glucose catabolic process	7	3.6E-04
	GO:0019320~hexose catabolic process	7	6.7E-04
	GO:0006094~gluconeogenesis	5	8.0E-04
	GO:0046365~monosaccharide catabolic process	7	8.4E-04
	GO:0046164~alcohol catabolic process	7	1.6E-03
	GO:0016052~carbohydrate catabolic process	8	1.6E-03
	GO:0019319~hexose biosynthetic process	5	2.3E-03
	GO:0046364~monosaccharide biosynthetic process	5	5.2E-03
	GO:0034637~cellular carbohydrate biosynthetic process	6	7.8E-03
	GO:0046165~alcohol biosynthetic process	5	9.0E-03
	GO:0006091~generation of precursor metabolites and energy	13	1.2E-02
	GO:0016051~carbohydrate biosynthetic process	6	4.6E-02
	Cluster 3		
2.4	GO:0007010~cytoskeleton organization	20	2.8E-04
	GO:0030029~actin filament-based process	12	5.1E-03
	Cluster 4		
1.8	GO:0030048~actin filament-based movement	5	6.4E-04
	GO:0033275~actin-myosin filament sliding	3	1.0E-02
	GO:0030049~muscle filament sliding	3	1.0E-02
	GO:0070252~actin-mediated cell contraction	3	1.0E-02
	GO:0030705~cytoskeleton-dependent intracellular transport	5	1.7E-02
	Cluster 5		
1.6	GO:0007517~muscle organ development	14	1.3E-04
	GO:0060537~muscle tissue development	10	4.7E-04
	GO:0014706~striated muscle tissue development	9	1.4E-03
	GO:0031032~actomyosin structure organization	4	1.7E-02
	GO:0010927~cellular component assembly involved in morphogenesis	4	2.3E-02
	GO:0060538~skeletal muscle organ development	5	4.4E-02
	GO:0007519~skeletal muscle tissue development	5	4.4E-02
	Cluster 6		
1.5	GO:0001525~angiogenesis	10	1.1E-03
	GO:0048514~blood vessel morphogenesis	12	1.2E-03
	GO:0001568~blood vessel development	12	4.2E-03
	GO:0001944~vasculature development	12	4.7E-03
	GO:0001502~cartilage condensation	3	4.5E-02
	GO:0048705~skeletal system morphogenesis	6	4.6E-02

[1]An enrichment score (ES) of 1.3 is equivalent to a nonlog scale value of 0.05. Count refers to the gene members that belong to an annotation term. Fisher Exact *P*-value represents the degree of enrichment of the Gene Ontology (GO) term

Supplementary Table 1. Primers used for RT qPCR analysis

Gene Name	Forward primer	Reverse primer	Design RefSeq
ACSL4	GCCCCTCTTATTTGCTGTGAA	GGGTTTGGCTTGTCGTGAA	NM_001038694.1
AGL	GGAGAAAGTGACAGAGCCAGAA	ACTCAGGCCCACAATTTCCA	FLDM-003439.1
AMPD1	GTGTTTGAGGCCACCATCAAC	CACGCTGTCGAAGCCAGTA	NM_001123076.1
APIP	GTCTACGTCTGGGGAGAGAC	TGATACGGCAACATCGAACAA	XM_003122870.1
AQP4	CTGGTCATGGTCTCCTGGTA	TTGGAATCACAGCTGGCAAA	NM_001110423.1
BNIP3	CGCACACAGTGTTGGAGAAA	TCCCTCCTCCTCTCCATGTAA	XM_003359404.1
BTG2	TTTTCAGCGGGGCTCTCC	AGCCCTTGGATGGCTTTTCA	NM_001097505.2
CA3	AGTTCCAACTAGTCCTTGATGCA	AACAGGCAGGATGGGTTGAA	NM_001008688.1
CCDC88C	TGCACGACGTGGACTTCTA	CCAACATGGCCTTGGTTTCA	XM_001928733.1
CDKN1B	GACAGCCAGACGGGGTTA	TCGGGGAACCGTCTGAAAC	NM_214316.1
CIRBP	GAGTCAGGGTGGCAGCTA	AACTGTCGTAGCTGTCTCTGTA	XM_003122988.3
CTGF	AAGATCTCCAAGCCCGTCAA	TGCATACCCCGCAGAACTTA	U83916.1
DDIT4L	AGTTGCTAGACCGTAGCTTCC	TTGGGTTCAGGGACAACGTAA	XM_003129299.2
EIF4EBP1	TGGAGTGTCGGAACTCACCT	ATCACCCACAGGGCTGGT	NM_001244225.1
FBXO30	GGGGAAAAGGAAGTATCCAGA	AGTGGTCAGCCATACTTAGGA	XM_003121151.1
FBXO32	GAAGGACATGCTGAACAGCAAA	AGTACTTCCTTTGTGAACATAGATCCA	NM_001044588.1
FOXO1	CTCACGCTCTCGCAGATCTA	AGCTGTTGCTGTCACCCTTA	NM_214014.2
GSTK1	CGGACCGTGGAGCTTTTCTA	AGCTGCAGGTTGACATTCCA	XM_003134573.1
HSPA2	ACGACAAAGGTCGTCTAAGCA	GGCCTCATCTTCCGACTTGTA	XM_003356734.1
HSPA9	ACAGGAACACCACCATTCCA	CTCCACTTGAGTCTGTCCATCA	XM_003123976.2
IGF2	CAGCCCACAGCGATTCCAA	GAGGCCAAGGCCAAGAAGAC	NM_213883.2
IVNS1ABP	AAAAGCCACCACGTGAGAA	TCCGAAGCAACGATTTTCCA	NM_001161649.1
LIPE	CTGGATGTGCACTTCTGGAAA	GCCGATGCCATGTTTGCTA	NM_214315.1
MAX	AAAACCACACACACCAGCAA	CTGTTGTCTGAGGAGGGGTA	FLDM-041055.1
NDRG2	CCAGCTTGCAGACATGATCC	CAGCTCCAACACCAATTCCAA	NM_001078683.1
PHB2	AGTGTGGTGGCCAAGTTCA	GTCAGCTCCCTTCGGATCAA	NM_001243556.1
PSMC3	GGGTTGGTGGATGCTGAAAA	AGGGTCTCCAGGATCAGGTA	XM_003122809.1
PSMD3	CTCCCAAATCGTACAACAAGGAC	TCTGCCATCTCCTTGGCAAA	XM_003131496.2
PYGM	TCCACTCCGAGATCCTCAAGAA	GGGTGATGCCGTTGGTCTTA	XM_003122588.2
RBM3	TCGACCTGGAGGATATGGCTA	TCCTCCTGAGTAGCGGTCATA	NM_001243419.1
RORC	GGATCCACTACGGGGTTATCA	AGGAGTAGGCCACGTTACAC	XM_003355171.1
SELENBP1	GCGGCTCCCAATGTCTTAC	ACGTGTAAGCAGCTCCCATA	XM_001929643.2
SLC37A4	ATCCATGTACCTCTTCCGGGTA	ACCAAACACAGCTCCCAACA	NM_001199719.1
TOP2B	CGGCAGGAGAACATCCAAAA	GGGAAGAGGTCCACATCTGAA	NM_001258386.1

Supplementary table 2. Verification of RNAseq results using real-time PCR for select genes.

	RNAseq			RT-PCR	
Gene name	Fold change	*P*-value	q-value	Fold change	*P*-value
Higher expression in low tADG					
RORC	2.53	1.3E-06	3.7E-03	3.23	9.0E-04
GSTK1	2.01	6.5E-06	8.3E-03	2.08	4.3E-03
PSMC3	1.70	5.3E-05	2.1E-02	1.58	2.7E-03
IVNS1ABP	2.00	1.3E-04	2.7E-02	2.30	1.8E-03
BNIP3	2.07	1.4E-04	2.7E-02	2.40	1.8E-03
CIRBP	2.02	1.4E-04	2.7E-02	1.66	3.0E-03
FOXO1	2.38	2.0E-04	3.1E-02	3.99	9.9E-03
SELENBP1	2.14	2.6E-04	3.4E-02	2.23	6.0E-04
APIP	2.07	3.6E-04	3.5E-02	2.34	9.3E-03
HSPA2	1.96	3.6E-04	3.5E-02	2.42	2.2E-03
NDRG2	1.67	2.9E-04	3.5E-02	1.71	6.0E-03
PSMD3	1.59	3.5E-04	3.5E-02	1.61	5.3E-02
ACSL4	1.64	8.3E-04	4.6E-02	1.82	4.0E-03
LIPE	2.29	1.0E-03	5.1E-02	5.22	1.2E-02
AQP4	2.42	1.2E-03	5.3E-02	2.38	6.2E-03
EIF4EBP1	1.72	1.4E-03	5.5E-02	2.24	1.9E-02
DDIT4L	2.25	1.5E-03	5.7E-02	2.39	7.0E-04
FBXO32	6.43	1.7E-03	5.9E-02	13.29	2.3E-02
PHB2	1.43	2.7E-03	6.9E-02	1.43	1.4E-02
MAX	1.81	2.9E-03	7.1E-02	1.75	4.2E-02
RBM3	2.37	3.9E-03	8.0E-02	1.43	1.2E-01
HSPA9	1.58	5.8E-03	9.3E-02	1.31	1.8E-02
CDKN1B	1.44	6.4E-03	9.8E-02	1.38	6.9E-02
Lower expression in low tADG					
CCDC88C	-1.70	1.3E-04	2.7E-02	-2.18	1.0E-04
SLC37A4	-1.57	1.2E-04	2.7E-02	-2.35	8.0E-04
CA3	-1.73	1.7E-04	2.8E-02	-2.55	1.0E-04
CTGF	-1.71	2.0E-04	3.1E-02	-2.70	4.1E-02
IGF2	-1.35	5.9E-04	4.2E-02	-1.10	7.0E-01
AGL	-1.45	6.4E-04	4.2E-02	-1.80	2.0E-04
PYGM	-1.25	4.2E-03	8.2E-02	-1.37	2.3E-02
AMPD1	-1.25	4.3E-03	8.2E-02	-1.30	1.2E-01
BTG2	-1.79	6.1E-03	9.6E-02	-1.76	3.8E-02

Supplementary Table 3. Overrepresented GO biological process terms by the higher expressed genes ($q \leq 0.10$, fold-change ≥ 1.2) in logissimus dorsi tissue of low transition ADG pigs[1]

GO Biological Process term	Count	*P*-value
GO:0006511~ubiquitin-dependent protein catabolic process	12	6.8E-03
GO:0030163~protein catabolic process	22	7.2E-03
GO:0051436~negative regulation of ubiquitin-protein ligase activity during mitotic cell cycle	6	9.3E-03
GO:0031145~anaphase-promoting complex-dependent proteasomal ubiquitin-dependent protein catabolic process	6	9.3E-03
GO:0051444~negative regulation of ubiquitin-protein ligase activity	6	1.0E-02
GO:0051352~negative regulation of ligase activity	6	1.0E-02
GO:0032269~negative regulation of cellular protein metabolic process	10	1.0E-02
GO:0051603~proteolysis involved in cellular protein catabolic process	21	1.1E-02
GO:0051437~positive regulation of ubiquitin-protein ligase activity during mitotic cell cycle	6	1.1E-02
GO:0006446~regulation of translational initiation	5	1.1E-02
GO:0044257~cellular protein catabolic process	21	1.1E-02
GO:0051248~negative regulation of protein metabolic process	10	1.1E-02
GO:0044265~cellular macromolecule catabolic process	24	1.2E-02
GO:0051443~positive regulation of ubiquitin-protein ligase activity	6	1.2E-02
GO:0043632~modification-dependent macromolecule catabolic process	20	1.3E-02
GO:0019941~modification-dependent protein catabolic process	20	1.3E-02
GO:0009057~macromolecule catabolic process	25	1.3E-02
GO:0051439~regulation of ubiquitin-protein ligase activity during mitotic cell cycle	6	1.3E-02
GO:0051351~positive regulation of ligase activity	6	1.5E-02
GO:0031397~negative regulation of protein ubiquitination	6	1.6E-02
GO:0032268~regulation of cellular protein metabolic process	17	1.7E-02
GO:0010885~regulation of cholesterol storage	3	1.8E-02
GO:0043434~response to peptide hormone stimulus	8	1.9E-02
GO:0051438~regulation of ubiquitin-protein ligase activity	6	1.9E-02
GO:0016578~histone deubiquitination	3	2.2E-02
GO:0051340~regulation of ligase activity	6	2.3E-02
GO:0031398~positive regulation of protein ubiquitination	6	2.6E-02
GO:0006869~lipid transport	7	2.8E-02
GO:0006350~transcription	43	3.2E-02
GO:0022613~ribonucleoprotein complex biogenesis	9	3.3E-02
GO:0042254~ribosome biogenesis	7	3.3E-02
GO:0045449~regulation of transcription	51	3.4E-02
GO:0045947~negative regulation of translational initiation	3	3.6E-02
GO:0042157~lipoprotein metabolic process	5	4.0E-02
GO:0010876~lipid localization	7	4.2E-02
GO:0010608~posttranscriptional regulation of gene expression	9	4.6E-02
GO:0031396~regulation of protein ubiquitination	6	5.0E-02

[1]Count refers to the gene members that belong to an annotation term. Fisher Exact *P*-value represents the degree of enrichment of the Gene Ontology (GO) term

Supplementary Table 4. Overrepresented GO biological process terms by the lower expressed genes ($q \leq 0.10$, fold-change ≥ 1.2) in logissimus dorsi tissue of low transition ADG pigs[1]

GO Biological Process term	Count	*P*-value
GO:0006936~muscle contraction	15	1.1E-07
GO:0006006~glucose metabolic process	15	3.4E-07
GO:0003012~muscle system process	15	4.2E-07
GO:0019318~hexose metabolic process	15	7.2E-06
GO:0005996~monosaccharide metabolic process	15	3.5E-05
GO:0006096~glycolysis	7	9.0E-05
GO:0006941~striated muscle contraction	7	1.1E-04
GO:0006090~pyruvate metabolic process	7	1.3E-04
GO:0007517~muscle organ development	14	1.3E-04
GO:0007010~cytoskeleton organization	20	2.8E-04
GO:0043434~response to peptide hormone stimulus	11	3.0E-04
GO:0044275~cellular carbohydrate catabolic process	8	3.4E-04
GO:0006007~glucose catabolic process	7	3.6E-04
GO:0032868~response to insulin stimulus	9	4.4E-04
GO:0060537~muscle tissue development	10	4.7E-04
GO:0030048~actin filament-based movement	5	6.4E-04
GO:0019320~hexose catabolic process	7	6.7E-04
GO:0006094~gluconeogenesis	5	8.0E-04
GO:0046365~monosaccharide catabolic process	7	8.4E-04
GO:0010564~regulation of cell cycle process	9	9.0E-04
GO:0001525~angiogenesis	10	1.1E-03
GO:0048514~blood vessel morphogenesis	12	1.2E-03
GO:0014706~striated muscle tissue development	9	1.4E-03
GO:0033043~regulation of organelle organization	12	1.4E-03
GO:0046164~alcohol catabolic process	7	1.6E-03
GO:0016052~carbohydrate catabolic process	8	1.6E-03
GO:0019319~hexose biosynthetic process	5	2.3E-03
GO:0001568~blood vessel development	12	4.2E-03
GO:0001944~vasculature development	12	4.7E-03
GO:0030029~actin filament-based process	12	5.1E-03
GO:0046364~monosaccharide biosynthetic process	5	5.2E-03
GO:0034637~cellular carbohydrate biosynthetic process	6	7.8E-03
GO:0007605~sensory perception of sound	6	7.8E-03
GO:0032869~cellular response to insulin stimulus	6	7.8E-03
GO:0007049~cell cycle	25	8.5E-03
GO:0046165~alcohol biosynthetic process	5	9.0E-03
GO:0050954~sensory perception of mechanical stimulus	6	9.7E-03
GO:0030049~muscle filament sliding	3	1.0E-02
GO:0070252~actin-mediated cell contraction	3	1.0E-02
GO:0033275~actin-myosin filament sliding	3	1.0E-02
GO:0006091~generation of precursor metabolites and energy	13	1.2E-02
GO:0007017~microtubule-based process	11	1.3E-02
GO:0051130~positive regulation of cellular component organization	9	1.4E-02
GO:0000278~mitotic cell cycle	15	1.4E-02
GO:0051783~regulation of nuclear division	5	1.4E-02
GO:0007088~regulation of mitosis	5	1.4E-02
GO:0051329~interphase of mitotic cell cycle	7	1.5E-02
GO:0051325~interphase	7	1.6E-02
GO:0010638~positive regulation of organelle organization	6	1.7E-02
GO:0030705~cytoskeleton-dependent intracellular transport	5	1.7E-02
GO:0031032~actomyosin structure organization	4	1.7E-02

GO:0007346~regulation of mitotic cell cycle	8	1.9E-02
GO:0009725~response to hormone stimulus	13	1.9E-02
GO:0001501~skeletal system development	12	1.9E-02
GO:0007169~transmembrane receptor protein tyrosine kinase signaling pathway	10	2.0E-02
GO:0010927~cellular component assembly involved in morphogenesis	4	2.3E-02
GO:0031344~regulation of cell projection organization	6	2.3E-02
GO:0000082~G1/S transition of mitotic cell cycle	5	2.6E-02
GO:0044042~glucan metabolic process	4	2.6E-02
GO:0005977~glycogen metabolic process	4	2.6E-02
GO:0006073~cellular glucan metabolic process	4	2.6E-02
GO:0042325~regulation of phosphorylation	15	3.4E-02
GO:0043462~regulation of ATPase activity	3	3.5E-02
GO:0009719~response to endogenous stimulus	13	3.6E-02
GO:0006112~energy reserve metabolic process	4	3.6E-02
GO:0010975~regulation of neuron projection development	5	3.7E-02
GO:0046700~heterocycle catabolic process	5	3.7E-02
GO:0048562~embryonic organ morphogenesis	6	4.2E-02
GO:0000165~MAPKKK cascade	8	4.4E-02
GO:0060538~skeletal muscle organ development	5	4.4E-02
GO:0007519~skeletal muscle tissue development	5	4.4E-02
GO:0001502~cartilage condensation	3	4.5E-02
GO:0048568~embryonic organ development	7	4.6E-02
GO:0048705~skeletal system morphogenesis	6	4.6E-02
GO:0016051~carbohydrate biosynthetic process	6	4.6E-02
GO:0051048~negative regulation of secretion	4	4.6E-02
GO:0051174~regulation of phosphorus metabolic process	15	4.6E-02
GO:0019220~regulation of phosphate metabolic process	15	4.6E-02
GO:0022403~cell cycle phase	14	4.9E-02

[1]Count refers to the gene members that belong to an annotation term. Fisher Exact *P*-value represents the degree of enrichment of the Gene Ontology (GO) term