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Identification of differentially expressed genes in early-postmortem Semimembranosus muscle of Italian Large White heavy pigs divergent for glycolytic potential

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1	Identification of differentially expressed genes in early-postmortem
2	Semimembranosus muscle of Italian Large White heavy pigs divergent for
3	glycolytic potential
4	
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17	board. The other co-authors declare that they have no competing interests.

19 Abstract

Glycolytic potential (GP) is one of the *postmortem* traits utilized to predict the quality of the final 20 meat products. Despite that, the knowledge of the molecular and metabolic pathways controlling 21 this trait is still not complete. To add some information on this field we used two pools of Italian 22 Large White heavy pigs divergent for GP to investigate through a microarray the differences of 23 gene expressions between the two pools. On the whole, 32 genes were differentially expressed, and 24 25 among them 31 were overexpressed in low GP pool. These genes were involved in mitochondrial functions and ATP biosynthetic processes, in calcium homeostasis, and in lipid metabolism, with 26 27 PPAR signaling being a possible master regulator of the molecular differences observed between the two pools. The different GP levels between the two pools could have determined in low GP 28 29 muscles a more rapid occurrence of the molecular cascade related to the events triggering cell death.

30

31 Keywords

32 Swine; skeletal muscle; gene expression; glycogen; pHu; meat quality.

33

34 **1. Introduction**

Glycolytic potential (GP) is a biochemical parameter that defines in the skeletal muscle the amount 35 of carbohydrates susceptible to conversion into lactate during the postmortem phase (Monin & 36 37 Sellier, 1985). The major component of GP and the prevalent storage form of carbohydrate in skeletal muscle is glycogen, whose content and breakdown play a major role in the biochemical 38 changes occurring *postmortem* in the muscle tissue. Changes in the GP of a muscle affect the pH 39 40 decline: after slaughtering, the oxygen level decreases and, to satisfy the energy demand of the cells, muscle glycogen is mobilized towards the formation of glucose and lactate via glycogenolysis 41 and anaerobic glycolysis pathway (Nanni Costa et al., 2009). As anaerobic glycolysis is undeniably 42 the dominant pathway *postmortem*, muscles with high GP levels usually show a lower ultimate pH 43

that, in turn, affects other quality traits such as meat color, water holding capacity (WHC), drip loss, 44 tenderness, and processing yield (Enfält, Lundström, Hansson, Johansen, & Nyström, 1997; Nanni 45 Costa et al., 2009). Hence, GP at 24 hours (h) after slaughtering may be considered, together with 46 glycogen and lactate contents separately, among the muscle parameters capable of predicting the 47 final meat quality (Monin, Mejenes-Quijano, & Talmant, 1987; Monin, 1988; Henckel, Karlsson, 48 Mogens, Oksbjerg & Petersen, 2002; Boler et al., 2010; Chauhan & England, 2018). Even though 49 this parameter is often used to explain or predict ultimate pH (pH_u), Scheffler, Scheffler, Kasten, 50 Sosnicki & Gerrard (2013) reported that complex metabolic properties of muscle likely play a more 51 critical role than glycogen content and *postmortem* glycolysis in determining the course and 52 53 culmination of the pH decline. Certainly, lactate production is not the only metabolic process 54 influencing *postmortem* pH decline because some reports show that mitochondria may contribute to muscle *postmortem* metabolism influencing pH drop. Several studies have indicated that 55 mitochondria, to sustain an increase in ATP demand early after slaughter, may enhance the muscle 56 glycogen degradation with aerobic glucose oxidation by restoring ATP levels (England et al., 2018; 57 Matarneh, England, Scheffler, Yen, & Gerrard, 2017; Matarneh, Beline, de Luz e Silva, Shi, & 58 Gerrard, 2018). Afterward, once oxygen is extremely depleted after slaughtering, ATP hydrolysis 59 contributes the most to the H⁺ production, lowering pH, and influencing meat quality. However, the 60 61 same Authors showed that the exact underlying mechanism of how mitochondria contribute to 62 muscle metabolism and biochemical changes in the *postmortem* period is not yet clear (England, Scheffler, Kasten, Matarneh, & Gerrard, 2013; Matarneh, Yen, Bodmer, El-Kadi, &. Gerrard, 63 64 2021). The amount of glycogen stored in muscle tissue depends on several factors, such as the animal 65

species, the breed within a given species, the genetics of the animal within a breed, the muscle fiber
type composition (Shen et al., 2015), the finishing diet, and the occurrence of stressful events before
and during slaughter (reviewed in Xing et al., 2019). Concerning the animals' genetics, several

studies have focused on the discovery of genes and Quantitative Trait Loci (QTL) related to 69 70 glycogen and GP. Even though many QTLs have been detected and markers identified in some 71 candidate genes associated with GP (Kaminski et al., 2010), the knowledge of genes affecting GP is still incomplete. To date, the gene Protein Kinase AMP-Activated Non-Catalytic Subunit Gamma 3 72 (PRKAG3) has been indicated as one of the major molecular players affecting GP levels in pig 73 muscles (Milan et al., 2000; Ciobanu et al., 2001) but other genes have been investigated as 74 75 candidates influencing GP, meat color and WHC in different pig breeds (Ma et al., 2014; Zappaterra, Sami, & Davoli, 2019). However, the knowledge of the gene expression framework 76 associated with porcine muscle *postmortem* metabolism and glycogen degradation is still scant. In 77 78 this scenario, the present study utilizes two pools of Italian Large White (ILW) pigs divergent for 79 pHu, glycogen and GP measured at 24 h postmortem and aims to investigate early postmortem differences in the gene expression profile between Semimembranosus muscle (SM) samples 80 81 belonging to the two pools. The identification of differentially expressed genes (DEGs) may provide insight into molecular events occurring in early *postmortem* muscle and likely influencing 82 the metabolic and biochemical processes during the conversion of muscle to meat in samples 83 characterized by the presence of divergent amounts of muscle residual glycogen. To our knowledge, 84 this is the first study investigating the DEGs related to GP content 24 h postmortem in ILW heavy 85 86 pigs intended for the production of high-quality seasoned pork products.

87

88 2. Material and methods

89 **2.1 Animals and phenotypes**

Eight purebred ILW pigs (four females and four barrows) were selected from a larger group of 277
sib-tested pigs for their GP value measured in SM. The 277 pigs were reared at the Sib-Test genetic
station managed by the Italian Pig Breeders National Association (Associazione Nazionale
Allevatori Suini; ANAS; <u>http://www.anas.it</u>). Italian Sib-test relies on the use of triplets (two gilts

and one barrow) of full sibs of candidate boars tested to become breeders. The 277 pigs (183 gilts 94 95 and 94 barrows) were therefore subjects belonging to triplets coming from 154 litters, originated from 154 dams and 80 sires. The 277 pigs were reared in a unique testing station, and thus in a 96 standard environment, in order to avoid any environmental effects on the phenotypes of the sib-97 tested population. The testing period lasted from 30-45 days of age to about 9 months of age, up to 98 a final live weight of 155 kg, in line with the specifications reported on the document describing the 99 100 rules and the procedures to obtain Protected Designation of Origin Parma dry-cured hams. During the testing period, siblings were kept separated and all animals were fed the same growing and 101 finishing diets in order to avoid any possible effects related to diet composition. The pigs were fed 102 103 with a quasi ad libitum nutrition level, which means that about 60% of pigs were able to ingest the 104 entire supplied ration. All animals used in this study were reared according to Italian and European law for pig production and all slaughter procedures complied with national and European Union 105 106 regulations for animal care and slaughtering. At the end of the testing period, pigs were fasted for about 12 h before being transported and immediately slaughtered at a commercial abattoir located 107 near the genetic station. Pigs were transported and slaughtered in compliance with European rules 108 on the protection of animals during transport and at slaughtering (Council Regulation (EC) No. 109 110 1/2005 and Council Regulation (EC) No. 1099/2009). At the slaughterhouse, the pigs were stunned 111 by CO₂ at a concentration of 87% in the air (Butina, Holbaek, Denmark) and bled in a supine position. Slaughter procedures were monitored by the veterinary team appointed by the Italian 112 Ministry of Health. Pigs were slaughtered during six slaughtering days. The date of slaughter and 113 114 sex were recorded for each animal. SM muscle samples were gathered from the whole population of 277 pigs. One aliquot of SM samples was collected from the 277 pigs approximately 1 h after 115 slaughtering, immediately frozen in liquid nitrogen, then stored in a deep freezer at -80°C until 116 DNA or RNA extraction. From the same thigh used to collect SM samples for DNA and RNA 117 extraction, a portion of SM muscle was also gathered at 24 h postmortem from the 277 pigs, frozen 118

in liquid nitrogen and stored in a deep freezer at -80°C until performing the analyses for the
determination of glycogen, lactic acid and GP content.

Meat pH₁ (measured about 1 h postmortem) and pH_u (measured 24 h postmortem) were measured 121 directly on the SM of the left thigh of each animal. Glycogen, lactic acid content, and GP were 122 123 measured in a portion of SM collected from the same thigh at 24 h postmortem. All samples were processed within 4 months after sampling. Two separate analyses were carried out: one for the 124 125 lactate and the other for the sum of glycogen (the main component of GP degraded to glucose with amyloglucosidase), glucose, and glucose-6-phosphate using the L-Lactic acid and D-Glucose 126 Enzymatic Bio-Analysis kits (R-Biopharma, Milan, Italy), respectively. GP was calculated 127 128 according to Monin & Sellier (1985) by summing: 2[glycogen + glucose + glucose-6-phoshate] + 129 [lactate] and expressed as micro-moles (µmoles) of lactic acid equivalent per gram of fresh muscle. The animals used to perform the microarray analysis were chosen avoiding full-sib pigs and 130 selecting eight animals with extreme and divergent GP values: Pool L, four pigs with a GP value 131 less than the mean - 2 standard deviations (S.D.); Pool H with four pigs with a GP value greater 132 than the mean + 2 S.D. The pigs comprised in each group (Pool H and Pool L) were selected by 133 balancing for the animals' sex, thus selecting two gilts and two barrows for their inclusion in each 134 group. Kruskal-Wallis test, performed in the R environment (R Core Team, 2020), was then applied 135 136 to test whether the samples used for the two pools had statistically different values for the measured phenotypes. Data of the two pools were considered significantly different for *P*-values < 0.05. 137

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139 2.2 Genotyping for the mutations *PRKAG3* p R200Q and *RYR1* c.1843C>T

The stored samples of SM muscle gathered from the whole population of 277 pigs were submitted
to DNA extraction using a standard Phenol:Chloroform protocol (Sambrook and Russell, 2006).
After the extraction, the quality of the DNA was tested with an ND-1000 spectrophotometer

143 (NanoDrop Technologies, Willmington, DE, USA). The 277 samples were then genotyped for the

144	major mutations affecting pH decline and meat quality. In particular, the samples were genotyped
145	for i) the p.R200Q mutation (rs1109104772) of the PRKAG3 gene (Milan et al., 2000), causative for
146	the acid meat defect; ii) for the c.1843C>T mutation (rs344435545) of the <i>Ryanodine receptor 1</i>
147	(<i>RYR1</i>) gene (Fujii et al., 1991), responsible of the meat quality defect Pale, Soft, Exudative (PSE).
148	PRKAG3 genotyping was carried out with Polymerase Chain Reaction- Restriction Fragment
149	Length Polymorphism (PCR-RFLP) as described by Fontanesi, Davoli, Nanni Costa, Scotti, &
150	Russo (2003) and Fontanesi et al. (2008); RYR1 genotyping was performed as reported by Russo,
151	Fontanesi, Davoli, Chiofalo, Liotta, & Zumbo (2004) with PCR-RFLP.
152	

153 2.3 Total RNA extraction

154 From the total population of 277 pigs, eight pigs (four gilts and four barrows) were chosen for their extreme and divergent values of GP, assessed on the SM muscles gathered 24 h after slaughter. The 155 156 SM samples of the eight pigs, which were stored at -80°C, were submitted to total RNA extraction using the Ribo pure kit (Ambion, Thermo Fisher Scientific Inc., Waltham, MA, USA), following 157 the manufacturer's instructions. The RNA quality and integrity were checked with the ND-1000 158 Spectrophotometer (NanoDrop Technologies, Willmington, DE, USA), and by visualization on 1% 159 agarose gel. The RNA samples were treated with DNaseI (Invitrogen, Thermo Fisher Scientific 160 161 Inc., Waltham, MA, USA) and for each sample, an equal amount of total RNA (2,500 ng) was mixed to obtain two pools of 10,000 ng of RNA each utilized for the next steps of analysis. 162

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164 **2.4 Oligonucleotides microarray analysis**

The DIPROVAL-OPERON *Sus scrofa* AROS V1.0 oligonucleotide microarray is described in the GEO database with the accession number GPL5468. The microarray is composed of 10,665 70-mer oligonucleotides (probes). Each oligonucleotide was spotted twice on each slide. The hybridization and post-hybridization protocols are detailed on the same GEO entry indicated above. For the

present research, six slides were used: Pool H was labeled with Cy3 (green) on slides 1, 2, and 5, 169 170 while Pool L was labeled with Cy5 (red) on the same three slides. Opposite labeling of the two pools was used for the hybridization of slides 3, 4, 6. For the cDNA synthesis and the labeling 171 procedure, the SuperScript Plus Indirect cDNA Labelling System (Invitrogen, Thermo Fisher 172 173 Scientific Inc. Waltham, MA, USA) was used according to the manufacturer's recommendations. The obtained cDNA samples were labeled with the fluorochromes Cy3 and Cy5 overnight and in 174 175 the dark. After hybridization and washing, the slides were scanned with ScanArray Gx scanner (Perkin Elmer, Waltham, MA, USA) and the images were processed using ScanArray Express 176 software. 177

178

179 **2.5 Oligonucleotides re-annotation**

The original annotation of the oligonucleotides was updated for the present work using the 180 181 annotation available for Sus scrofa, Sscrofa 11.1 genome assembly in Ensembl (Howe et al., 2021) and the most updated genome annotation available for Sus scrofa, Homo sapiens and other species 182 using Nucleotide BLAST (Zhang, Schwartz, Wagner, & Miller, 2000) were used to search for 183 sequences similar to the probes. The parameters to select sequences resulting from the local 184 alignments were percent of similarity of at least 80% and length of the alignment between 64 and 185 186 72 nucleotides. This type of analysis was carried out first in the Sus scrofa species, using both "Nucleotide collection (nr/nt)" and "Expressed sequence tags (est) "databases, subsequently, the 187 oligonucleotides that were not identified were aligned against Homo sapiens sequences. After those 188 189 two steps, the oligonucleotides that were still lacking an identification were used to carry out another alignment step against the Nucleotide collection (nr/nt) of all other animal species. The 190 GenBank codes identified for the oligonucleotides were then entered in the DAVID Bioinformatic 191 Resources v. 6.8 Gene ID Conversion Tool (Huang, Sherman, & Lempicki, 2009) and in Ensembl 192 BioMart (Howe et al., 2021) to assign a gene to each oligonucleotide. 193

194

2.6 Identification of differentially expressed genes and functional analysis

Microarray data were analyzed using the *limma* R package (v. 3.46.0) (Ritchie et al., 2015). Both 196 mean and median data were submitted to background correction using two different methods: 197 198 Subtract and Edwards (Edwards, 2003). The first is the traditional background correction method where the background fluorescence intensity is subtracted from the foreground one for each spot. 199 200 The latter method uses a log-linear interpolation method to adjust lower intensities as described in Edwards (2003): the background is corrected only if the difference between foreground and 201 background is larger than a small threshold value. If this difference is lower than the threshold, a 202 203 smooth monotonic function which is linear with respect to background intensity on the log scale is 204 used instead of subtraction (Edwards, 2003). The main difference between these two methods is that Edwards avoids negative intensities which lead to loss of information and bias (since it is most 205 206 likely to occur when the expression levels are low) in the subsequent logarithmic transformation. The obtained four datasets (Mean subtract- MNS; mean Edwards- MNE; median subtract- MDS; 207 median Edwards- MDE) were submitted to the within and the between arrays normalization by 208 using the "loess" (Yang et al., 2002) and the "scale" methods (Bolstad, Irizarry, Astrand, & Speed, 209 210 2003), respectively.

211 The resulting data were analyzed with the *lmFit* and *eBayes* functions included in *limma* package. Both models were performed using the "robust" method that allows reducing the changes to 212 consider a hypervariable gene as a DEG and increase statistical power for the other genes (Phipson, 213 214 Lee, Majewski, Alexander, & Smyth, 2016). Raw P-values were adjusted applying the False Discovery Rate (FDR) correction method (Benjamini & Hochberg, 1995). The cut-off criteria for 215 defining a gene as differentially expressed (DE) were the adjusted *P*-value < 0.05 and a fold change 216 (FC), expressed as the ratio between the normalized intensities of pool H and pool L, > 1. At the 217 end of the procedure, four different sets of DEGs were obtained (two for the mean intensity and two 218

for the median intensity). The online web tool Bioinformatics and Evolutionary Genomics 219 220 (Bioinformatics and Evolutionary Genomics, 2021) was then used to graphically represent with a Venn diagram the DEGs found, and the intersection obtained with each statistical approach. 221 The functional annotation was obtained using Cytoscape 3.8.2 (Shannon et al., 2003) with the 222 ClueGO app (Bindea et al., 2009). Functional characterization was performed selecting human 223 database as a reference and the DEGs were significantly enriched in KEGG (Kyoto Encyclopedia of 224 225 Genes and Genomes) Pathway, biological process (BP), cellular component (CC), and molecular function (MF). The P-values of the obtained functional categories were adjusted with the 226 Bonferroni step-down method. The adjusted *P*-values were considered significant for P < 0.10, 227 228 following the significance threshold used in other studies (such as Lorenz et al., 2019), and the 229 significant functional categories were graphically displayed with Cytoscape tools.

230

231 **2.7 Validation with quantitative Real-time PCR**

The expression levels of some of the DEGs were also analyzed using the relative quantitative Real-232 Time polymerase chain reaction (qRT-PCR) standard curve method (Pfaffl, Tichopad, Prgomet, & 233 Neuvians, 2004). Six out of the found DEGs were chosen for the validation, and their expression 234 levels were normalized against the geometric mean for the transcription level of the normalizing 235 236 genes Beta-2-microglobulin (B2M) and RNA polymerase II subunit A (POLR2A). Those two normalizing genes were used because they were found to be the most stable in previous studies 237 (Zappaterra, Braglia, Bigi, Zambonelli, & Davoli, 2015) and were chosen following GENORM 238 239 software based on the method proposed by Vandesompele et al. (2002). The total RNA of the eight samples used also to create the two pools was individually reverse-transcribed. For each sample, 1 240 ug of total RNA was retrotranscribed to cDNA, according to the manufacturer's instructions, using 241 the Improm-IITM Reverse Transcription System and Oligo-dT primers (Promega Corporation, 242 Madison, WI, USA). Then, the obtained cDNA was diluted 1:10 and used to perform the qRT-PCR 243

on Light Cycler 1.0 System (Roche Diagnostics, Mannheim, Germany) using SYBR Premix Ex 244 TaqTM (TAKARA Bio INC, Olsu, Shiga, Japan), 10 pmol of each primer, and 2 µl of cDNA 245 template diluted 1:10, for a total volume of 10 µl. The Light Cycler protocol was optimized using 246 specific annealing temperatures for each primer pair as shown in Supplementary Table S1. The 247 same protocol reported in Zappaterra, Braglia, Bigi, Zambonelli, & Davoli (2015) for standard 248 curve creation and calculation of qRT-PCR efficiency was applied. The samples were quantified in 249 250 triplicate and the quantitation for each sample was retained if the coefficient of variation was less 251 than or equal to 0.20.

The obtained normalized quantitation for the samples was then used to calculate the mean for each 252 253 pool and to obtain for each gene the \log_2 of the FC value (\log_2 FC), which was obtained by dividing the average expression level of the Pool H for the average expression identified in Pool L. The 254 log₂FC values of the gene expression quantified with qRT-PCR and with microarray analysis were 255 256 used to perform Spearman correlations and test whether the trend of the gene expression levels was maintained between the qRT-PCR and the four statistical procedures applied to microarray data to 257 find DEGs (MNE, MNS, MDE, MDS). Spearman correlations were estimated with Hmisc R 258 package (v 4.5.0; Harrell Jr, 2021). The correlations were considered significant for P < 0.05. 259

260

261 **3. Results**

3.1 Description of the two pools

The samples were grouped in two pools selected according to the divergence of GP level measured in SM. The mean value of GP in the whole sample of 277 ILW pigs was 103.50 µmoles/g while the two pools were characterized by an extreme and divergent mean value of this parameter with significant differences between the two pools, as reported in Table 1.

267

Table 1. Descriptive statistics (Mean and Standard Deviation, S.D.) for the phenotypic parameters
measured on the pigs of Pool H (high glycolytic potential), of Pool L (low glycolytic potential) and
on the whole sample of 277 Italian Large White heavy pigs. For the Pool H *vs.* Pool L comparison,

271 Kruskal-Wallis test statistics ($\chi 2$ and *P*-value) were also reported.

			/	Krusk	al-Wallis	
Phenotypes	Total sample $(N = 277)$	Pool H $(n = 4)$	Pool L (n = 4)	(Pool H vs. Pool L)		
			,	χ^2	<i>P</i> -value	
pH_1^1	5.94 ± 0.24	6.14 ± 0.14	6.21 ± 0.15	0.33	0.564	
$p{H_u}^2$	5.67 ± 0.21	5.48 ± 0.07	6.08 ± 0.16	5.33	0.021	
Glycogen (µmoles/g) ³	47.33 ± 22.79	114.39 ± 12.98	13.15 ± 6.38	5.33	0.021	
Lactate $(\mu moles/g)^3$	56.30 ± 15.16	47.65 ± 7.35	42.88 ± 5.72	0.33	0.564	
Glycolitic Potential	102 (0) 22 15	162.02 + 12.62	56.02 . 5.72	5.22	0.001	
(GP) $(\mu moles/g)^3$	103.68 ± 23.15	162.03 ± 12.62	56.03 ± 5.73	5.33	0.021	

272 $\overline{}^{1}$ pH₁ was measured about 1 h *postmortem* directly on the *Semimembranosus* muscle of the left thigh.

 2 pH_u was measured about 24 h *postmortem* directly on the *Semimembranosus* muscle of the left thigh.

274 ³ These parameters were measured 24 h *postmortem on Semimembranosus* muscle samples and were expressed as

275 micro-moles (µmoles) of lactic acid equivalent per gram of fresh muscle.

276

277 The characteristics of the eight selected samples are reported in Supplementary Table S2. The

average levels of glycogen and GP found at 24 h after slaughter in the Pool L were significantly

lower (13.15 \pm 6.38 µmoles/g and 56.03 \pm 5.73 µmoles/g, respectively) than the average of the

- whole group of 277 pigs, whereas the mean values of the glycogen and GP levels in the Pool H
- were $114.39 \pm 12.98 \ \mu moles/g$ and $162.03 \pm 12.62 \ \mu moles/g$, respectively.

282 The average pH_1 values of the two groups did not differ significantly, while the pH_u values showed

a significant difference (P = 0.021) between the samples of the two pools. This result for pH_u agrees

with the result of the comparison of the GP values between the two pools: the pool with the lowest pH_u mean values also showed the highest values of GP (Table 1). Similarly, the glycogen level differed too between the two pools (P = 0.021) with divergent GP. In Table 1 the lactate level of both pools is also shown. We did not observe different lactate content between the two groups of samples.

The pigs of the two pools did not display the alleles of the two major genes influencing negatively porcine meat quality traits so far detected: p.200Q of the *PRKAG3* gene (Milan et al., 2000) and c.1843T of the *RYR1* gene (Fujii et al., 1991).

292

3.2 Microarray results and functional analysis

Supplementary Table S3 reports the oligonucleotides identified as DE with each statistical method and the complete output of each analytical procedure used to identify sequences differentially expressed between pools. The complete information concerning oligonucleotides sequence and their relative gene identification is reported in Supplementary Table S4. Table 2 shows the DE oligonucleotides identified by each method and Figure1 graphically displays the number of DE oligonucleotides retained in the different considered methods.

300

Figure 1. VENN diagram showing the number of differentially expressed oligonucleotides found
with the four statistical methods (MNE = Mean Edwards; MNS = Mean Subtract; MDE = Median
Edwards; MDS = Median Subtract) and the number of oligonucleotides obtained by the
intersections of the four methods. On the central part of the diagram are indicated the 17
differentially expressed oligonucleotides identified by all four statistical methods.

Table 2. The 35 oligonucleotides (oligo ID) found differentially expressed with the four statistical methods (MNE = Mean Edwards; MNS = Mean

Subtract; MDE = Median Edwards; MDS = Median Subtract) and their gene identification. Data are reported with the log_2 of the fold change

 $(\log_2 FC)$ and the adjusted *P*-value.

	Cono	MNE			MNS		MDE	MDS		
Oligo ID	symbol	Log ₂ FC	Adjusted <i>P</i> -value	Log ₂ FC	Adjusted <i>P</i> -value	Log ₂ FC	Adjusted <i>P</i> -value	Log ₂ FC	Adjusted <i>P-</i> value	
SS0000291	МҮН3	ns	ns	1.8410	1.63E-03	1.7273	2.37E-02	2.2504	1.43E-03	
SS00000459	NTRK3	ns	ns	ns	ns	-1.1864	3.96E-04	-1.1706	1.01E-04	
SS00000539	HBB	-1.0303	9.71E-04	-1.0394	1.15E-03	ns	ns	ns	ns	
SS00000679	SCD	-2.5490	1.53E-02	-2.5348	1.48E-02	-2.9639	1.30E-02	-2.9573	1.01E-02	
SS00000695	PLN	-2.1164	5.01E-03	-2.0850	4.76E-03	-2.0953	6.58E-03	-2.0634	5.76E-03	
SS00000696	GOT1	ns	ns	ns	ns	-1.0043	9.03E-04	-1.0245	2.48E-03	
SS00000767	UGP2	-1.0962	9.58E-03	-1.0726	8.32E-03	-1.1868	4.31E-03	-1.1674	3.09E-03	
SS00001044	FABP3	-1.3071	9.71E-04	-1.3264	1.63E-03	-1.3513	4.46E-04	-1.3737	7.45E-04	
SS00001123	PPARA	-1.0346	1.72E-02	-1.0460	3.00E-02	-1.0382	5.95E-03	-1.0405	7.36E-03	
SS00001972	FABP5	-1.2372	2.15E-03	-1.2114	1.20E-03	-1.2275	7.55E-04	-1.1894	2.39E-04	

SS00002255*		ns	ns	ns	ns	-1.4485	3.22E-02	ns	ns
SS00002454	DVL3	-1.5981	2.14E-02	-1.3220	1.41E-02	-1.4296	1.19E-02	-1.3706	8.89E-03
SS00002786	ATP5F1A	-1.0630	4.21E-02	ns	ns	ns	ns	ns	ns
SS00002870	MYOZ2	-1.9427	3.08E-02	-1.9236	2.81E-02	-2.0071	2.12E-02	-1.9818	1.85E-02
SS00003271	HSPB6	-1.1909	7.50E-03	-1.1810	6.37E-03	ns	ns	ns	ns
SS00003290	EIF2B2	ns	ns	ns	ns	-1.1900	8.35E-03	-1.0254	1.20E-02
SS00003487*		ns	ns	ns	ns	-1.1355	4.98E-02	ns	ns
SS00004032	C1QBP	ns	ns	ns	ns	-1.0172	5.06E-03	ns	ns
SS00004323	ATP5MC3	-1.0196	1.02E-02	-1.0071	8.30E-03	-1.0124	1.83E-03	-1.0072	9.42E-04
SS00004770	HSPB7	-1.3000	1.18E-02	-1.2853	1.01E-02	-1.2715	1.35E-02	-1.2675	1.32E-02
SS00005142	FCHSD1	ns	ns	ns	ns	-1.0535	4.43E-03	-1.0215	3.43E-03
SS00005172	POLA2	-1.7040	1.88E-02	-1.6282	2.04E-02	ns	ns	-1.4361	4.55E-02
SS00005914	HOMER2	-1.1198	3.76E-03	-1.1020	3.00E-03	-1.1156	6.46E-03	-1.0967	5.76E-03
SS00005997	AP1G2	-1.2248	4.29E-02	ns	ns	-1.3253	1.35E-02	-1.0503	1.83E-02
SS00006189	CSRP3	-1.6951	1.42E-02	-1.6674	1.10E-02	-1.6566	7.10E-03	-1.6071	4.76E-03
SS00006369	TSSK6	ns	ns	ns	ns	-1.7502	4.05E-02	ns	3.40E-04

SS00006853	SCD	-2.0247	3.02E-03	-2.0062	2.85E-03	-1.9751	5.02E-04	-1.9667	ns
SS00006888	LPL	-1.5160	1.40E-03	-1.4980	1.06E-03	-1.4952	2.80E-04	-1.4807	1.74E-04
SS00006966	RNF220	ns	ns	ns	ns	-2.7826	4.08E-03	ns	ns
SS00007208	COQ9	-1.8260	3.92E-02	ns	ns	-3.2519	2.69E-04	-3.7321	4.59E-02
SS00007292	MCU	ns	ns	ns	ns	-1.1740	1.30E-02	ns	ns
SS00008224	DNAJB1	-1.1845	3.73E-02	-1.1640	3.56E-02	-1.1004	3.84E-02	-1.0803	3.66E-02
SS00008616	ACSL1	-1.0801	5.40E-03	-1.0681	5.55E-03	-1.1553	7.64E-03	-1.1440	5.76E-03
SS00009114	HSPB2	Ns	ns	ns	ns	-1.2000	2.39E-02	ns	ns
SS00009459	SPARCL1	-1.0378	2.89E-02	-1.1434	1.10E-02	-1.0704	9.18E-03	-1.1538	6.33E-03

 $310 \quad ns= not significant$

311 *not further considered

313 The four statistical approaches allowed the identification of a total of 35 oligonucleotides

differentially expressed between the two pools. Among them, two oligonucleotides (SS00002255

and SS00003487) correspond to Expressed Sequence Tags (ESTs). The sequence of the EST

corresponding to the oligonucleotide SS00002255 was found in *Sus scrofa* ovary

317 (OVRM10048C10) and the oligonucleotide SS00003487 partially recognizes the cDNA of the Sus

scrofa gene Enhancer of mRNA decapping (EDC4). Both these oligonucleotides were not further

considered. The thirty-three remaining oligonucleotides corresponded to 32 unique genes as the

sequence of two oligonucleotides (SS00000679 and SS00006853) targeted the gene *Stearoyl-CoA*

321 *Desaturase delta* 9 (SCD) (Table 2 and Supplementary Table S4).

All but one of the DEGs were over-expressed in the Pool L. The oligo SS00000291 coding for a

323 putative Myosin heavy chain isoform (MYH3) was the only one overexpressed in Pool H. However,

to utilize microarray data more comprehensively, all 32 unique genes identified with the four

methods were submitted to functional analysis and the results are reported in Table 3 and Figure 2.

Table 3. Gene ontology (GO) terms and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways significantly enriched for the genes

328 differentially expressed between Pool L and Pool H. Functional categories are sorted by decreasing Bonferroni adjusted *P*-values.

					Bonferroni
Associated Genes Found	GO/KEGG ID	GO Term/KEGG pathway	N. Genes	<i>P</i> -value	adjusted P-
					value
ACSL1, FABP3, FABP5, LPL, PPARA, SCD	KEGG:03320	PPAR signaling pathway	6	3.41E-09	1.64E-07
CSRP3, FBXO32, HSPB6, MYH3, MYOZ2, PLN,					
PPARA	GO:0003012	muscle system process	7	1.12E-05	5.27E-04
CSRP3, EIF2B2, FABP3, GOT1, LPL, PLN, PPARA	GO:0043434	response to peptide hormone	7	1.60E-05	7.37E-04
ACSL1, ATP5F1A, ATP5MC3, PPARA, SCD	GO:0006164	purine nucleotide biosynthetic process	5	3.26E-05	1.47E-03
ACSL1, ATP5F1A, ATP5MC3, PPARA, SCD	GO:0009260	ribonucleotide biosynthetic process	5	3.64E-05	1.60E-03
CSRP3, MYH3, MYOZ2	GO:0045214	sarcomere organization	3	3.88E-05	1.67E-03
ACSL1, FABP3, FABP5, LPL	GO:0006641	triglyceride metabolic process	4	5.40E-05	2.27E-03
CSRP3, FABP5, FBXO32, HSPB6, HSPB7, PLN,					
PPARA	GO:0044057	regulation of system process	7	7.69E-05	3.15E-03
ACSL1, FABP3, FABP5, LPL, PPARA, SCD	GO:0006631	fatty acid metabolic process	6	7.94E-05	3.18E-03

ACSL1, ATP5F1A, ATP5MC3, FABP3, FABP5,

	GO:0090407	organophosphate biosynthetic process	7	1.18E-04	4.61E-03
PPARA, SCD					
ACSL1, FABP3, PPARA	GO:2000191	regulation of fatty acid transport	3	1.25E-04	4.75E-03
ACSL1, FABP3, FABP5, PPARA	GO:0015908	fatty acid transport	4	1.35E-04	4.87E-03
ATP5F1A, ATP5MC3, PPARA	GO:0006754	ATP biosynthetic process	3	1.32E-04	4.87E-03
CSRP3, MYH3, MYOZ2, PPARA	GO:0055001	muscle cell development	4	2.37E-04	8.31E-03
ATP5F1A, ATP5MC3, COQ9, MYH3, PPARA	GO:0046034	ATP metabolic process	5	2.49E-04	8.45E-03
FABP5, GOT1, LPL, SCD, UGP2	GO:0046394	carboxylic acid biosynthetic process	5	2.78E-04	9.16E-03
ACSL1, FABP3, LPL, PPARA	GO:1905952	regulation of lipid localization	4	3.51E-04	1.12E-02
CSRP3, HSPB7, PLN	GO:0060047	heart contraction	3	1.28E-02	1.28E-02
ATDEFIA ATDEMC2 DDADA	CO-0000144	purine nucleoside triphosphate	2	5 25E 04	1.525.02
ATPSFTA, ATPSMCS, PPAKA	GO:0009144	metabolic process	3	5.25E-04	1.52E-02
	CO.0000142	nucleoside triphosphate biosynthetic	2	5 00E 04	1.525.02
ATPSFTA, ATPSMC3, PPARA	GO:0009142	process	3	5.09E-04	1.53E-02
	GO 0000100	ribonucleoside triphosphate metabolic	2		1 (75.00
AIPSFIA, AIPSMU3, PPARA	GO:0009199	process	3	3.96E-04	1.6/E-02

HOMER2, MCU, MYOZ2, PLN	GO:0019722	calcium-mediated signaling	4	7.90E-04	1.98E-02
ACSL1, FABP5, SCD	GO:0120162	positive regulation of cold-induced thermogenesis	3	8.42E-04	2.02E-02
FBXO32, HSPB6, PLN, PPARA	GO:0090257	regulation of muscle system process	4	7.78E-04	2.02E-02
FABP3, FABP5, PPARA	GO:0019217	regulation of fatty acid metabolic process	3	7.54E-04	2.04E-02
ATP5F1A, ATP5MC3, MCU	GO:1990542	mitochondrial transmembrane transport	3	1.01E-03	2.13E-02
FABP3, FABP5, LPL, PPARA	GO:0044242	cellular lipid catabolic process	4	9.32E-04	2.14E-02
FBXO32, MYOZ2, PPARA	GO:0043500	muscle adaptation	3	9.87E-04	2.17E-02
ACSL1, ATP5F1A, ATP5MC3, PPARA, SCD	GO:0009150	purine ribonucleotide metabolic process	5	1.16E-03	2.33E-02
CSRP3, FABP5, MCU	GO:0042593	glucose homeostasis	3	1.19E-02	2.39E-02
ACSL1, FABP5, SCD	GO:0033559	unsaturated fatty acid metabolic process	3	1.41E-03	2.54E-02
CSRP3, MYH3, MYOZ2, PPARA	GO:0051146	striated muscle cell differentiation	4	1.39E-03	2.65E-02

ATP5F1A, ATP5MC3, COQ9	GO:0006119	oxidative phosphorylation	3	2.31E-03	3.93E-02
C1QBP, MCU, NTRK3	GO:0050921	positive regulation of chemotaxis	3	2.31E-03	3.93E-02
ACSL1, FABP5, SCD	GO:0106106	cold-induced thermogenesis	3	2.49E-03	3.98E-02
CSRP3, MYOZ2, PLN, PPARA	GO:0014706	striated muscle tissue development	4	4.33E-03	4.33E-02
CSRP3, GOT1, LPL	GO:0032869	cellular response to insulin stimulus	3	9.03E-03	4.51E-02
FBXO32, GOT1, NTRK3	GO:0051384	response to glucocorticoid	3	3.01E-03	4.52E-02
DNAJB1, HSPB2, HSPB7	GO:0006986	response to unfolded protein	3	4.20E-03	4.62E-02
CSRP3, HSPB6, MYH3, PLN	GO:0006936	muscle contraction	4	3.66E-03	4.75E-02
FABP5, LPL, SCD	GO:0006633	fatty acid biosynthetic process	3	3.61E-03	5.05E-02
CSRP3, HSPB7, PLN	GO:0008016	regulation of heart contraction	3	8.83E-03	5.30E-02
FABP5, GOT1, PPARA	GO:0006006	glucose metabolic process	3	8.64E-03	6.05E-02
MCU, NTRK3, PLN	KEGG:04020	calcium signaling pathway	3	8.26E-03	6.61E-02
ACSL1, ATP5F1A, ATP5MC3	KEGG:04714	Thermogenesis	3	7.53E-03	6.77E-02

Among the DEGs upregulated in Pool L we have found the genes ATP Synthase Membrane Subunit 331 C Locus 3 (ATP5MC3), ATP Synthase F1 Subunit Alpha (ATP5F1A), Coenzyme Q9 (COQ9), 332 Mitochondrial Calcium Uniporter (MCU), F-box protein 32 (FXBO32), Glutamic-Oxaloacetic 333 Transaminase 1 (GOT1), and Complement C1q Binding Protein (C1QBP), which are expressed in 334 mitochondria and are involved in important functions mainly aimed to support the energy needs of 335 the cells. In particular, the genes ATP5F1A, ATP5MC3, and COQ9, together with Myosin Heavy 336 Chain 3 (MYH3), and Peroxisome Proliferator Activated Receptor Alpha (PPARA) were 337 significantly enriched in "ATP metabolic process" (adjusted P = 8.45E-03), and ATP5F1A, 338 ATP5MC3, and MCU significantly entered in "mitochondrial transmembrane transport" category 339 340 (adjusted P = 2.13E-02). This would suggest these genes are involved in determining mitochondrial 341 respiratory function and play pivotal roles to maintain the cellular energy state. The DEGs Acyl-CoA Synthetase Long Chain Family Member 1 (ACSL1), Fatty Acid Binding Protein 3 (FABP3), 342 Fatty Acid Binding Protein 5 (FABP5), Lipoprotein Lipase (LPL), PPARA, and Stearoyl-CoA 343 Desaturase (SCD) were significantly enriched in "fatty acid metabolic process" (adjusted P =344 3.18E-03); ACSL1, FABP3, FABP5, and PPARA significantly entered in "fatty acid transport" 345 (adjusted P = 4.87E-03). A group of DEGs (Heat Shock Protein Family B (Small) Member 2-346 HSBP2, DnaJ Heat Shock Protein Family (Hsp40) Member B1- DNAJB1, Heat Shock Protein 347 348 Family B (Small) Member 7- HSPB7) belonging to the Heat Shock Protein family were significantly clustered in the functional category "Response to unfolded proteins" (adjusted P = 4.62E-02). The 349 functional classification of the DEGs Cysteine And Glycine Rich Protein 3 (CSRP3), Myozenin 2 350 351 (MYOZ2), Phospholamban (PLN), and PPARA showed to be significantly involved in "Striated muscle tissue development" (adjusted P = 4.33E-02), Homer Scaffold Protein 2 (HOMER2), MCU, 352 MYOZ2, PLN were included in "Calcium-mediated signaling" (adjusted P = 1.98E-02), and MCU, 353 Neurotrophic Receptor Tyrosine Kinase 3 (NTRK3), PLN were significantly clustered in "Calcium 354 signaling pathway" (adjusted P = 6.61E-02). The gene UDP-Glucose Pyrophosphorylase 2 355

(*UGP2*), which is a precursor of the glycogen synthesis, was found included in the functional
category "Carboxylic acid biosynthetic process", together with the DEGs *FABP5*, *GOT1*, *LPL*, and *SCD* (adjusted *P* = 9.16E-03). Besides the functional classification of the DEGs, the main
connections among them were also evaluated (Table 3; Figure 2). The functional class "PPAR
signaling pathway" showed to be related with most of the functional categories containing the DEG
genes identified (Table 3; Figure 2), thus suggesting that *PPARA*, the main gene of the class, can be
considered the master regulator of the identified pathways.

363

Figure 2. Cytoscape functional analysis of the genes differentially expressed between Pool H andPool L.

Legend: hexagons = KEGG pathways; circles = biological processes (GO BP); shape size =

according to the *P*-value of the term in its own group; different colors are used to identify functional
categories pertaining to the same group of terms; interaction line thickness = according to Kappa
Score value, represents the strength of the interactions, lighter color corresponds to a lower strength
while darker color to a higher strength. The differentially expressed genes labelled with a frame are
those that will be further discussed.

372

373 **3.3 Validation of microarray results**

To validate the results obtained with the microarray we decided to analyze the expression level by qRT-PCR of six of the DEG found (*ATP5MC3*, *LPL*, *MYOZ2*, *PLN*, *SCD*, *UGP2*) after microarray hybridization; these six genes were selected for their relevant role in muscle metabolism. A high correlation value was found between the log₂FC found with qRT-PCR and the log₂FC values identified for the same genes with the four statistical methods (r = 0.94, *P* < 0.005 for all the correlations qRT-PCR *vs*. MNE, qRT-PCR *vs*. MNS, qRT-PCR *vs*. MDE, and qRT-PCR *vs*. MDS). The obtained correlations support the robustness of the results found with the four statisticalmethods used in this study.

382 **4. Discussion**

To our knowledge, this is the first study investigating by microarray the molecular frame of early *postmortem* muscle tissue in Italian Large White heavy pigs divergent for GP measured 24 h after slaughter. The results can help outline the molecular players participating in the metabolic processes involved in pH decline after slaughtering and influencing GP at 24 h *postmortem*.

387 In *postmortem* muscle, the energy demand of the tissue is supported by glycogen, glucose, and

lactate that are the main metabolites used to generate glycolytic substrates. It is widely known that

the metabolic and molecular processes related to muscle glycogen storage and glycogen degradation

to glucose can influence *postmortem* biochemical processes in the conversion of muscle to meat,

391 with particular reference to the rate of pH drop. Different Authors indicated that muscles with lower

392 glycogen and higher lactate show, early *postmortem*, a faster glycolytic rate than muscles with

higher glycogen and lower lactate level (Chauahn & England, 2018; England et al., 2016; Moreno,

Lipová, Ladero, García, & Cava, 2020; Henckel, Karlsson, Jensen, Oksbjerg & Petersen, 2002;

395 Matarneh, England, Scheffler, & Gerrard, 2017; Pösö & Puolanne, 2005).

Henckel, Karlsson, Jensen, Oksbjerg, &Petersen (2002) reported that the minimum amount of

397 glycogen required for a normal *postmortem* pH decline from 7.2 to 5.6, is 53 µmoles/g tissue. In the

present research, the amount of glycogen ($13.15 \pm 6.38 \,\mu$ moles/g) and the level of GP (56.03 ± 5.73

399 µmoles/g) found at 24 h *postmortem* in Pool L are much lower than the threshold indicated in

400 Henckel, Karlsson, Jensen, Oksbjerg, & Petersen (2002). This result could be consistent with the

- 401 limited pH decline at 24 h after slaughter observed in Pool L, which showed a quite elevated pH_u
- 402 value of 6.08 ± 0.16 compared to the glycogen and pH_u values of Pool H (pH_u = 5.48 ± 0.07 ;
- 403 glycogen = $114.39 \pm 12.98 \ \mu moles/g$; GP= $162.03 \pm 12.62 \ \mu moles/g$). The conversion of muscle to

meat is traditionally viewed as an anaerobic process that is largely governed by the degradation of 404 glycogen to lactate and H⁺. However, different Authors reported the evidence that glycolytic 405 capacity of muscle tissue is only partially associated with pH decline postmortem and that glycogen 406 content and *postmortem* glycolysis are not the unique factors responsible for pH drop (Scheffler & 407 Gerrard, 2007; Pösö & Puolanne, 2005; Scheffler, Scheffler, Kasten, Sosnicki, & Gerrard, 2013). 408 These Authors showed that other elements and aspects related to the complex metabolic properties 409 of muscle likely play a more critical role in determining the course and outcome of pH drop after 410 slaughter (Scheffler & Gerrard, 2007; Pösö & Puolanne, 2005; Scheffler, Scheffler, Kasten, 411 Sosnicki, & Gerrard, 2013). 412

The amount of lactate is considered by different Authors as an indicator of *postmortem* pH decline. 413 414 There is however lack of agreement in the scientific community with respect to the relationship linking lactate and *postmortem* pH decline. Other Authors have indeed reported that even though 415 there is a significant connection between muscle glycolysis, GP, lactate accumulation, and 416 *postmortem* pH, the relationship between glycogen content and pH_u is not complete and not linear, 417 as samples showing similar lactate values may display different pH_u (England et al., 2016; 418 Scheffler, Park, & Gerrard, 2011; Scheffler, Scheffler, Kasten, Sosnicki, & Gerrard, 2013; Pösö & 419 Puolanne, 2005; Chauhan & England, 2018; Huff-Lonergan, Baas, Malek, Dekkers, Prusa, & 420 421 Rothschild, 2002; Monin & Sellier, 1985). In agreement with results found in other studies (Van Laack & Kauffman, 1999; Van Laack, Liu, Smith, & Loveday, 2000; Choe et al., 2008), in the 422 present research, we noticed different pH_u values between the samples of the two pools but similar 423 424 values of lactate at 24h postmortem were present between the two pools. This result seems to indicate that the difference noticed between the pH_u values of the two pools may not be ascribed to 425 glycogen breakdown leading to lactate formation. The similar values of pH_u and lactate between the 426 two pools may indicate that the differences noticed in GP at 24 h postmortem should not be 427 attributed to different preslaughter handling conditions or different *premortem* environmental 428

factors. Previous research indicated higher levels of lactate at 24 h postmortem in muscles 429 belonging to animals that had higher exsanguination blood glucose and lactate and thus 430 experiencing more muscle activity and stress before slaughter (Choe et al., 2015). In the present 431 study, we hypothesize that other aspects (not related to pre-slaughter handling) may have caused the 432 observed differences between the two pools in the levels of glycogen, glycolytic potential, and pH_u. 433 Preslaughter stressing conditions or particular feeding protocols are known to reduce the content of 434 glycogen stored in muscles, producing lower pH_1 and pH_u values during the muscle to meat 435 transformation (Rosenvold & Andersen, 2003a; Pösö & Puolanne, 2005; England, Matarneh, 436 Oliver, Apaoblaza, Scheffler, Shi, & Gerrard, 2016; Rosenvold & Andersen, 2003b; Chauhan & 437 438 England, 2018). The pigs used in the present study were, however, reared in the same 439 environmental conditions, fed the same diet, and slaughtered in the same abattoir. Accordingly, we can infer that the observed phenotypic differences between the pigs of the two pools for the 440 indicated parameters (glycogen and GP), together with the similar lactate level and pH₁, could 441 likely depend on the effects of other factors, such as physiological conditions before slaughtering 442 related to muscle metabolism. It could be plausible also to hypothesize that in Pool L a limited 443 muscle glycogen reserve may have been already present in antemortem muscle and/or that 444 dysfunctions of enzymes related to glycogen metabolism may have caused in Pool L a rapid 445 446 glycogen breakdown. The hypothesized rapid glycogen degradation could have been caused by enzymatic altered activity represented by an up-regulation of proteins involved in glycogenolysis or 447 by the inhibition of the enzyme glycogen synthetase. However, neither glycogen phosphorylase nor 448 449 glycogen synthetase genes were found to be DE between the two pools. The only gene coding for an enzyme related to muscle glycogen synthesis found overexpressed in low GP samples is UGP2, 450 which gives origin to UDP-glucose, a direct precursor of glycogen. Nevertheless, the 451 overexpression of a single enzyme may change the level of a metabolite but may not be enough to 452 control the whole pathway of glycogen metabolism. Accordingly, Reynolds et al. (2005) reported 453

that the overexpression of UGP2 and an associated increase in the levels of UDP-glucose alone are 454 455 not able to affect glycogen synthesis in mouse skeletal muscles. The regulation of glycogen content is indeed a highly complex process and appears to be associated with several enzymatic proteins 456 and metabolic conditions (Mookerjee, Nicholls, & Brand, 2016; Roach, Depaoli-Roach, Hurley, & 457 Tagliabracci, 2012; Daran-Lapujade et al., 2007; Tanner et al., 2018). Several Authors showed that 458 altered functionality and activity of enzymes involved in glycogen synthesis and catabolism could 459 be hardly evidenced at the mRNA level (Mookerjee, Nicholls, & Brand, 2016; Roach, Depaoli-460 Roach, Hurley, & Tagliabracci, 2012; Daran-Lapujade et al., 2007; Tanner et al., 2018). The 461 glycogen metabolism was reported to be mainly regulated by a complex pattern of posttranslational, 462 conformational changes or phosphorylation and dephosphorylation of the main enzymes regulating 463 464 glycogen metabolism (Daran-Lapujade et al., 2007; Roach, 2002; Roach, Depaoli-Roach, Hurley, & Tagliabracci, 2012). Bouskila et al. (2010) showed that a reduced capacity to synthesize muscle 465 glycogen in glycogen synthase knockout mice may have led to metabolic adaptations and flexibility 466 resulting in the muscles of these animals being more efficient at utilizing extracellular glucose 467 and/or fatty acids as substrates for energy production. 468 Glucose is the major source of energy for cells. This carbohydrate represents the preferred energy 469 470 substrate used in muscle for ATP production. In normoxic cells, glucose is metabolized via 471 anaerobic glycolysis to pyruvate, which is then oxidatively metabolized to CO₂ in the tricarboxylic acid (TCA) cycle to generate large amounts of ATP through the mitochondrial oxidative 472

473 phosphorylation (Vamecq, Colet, Vanden Eynde, Briand, Porchet, & Rocchi, 2012; Lunt & Vander

474 Heiden, 2011; Matarneh, Yen, Bodmer, El-Kadi, & Gerrard, 2021).

475 In early *postmortem* muscle, when the level of oxygen starts to drop, the conversion of pyruvate to

476 lactate is important to support ATP production by enhancing glucose catabolism via the

477 mitochondrial TCA cycle in presence of an impaired or restrained oxidative phosphorylation due to

the limited supply of oxygen. The Lactate Dehydrogenase (LDH) reaction converting pyruvate to

lactate contributes to a continuous supply of NAD⁺ required for the glycolysis progression and the 479 transfer to lactate of glycolysis-generated NADH reducing equivalents (Arago, Formentini, & 480 Cueva, 2013; Gladden, 2004; Lunt, & Vander Heiden, 2011; England, Scheffler, Kasten, Matarneh, 481 & Gerrard, 2013). While in normoxic conditions, most pyruvate is oxidized to CO₂, when hypoxic 482 conditions are established, mitochondria respiration is arrested and the mitochondrial contribution 483 to glucose oxidation can be limited/blocked. In these conditions, cells develop only the glycolytic 484 contribution to glucose oxidation, and pyruvate, which is obtained from glycolysis, is reduced by 485 LDH to lactate. The latter tends to be accumulated as a byproduct of glycolysis under anaerobic 486 conditions and mitochondrial disfunction (Glancy, Kane, Kavazis, Goodwin, Willis, & Gladden, 487 488 2021; Ferguson, Rogatzki, Goodwin, Kane, Zachary, & Gladden, 2018; England et al., 2018; 489 Martinez-Reyes & Chandel, 2017; Matarneh, Yen, Bodmer, El-Kadi, & Gerrard, 2021). In this situation, the oxidation of one molecule of glucose would produce only two molecules of ATP 490 instead of the 36 (2 cytosolic and 34 mitochondrial) obtained through the glycolysis and subsequent 491 complete oxidation of one molecule of glucose in mitochondria. Anaerobic glucose catabolism does 492 not require oxygen, but it is much less efficient in generating ATP when compared with the TCA 493 cycle coupled to oxidative phosphorylation. Moreover, it is worth noting that in the reaction of 494 reduction of pyruvate to lactate, the latter can function as a regulator of cellular redox state 495 496 consuming H⁺ without contributing to the pH drop (Scheffler, Scheffler, Kasten, Sosnicki, & Gerrard, 2013; England et al., 2018; Glancy, Kane, Kavazis, Goodwin, Willis, & Gladden, 2021). 497 In the samples of the present research, it might be possible to assume that the glycogen degradation 498 499 and lactate could not be responsible for the different *postmortem* pH decrease between the two pools considering that 24 h after slaughtering lactate is accumulated in muscle postmortem of all 500 samples in a similar way. 501

502 Different mechanisms and processes related to the early *postmortem* muscle metabolism could be 503 considered to explain the different pH declines between the two pools later after slaughtering. A

graphical representation of the hypothesized events occurring in the early *postmortem* phases inPool L samples is reported in Figure 3.

506

Figure 3. Graphical representation of the hypothesized events occurring in the early postmortemphases in Pool L samples.

Legend: Genes overexpressed in Pool L samples are highlighted in red; those genes are included in
rectangular or oval shapes depending on whether they express respectively for enzymatic proteins
or other proteins; asterisks indicate genes whose expression is directly regulated by *Peroxisome Proliferator Activated Receptor Alpha (PPARA).*

513

514 In the present research, the condition of low glycogen level in Pool L pig muscles may have already been present before slaughter for some imbalance or disfunction regarding metabolic pathways of 515 lipids and/or carbohydrates. After slaughter, this altered metabolic state may have been worsened 516 by the development of *postmortem* crucial changes due to the cessation of blood flow (Chauhan & 517 England, 2018; England et al., 2018). In that condition, when the level of oxygen of the cells starts 518 to lower in the early *postmortem* period, substrates other than glucose can contribute to ATP 519 production. In particular, under glycogen-limiting conditions, the switch towards fatty acid 520 521 oxidation might have occurred as a compensatory mechanism in muscle energy metabolism (Muoio et al., 2002; Burri, Thoresen, & Berge, 2010; Lunt & Vander Heiden, 2011; Domenis, Bisetto, 522 Rossi, Comelli, & Mavelli, 2012; Phua, Wong, Liao, & Tan, 2018; Rakhshandehroo, Knoch, 523 524 Müller, & Kersten, 2010; Arago, Formentini, & Cueva, 2013). However, the conditions reported in the scientific literature are not always comparable with those found *postmortem* in muscles during 525 the biochemical processes leading to meat production. Therefore, despite being supported by the 526 literature, the hypothesized switch towards fatty acid oxidation in low GP muscles would need to be 527

validated with ad hoc studies specifically addressed to better understand biochemical processes 528 taking place *postmortem* in muscles coming from animals reared for meat purposes. 529 The hypothesized metabolic switch towards fatty acid oxidation may also be supported by the 530 upregulation in Pool L samples of some genes that have a direct impact on cellular metabolism and 531 energy production, such as PPARA. PPARA is a master regulator that controls the expression of 532 numerous target genes involved in many pathways related to lipid metabolism, including fatty acid 533 uptake. The upregulation of *PPARA* gene can activate mitochondrial fatty acid oxidation in muscle 534 tissue (Muoio et al., 2002; Burri, Thoresen, & Berge, 2010; Goto et al., 2011; Fan & Evans, 2015; 535 Bougarne et al., 2018; Phua, Wong, Liao, & Tan, 2018). Furthermore, several genes found DE in 536 537 the present study have been previously identified to be regulated by PPARA. For example, a large 538 body of literature reports that PPARA regulates genes involved in fatty acid uptake (such as the DE gene LPL), fatty acid intracellular transport (such as FABP3), and fatty acid phosphorylative 539 oxidation (such as mitochondrial ATPase complex V) (Hue & Taegtmeyer, 2009; Rakhshandehroo, 540 Knoch, Müller, & Kersten, 2010; Fan & Evans, 2015; Phua, Wong, Liao, & Tan, 2018; Boungarne 541 et al., 2018). Moreover, PPARA can also activate SCD gene, which codes for a key lipogenic 542 enzyme. The SCD overexpression in turn promotes the release of monounsaturated fatty acids 543 (oleate and palmitoleate). These fatty acids can induce an increase in PPARA expression and 544 545 mitochondrial fatty acids oxidative phosphorylation, in order to avoid excessive triglyceride accumulation (Goto et al., 2011). Accordingly, in the present research, the upregulation of PPARA 546 gene may have promoted the transcription of genes overexpressed in Pool L that are related to fatty 547 548 acid synthesis, transport, and oxidative metabolism (FABP3, FABP5, ACSL1, LPL, SCD), or that drive ATP production through fatty acid β -oxidation and oxidative phosphorylation (Wilson, 2017; 549 Teodoro et al., 2017; Young et al., 2018). The upregulation of the target genes activated by PPARA 550 could have triggered, in Pool L samples, the oxidation of fatty acids instead of glucose for cellular 551 energy production. This result is consistent with the early *postmortem* compensatory events that 552

may take place in response to hypoxia and low glycogen conditions (Smolkova et al., 2010; Wilson,
2017). The observed up-regulation of the *PPARA* gene may have, therefore, regulated the
expression of several other genes involved in energy metabolism, promoted mitochondrial
functions, and may have created conditions favorable to establish a pro-apoptotic *postmortem*condition, as will be further discussed later.

Among the genes found DE in the present study, several mitochondrial genes were up-regulated in 558 Pool L samples (such as ATP5F1A, ATP5MC3, C1QBP, GOT1, FBXO32, and MCU), likely 559 suggesting a *postmortem* functionality of mitochondria in these samples. The genes C1QBP, GOT1, 560 561 FBXO32, and MCU are involved in mitochondrial activity and oxidative phosphorylation of fatty acids, which are activities that need oxygen supply since oxygen is the final electron acceptor in the 562 electron transport chain (ETC) (Matarneh, Beline, de Luz e Silva, Shia, & Gerrard, 2018). This 563 564 result may appear to be surprising at first glance, as in *postmortem* muscle, oxygen is continuously decreasing due to hypoxic/anoxic conditions. However, different Authors reported that the muscle 565 oxygen concentration after slaughter declines gradually within the first 2 h *postmortem*, and thus 566 567 oxygen is not immediately depleted after exsanguination. In this condition, if energy substrates and oxygen are available, mitochondrial functionality still exists for some time after slaughter and 568 contributes to ATP production through oxidative phosphorylation (England et al., 2018; Matarneh, 569 Yen, Bodmer, El-Kadi, & Gerrard, 2021). As a consequence, in Pool L samples, mitochondria may 570 directly influence *postmortem* muscle metabolism sustaining ATP production in the first hours after 571 572 slaughter (Scheffler, Matarneh, England, & Gerrard, 2015; Matarneh, Yen, Bodmer, El-Kadi, & Gerrard, 2021). Matarneh, Yen, Bodmer, El-Kadi, & Gerrard (2021) carried out a study aimed to 573 analyze mitochondrial functionality relying on *in vitro* conditions simulating *postmortem* 574 metabolism in porcine Longissimus lumborum and masseter muscles. These Authors reported that, 575 under those conditions, mitochondria are capable of mobilizing pyruvate originated from glycolysis 576 (Matarneh, Yen, Bodmer, El-Kadi, & Gerrard, 2021). These data, together with the gradual 577

578 reduction in oxygen reported in their previous study (England et al., 2018), strongly support the 579 evidence that mitochondrial activity is maintained *postmortem*, albeit at reduced levels. It could be 580 argued that in our samples, in presence of low glycogen and low GP, the mitochondria functionality 581 and fatty acid oxidation could have played a noticeable role.

Domenis, Bisetto, Rossi, Comelli, & Mavelli (2012) and Arago, Formentini, & Cueva (2013) 582 reported that the expression of the enzymatic complex V of mitochondrial ATP synthase (F1F0 583 ATP synthase) is directly related to the activity of oxidative phosphorylation, whereas it inversely 584 correlates with the rate of glucose utilization by aerobic glycolysis. In Pool L samples we have 585 found a similar situation, with the overexpression of the ATP5F1A and ATP5MC3 and the 586 upregulation of genes coding for proteins involved in fatty acids uptake and activation of 587 588 mitochondrial oxidative phosphorylation. Different Authors reported that the mitochondrial membrane ATP synthase is a major determinant of muscle energy metabolism, has a pivotal role in 589 cell physiology, and can influence the *postmortem* metabolism of the muscle tissue (Senior, 590 Nadanaciva, & Weber, 2002; Matarneh, England, & Gerrard, 2015; England et al., 2018; Matarneh, 591 England, Scheffler, Yen, & Gerrard, 2017; Matarneh, Yen, Bodmer, El-Kadi, & Gerrard, 2021; 592 Aaon, Bhatt, & Cortassa, 2014; Junge & Nelson, 2015). In the present research, the overexpression 593 of genes coding for subunits of the mitochondrial ATPase complex V observed in the samples with 594 595 low glycogen, may allow us to suppose that the overexpression of these genes in Pool L may also 596 have influenced the *postmortem* skeletal muscle metabolism by regulating the expression of other genes related to energy metabolism. Mitochondrial membrane ATP synthase (F1F0 ATP synthase 597 598 or Complex V) can synthesize or hydrolyze ATP upon changes in cellular conditions in a reversible reaction and in particular, it produces ATP from ADP and inorganic phosphate in the presence of an 599 600 electrochemical gradient of protons across the inner membrane generated by electron transport complexes of the respiratory chain during oxidative phosphorylation (Walker, Lutter, Dupuis, & 601 Runswick, 1991; Boyer, 1997; Walker, 2013; Rühle & Leister, 2015). The flow of protons through 602

F1F0 ATP synthase (complex V) back to the mitochondrial matrix enhances ATP synthesis. The 603 description and characterization of the functions of the F1F0 ATPase complex V have been the 604 objectives of a large body of literature. This enzymatic complex consists of two structural domains, 605 the extra-membrane hydrophilic portion F1- which hosts the catalytic sites responsible for ATP 606 synthesis (and hydrolysis)-, and the membrane-embedded F0 domain- which contains the proton 607 channel (Walker, Lutter, Dupuis, & Runswick, 1991; Boyer, 1997; Walker, 2013; Rühle & Leister, 608 2015; Devenish, Prescott, & Rodgers, 2008; Zhou et al., 2015). ATP5MC3 is one of the three genes 609 that encode for the C-subunit of the proton channel of ATP synthase (Bonora et al., 2013). The C-610 subunit is produced from three nuclear genes, ATP5G1, ATP5G2, and ATP5G3 (ATP5MC3) 611 encoding identical copies of the mature protein (Yan, Lerner, Haines, & Gusella, 1994; He, Forda, 612 613 Carrolla, Dinga, Fearnleya, & Walkera, 2017). ATP5F1A codes for a subunit of the ATP synthase complex V domain F1 that protrudes in the mitochondrial matrix and is involved in the catalytic 614 activity of ATP synthesis and hydrolysis (He, Forda, Carrolla, Dinga, Fearnleya, & Walkera, 2017). 615 Despite their known importance in cell function and metabolism, the role of mitochondria and ATP 616 synthase in *postmortem* muscle remains mostly unknown. 617 The activation of mitochondrial oxidative phosphorylation in samples with low glycogen and low 618

GP can be also supported by the overexpression of the gene C1QBP that plays an important role in 619 620 mitochondrial metabolism (Fogal, Richardson, Karmali, Scheffler, Smith, & Ruoslahti, 2010; Chen et al., 2016; Gotoh et al., 2018; Noh et al. 2020). In particular, this gene is described as an important 621 promoter of fatty acid oxidation and oxidative phosphorylation and may be required for efficient 622 623 ATP production through oxidative phosphorylation (Fogal, Richardson, Karmali, Scheffler, Smith, & Ruoslahti, 2010; Aaon, Bhatt, & Cortassa, 2014). Gotoh et al. (2018) showed that loss of C1QBP 624 function in mice dendritic cells induced a metabolic reprogramming characterized by increased 625 glycolysis and impaired oxidative phosphorylation. In Pool L samples, with an extremely low level 626

of glycogen, the upregulation of the gene *C1QBP* could have supported the utilization of fatty acidsas an alternative energy substrate to produce ATP.

The hypothesis of an activation of oxidative phosphorylation to produce ATP in Pool L samples 629 may also be supported by the overexpression of the genes MCU and PLN found in that pool. MCU 630 codes for the inner mitochondrial membrane Ca^{2+} channel and promotes the increase of Ca^{2+} ions 631 levels in mitochondria (Shaikh, Sahoo & Periasamy, 2016). Mitochondria can receive Ca²⁺ ions from 632 the sarcoplasmic reticulum, the main calcium depot of the cell (Baughman et al., 2012; Giorgi, 633 Marchi, & Pinton, 2018). In Pool L samples, it may be suggested that the overexpression of the 634 gene *PLN* may inhibit the activity of the sarcoplasmic reticulum calcium pumps Ca²⁺-ATPases 635 636 (SERCAs), and the flux of calcium ions into the sarcoplasmic reticulum. This condition, together 637 with the upregulation of the mitochondrial gene MCU, may, in turn, have promoted the increase of calcium uptake into mitochondria (Fajardo et al., 2013; De Stefani, Patron, & Rizzuto, 2015). As a 638 consequence, it is possible to hypothesize that the MCU-mediated Ca²⁺ uptake within mitochondria 639 may have produced regulatory effects on the functions of these organelles and may also have 640 contributed to stimulating ATP production by oxidative phosphorylation. 641

Several studies described that when sarcoplasmic reticulum Ca^{2+} pumps are blocked and Ca^{2+} ions 642 are sequestered by the mitochondria, the first effect is a stimulation of the aerobic metabolism with 643 644 the activation of the F1F0 ATP synthase complex V to produce ATP, followed by parallel activation of ATP-consuming processes in the cytosol, which are aimed to prevent significant 645 alterations in the energy balance of the cell (Giorgi, Marchi, & Pinton, 2018; Martínez, Marmisolle, 646 Tarallo, & Quijano, 2020; Nath, 2020). Ca²⁺ ions overload and accumulation in mitochondria 647 trigger mitochondrial necrosis and can lead to the opening of the mitochondrial permeability 648 649 transition pore (mPTP), with a consequent dissipation of mitochondrial membrane potential and activation of the events leading to cell death (Rasola & Bernardi, 2007; Nesci, Trombetti, Ventrella, 650 & Pagliarani, 2018; Tait & Green, 2010; Arago, Formentini, & Cueva, 2013; England et al., 2018). 651

In consideration of the significant differences between pools for the glycogen and GP levels, the high glycogen content in Pool H may have allowed muscle cells to utilize carbohydrates as a rapidly available source of energy, contributing to a delay in the biological cascade leading to cell death.

656 **5. Conclusions**

The molecular patterns highlighted in the present research allow drawing some hypotheses bringing 657 out biological events that characterize the different *postmortem* cellular metabolism in the samples 658 belonging to the two pools. In particular, the results obtained in the Pool L in animal tissues 659 collected postmortem evidenced the upregulation of groups of genes related to mitochondrial 660 activity, lipid metabolism, ATP synthase complex V function and mitochondrial calcium uptake. 661 The overexpression of these genes in Pool L samples seems to be implicated in metabolic processes 662 taking place in *postmortem* muscle cells. The different GP levels between the two pools could have 663 determined in Pool L a more rapid occurrence of the molecular cascade related to events triggering 664 cell death. In conditions of reduced glycogen and GP, the overexpression of genes related to 665 mitochondrial activity may have influenced the pH drop after slaughtering. 666 667 To date, the knowledge of the muscle cell *postmortem* metabolism is still scant, and the exact role

played by mitochondria in the muscle-to-meat transformation remains largely unknown. The results
obtained in the present research could be helpful to add pieces of information to these complex
metabolic processes.

671

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678	Supplementary material
679	Supplementary Table S1. Primer sequences and amplification conditions used for the quantitative
680	real-time PCR analysis of the porcine genes found differentially expressed between the two pools.
681	Supplementary Table S2. The description of the pigs selected for their extreme and divergent
682	glycolytic potential (GP) and used to obtain the two pools (Pool L and Pool H).
683	Supplementary Table S3. Lists of oligonucleotides identified as differentially expressed between
684	pools with the four statistical methods.
685	Supplementary Table S4. Complete information of oligonucleotide sequences and their relative
686	gene identification.
687	
688	References
689	Aaon, M., Bhatt, N., & Cortassa, S. C. (2014). Mitochondrial and cellular mechanisms for
690	managing lipid excess. Frontiers in Physiology, 5, Article 282.
691	https://doi.org/10.3389/fphys.2014.00282.
692	Arago, M. S., Formentini, L., & Cueva, J. M. (2013). Mitochondria-mediated energy adaption in
693	cancer: the H^+ ATP Synthase geared switch of metabolism in human tumors. Antioxidants &
694	Redox Signaling, 19, 285-298. https://doi.org/10.1089/ars.2012.4883.
695	Baughman, J. M., Perocchi, F., Girgis, H. S., Plovanich, M., Belcher-Timme, C. A., Sancak, Y.,
696	Bao, X. R., Strittmatter, L., Goldberger, O., Bogorad, R. L., Koteliansky, V., & Mootha, V. K.
697	(2011). Integrative genomics identifies MCU as an essential component of the mitochondrial
698	calcium uniporter. Nature, 476, https://doi.org/341-345. 10.1038/nature10234.

- Benjamini, Y., & Hochberg, Y. (1995). Controlling the False Discovery Rate: A Practical and
- 700 Powerful Approach to Multiple Testing. *Journal of the Royal Statistical Society. Series B*
- 701 (*Methodological*), 57, 289-300. http://www.jstor.org/stable/2346101.
- 702 Bindea, G., Mlecnik, B., Hackl, H., Charoentong, P., Tosolini, M., Kirilovsky, A., Fridman, W. H.,
- 703 Pagès, F., Trajanoski, Z., & Galon, J. (2009). ClueGO: A Cytoscape plug-in to decipher
- functionally grouped gene ontology and pathway annotation networks. *Bioinformatics*, 25, 1091–
- 705 1093. https://doi.org/10.1093/bioinformatics/btp101.
- 706 Bioinformatics and Evolutionary Genomics (2021). Retrieved from
- http://bioinformatics.psb.ugent.be/webtools/Venn/. Accessed May 10, 2021.
- 708 Boler, D. D., Dilger, A. C., Bidner, B. S, Carr, S. N., Eggert, J. M., Day, J. W., Ellis, M., McKeith,
- F, K. & Killefer, J. (2010). Ultimate pH explains variation in pork quality traits. *Journal of*

710 *Muscle Foods*, 21, 119-130. https://doi.org/10.1111/j.1745-4573.2009.00171.x

- 711 Bolstad, B. M., Irizarry, R. A., Astrand, M., & Speed, T. P. (2003). A comparison of normalization
- methods for high density oligonucleotide array data based on variance and bias. *Bioinformatics*,
- 713 *19*, 185–193. https://doi.org/10.1093/bioinformatics/19.2.185.
- Bonora, M., Bononi, A., De Marchi, E., Giorgi, C., Lebiedzinska, M., Marchi, S., Patergnani, S.,
- Rimessi, A., Suski, J. M., Wojtala, A., Wieckowski, M. R., Kroemer, G., Galluzzi, L., & Pinton,
- P. (2013). Role of the c subunit of the FO ATP synthase in mitochondrial permeability transition.
- 717 *Cell Cycle*, *12*, 674–683. http://dx.doi.org/10.4161/cc.23599.
- Bougarne N., Weyers, B., Desmet, S. J., Deckers, J., Ray, D. W., Staels, B., & Bosscher, K.
- 719 D.(2018). Molecular Actions of PPAR α in Lipid Metabolism and Inflammation. *Endocrine*
- 720 *Reviews*, *39*, 760–802. https://doi.org/10.1210/er.2018-00064.
- Bouskila, M., Hunter, R. W., Ibrahim, A. F. M., Delattre, L., Peggie, M., van Diepen, J. A., Voshol,
- P. J., Jensen, J., & Sakamoto, K. (2010). Allosteric Regulation of Glycogen Synthase Controls

- Glycogen Synthesis in Muscle. *Cell Metabolism*, *12*, 456-466.
- 724 https://doi.org/10.1016/j.cmet.2010.10.006.
- Boyer, P. D. (1997). The ATP Synthase— A splendid molecular machine. Annual Review of
- 726 *Biochemistry*, 66, 717.749. https://doi.org/10.1146/annurev.biochem.66.1.717.
- 727 Burri, L., Thoresen, G. H., & Berge, R. K. (2010). The Role of PPARα Activation in Liver and
- 728 Muscle. *PPAR Research*, 2010, Article 542359. https://doi.org/10.1155/2010/542359.
- 729 Chauhan, S. S., & England, E. M. (2018). Postmortem glycolysis and glycogenolysis: insights from
- species comparisons. *Meat Science*, *144*, 118-126. https://doi.org/10.1016/j.meatsci.2018.06.021.
- 731 Chen, R., Xiao, M., Gao, H., Chen, Y., Li, Y., Liu, Y., & Zhang, N. (2016). Identification of a
- novel mitochondrial interacting protein of C1QBP using subcellular fractionation coupled with
- 733 CoIP-MS. Analytical & Bioanalytical Chemistry, 408, 1557–1564.
- 734 <u>https://doi.org/10.1007/s00216-015-9228-7</u>.
- 735 Choe, J. H., Choi, Y. M., Lee, S. H., Shin, H. G., Ryu, Y. C., Hong, K. C., & Kim, B. C. (2008).
- The relation between glycogen, lactate content and muscle fiber type composition, and their
- influence on postmortem glycolytic rate and pork quality. *Meat Science*, *80*, 355-362. doi:

738 https://doi.org/10.1016/j.meatsci.2007.12.019

- 739 Choe, J., Choi, M., Ryu, Y., et al. (2015). Estimation of Pork Quality Traits Using Exsanguination
- 740 Blood and Postmortem Muscle Metabolites. *Asian-Australasian Journal of Animal Sciences*
- 741 (*AJAS*), 28(6), 862-869. doi: https://doi.org/10.5713/ajas.14.0768
- 742 Ciobanu, D., Bastiaansen, J., Malek, M., Helm, J., Woollard, J., Plastow, G., & Rotschild, M.
- (2001). Evidence for New Alleles in the Protein Kinase Adenosine Monophosphate Activated
- 745 quality. *Genetics*, 159, 1151–1162.

- 746 Daran-Lapujade, P., Rossell, S., van Gulik, W. M., Luttik, M. A., de Groot, M. J., Slijper, M., Heck,
- 747 A. J., Daran, J. M., de Winde, J. H., Westerhoff, H. V., Pronk, J. T., & Bakker, B. M. (2007).
- The fluxes through glycolytic enzymes in Saccharomyces cerevisiae are predominantly regulated
- at posttranscriptional levels. *Proceedings of the national academy of sciences*, 104, 15753–
- 750 15758. www.pnas.org_cgi_doi_10.1073_pnas.0707476104.
- 751 De Stefani, D., Patron, M., & Rizzuto, R. (2015). Structure and function of the mitochondrial
- calcium uniporter complex. *Biochimica et Biophysica Acta*, *1853*, 2006–2011.
- 753 http://dx.doi.org/10.1016/j.bbamcr.2015.04.008.
- Devenish, R. J., Prescott, M., & Rodgers, A. J. W. (2008). The Structure and Function of
- 755 Mitochondrial F1F0-ATP Synthases. *International Review of Cell and Molecular Biology*, 267,
- 756 1-58. https://doi.org/10.1016/S1937-6448(08)00601-1.
- 757 Domenis, R., Bisetto, E., Rossi, D., Comelli, M., & Mavelli, I. (2012). Glucose-Modulated
- 758 Mitochondria Adaptation in Tumor Cells: A Focus on ATP Synthase and Inhibitor Factor 1
- 759 *International Journal of Molecular Sciences*, *13*, 1933-1950.
- 760 https://doi.org/10.3390/ijms13021933.
- 761 Edwards, D. (2003). Non-linear normalization and background correction in one channel cDNA
- microarray studies, *Bioinformatics*, 19, 825-833. https://doi.org/10.1093/bioinformatics/btg083.
- 763 Enfält, A. C., Lundström, K., Hansson, I., Johansen, S., & Nyström, P. E. (1997). Comparison of
- non-carriers and heterozygous carriers of the RN-allele for carcass composition, muscle
- distribution and technological meat quality in Hampshire sired pigs. *Livestock Production*
- *Science*, *47*, 221-229. https://doi.org/10.1016/S0301-6226(96)01409-1.
- 767 England, E. M., Matarneh, S. K., Mitacek, R. M., Abrahamb, A., Ramanathan, R., Wicks, J. C., Shi
- H., Scheffler, T. L., Oliver E. M., Helma, E. T., & Gerrard, D. E. (2018). Presence of oxygen and

- mitochondria in skeletal muscle early postmortem. *Meat Science*, 139, 97–106.
- 770 https://doi.org/10.1016/j.meatsci.2017.12.008.
- 771 England, E. M., Matarneh, S. K., Oliver, E. M., Apaoblaza, A., Scheffler, T. L., Shi, H., & Gerrard,
- D. E. (2016). Excess glycogen does not resolve high ultimate pH of oxidative muscle. *Meat*
- *Science*, *114*, 95–102. https://doi.org/10.1016/j.meatsci.2015.10.010.
- England, E. M., Scheffler, T. L., Kasten, S. C., Matarneh, S. K., & Gerrard, D.E. (2013). Exploring
- the unknowns involved in the transformation of muscle to meat. *Meat Science*, *95*, 837-843.
- 776 https://doi.org/10.1016/j.meatsci.2013.04.031.
- Fajardo, V. A., Bombardier, E., Vigna, C., Devji, T., Bloemberg, D., Gamu, D., Gramolini, A. O.,
- 778 Quadrilatero, J., Tupling, A. R. (2013). Co-Expression of SERCA Isoforms, Phospholamban and
- Sarcolipin in Human Skeletal Muscle Fibers. *PLoS ONE*, 8, Article e84304.
- 780 https://doi.org/10.1371/journal.pone.0084304.
- Fan, W., & Evans, R. (2015). PPARs and ERRs: molecular mediators of mitochondrial
- metabolism. *Current Opinion in Cell Biology*, *33*, 49-54.
- 783 https://doi.org/10.1016/j.ceb.2014.11.002.
- Ferguson, B. S., Rogatzki, M. J., Goodwin, M. L., Kane, D. K., Zachary, R., & Gladden, L. B.
- 785 (2018). Lactate metabolism: historical context, prior misinterpretations and current
- understanding. *European Journal of Applied Physiology*, *118*, 691–728.
- 787 https://doi.org/10.1007/s00421-017-3795-6.
- Fogal, V., Richardson, A. D., Karmali, P. P., Scheffler, I. E., Smith, J. W., & Ruoslahti, E. (2010).
- 789 Mitochondrial p32 Protein Is a Critical Regulator of Tumor Metabolism via Maintenance of
- 790 Oxidative Phosphorylation. *Molecular and Cellular Biology*, *30*, 1303–1318.
- 791 https://doi.org/10.1128/MCB.01101-09.

- 792 Fontanesi, L., Davoli, R., Nanni Costa, L., Beretti, F., Scotti, E., Tazzoli, M., Tassone, F., Colombo,
- 793 M., Buttazzoni, L., & Russo, V. (2008). Investigation of candidate genes for glycolytic potential
- of porcine skeletal muscle: association with meat quality and production traits in Italian Large
- 795 White pigs. *Meat Science*, 80, 780–787. https://doi.org/10.1016/j.meatsci.2008.03.022.
- 796 Fontanesi, L., Davoli, R., Nanni Costa, L., Scotti, E., & Russo, V. (2003). Study of candidate genes
- for glycolytic potential of porcine skeletal muscle: identification and analysis of mutations,
- ⁷⁹⁸ linkage and physical mapping and association with meat quality traits in pigs. *Cytogenetic and*
- *Genome Research*, *102*, 145–151. https://doi.org/10.1159/000075740.
- 800 Fujii, J., Otsu, K., Zorzato, F., de Leon, S., Khanna, V. K., Weiler, J. E., O'Brien, P. J., &
- MacLennan, D. H. (1991). Identification of a mutation in porcine ryanodine receptor associated
 with malignant hyperthermia. *Science*, *253*, 448-51.
- Giorgi, C., Marchi, S., & Pinton, P. (2018). The machineries, regulation and cellular functions of
 mitochondrial calcium. *Nature Reviews Molecular Biology*, *19*, 713-730.
- 805 https://doi.org/10.1038/s41580-018-0052-8.
- Gladden, L. B. (2004). Lactate metabolism: a new paradigm for the third millennium. Journal of
- 807 *Physiology*, 558.1, 5-30. https://doi.org/10.1113/jphysiol.2003.058701.
- Glancy, B., Kane, D. A., Kavazis, A. N., Goodwin, M. L., Willis, W. T., & Gladden, L. B. (2021).
- 809 Mitochondrial lactate metabolism: history and implications for exercise and disease. *Journal of*
- 810 *Physiology*, *599.3*, 863–888. https://doi.org/10.1113/JP278930.
- Goto, T., Joo-Young Lee, J.-Y., Teraminami, A., Yong-Il Kim, Y.-I., Hirai, S., Uemura, T., Inoue,
- H., Takahashi N., & Kawada, T. (2011). Activation of peroxisome proliferator-activated
- 813 receptor-alpha stimulates both differentiation and fatty acid oxidation in adipocytes. *Journal of*
- Lipid Research, 52, 873–884. https://doi 10.1194/jlr.M011320.

- Gotoh, K., Morisaki, T., Setoyama, D., Sasaki, K., Yagi, M., Igami, K., Mizuguchi, S., Uchiumi, T.,
- Fukui, Y., & Kang, D. (2018). Mitochondrial p32/C1qbp Is a Critical Regulator of Dendritic Cell
- 817 Metabolism and Maturation. *Cell Reports*, 25, 1800–1815.
- 818 https://doi.org/10.1016/j.celrep.2018.10.057.
- Harrell Jr, F. E. (2021). Hmisc: Harrell Miscellaneous. R package version 4.5-0. Retrieved from
- https://CRAN.R-project.org/package=Hmisc. Accessed May 8, 2021.
- He, J., Forda, H. C., Carrolla, J., Dinga, S., Fearnleya, I. M., & Walkera, J. E. (2017). Persistence of
- the mitochondrial permeability transition in the absence of subunit c of human ATP synthase.
- 823 Proceedings of the national academy of sciences of the United States of America, 114, 3409-
- 824 3414. https://doi.org/10.1073/pnas.1702357114.
- Henckel, P., Karlsson, A., Mogens, T. J., Oksbjerg, N., & Petersen, J. S. (2002). Metabolic
- conditions in Porcine longissimus muscle immediately pre-slaughter and its influence on peri-
- and post mortem energy metabolism. *Meat Science*, 62, 145-155. https://doi.org/10.1016/s03091740(01)00239-x.
- Howe, K. L., Achuthan, P., Allen, J., Allen, J., Alvarez-Jarreta, J., Amode, M. R., Armean, I. M.,
- Azov, A. G., Bennett, R., ... & Flicek, P. (2021). Ensembl 2021. Nucleic Acids Research, 49,
- 831 D884–D891. https://doi.org/10.1093/nar/gkaa942.
- Huang, da W., Sherman, B. T., & Lempicki, R. A. (2009). Systematic and integrative analysis of
- large gene lists using DAVID bioinformatics resources. *Nature Protocols*, *4*, 44-57.
- https://doi.org/10.1038/nprot.2008.211.
- Hue, L., & Taegtmeyer, H. (2009). The Randle cycle revisited: a new head for an old hat. *American Journal of Physiology, Endocrinology and Metabolism, 297*, E578–E591.
- 837 https://doi.org/10.1152/ajpendo.00093.2009.

- Huff-Lonergan, E., Baas, T. J., Malek, M., Dekkers, J. C., Prusa, K., & Rothschild, M. F. (2002).
- Correlations among selected pork quality traits. *Journal of Animal Science*, 80, 617–627.

840 https://doi.org/10.2527/2002.803617x

- Junge, W., & Nelson, N. (2015). ATP Synthase. Annual Review of Biochemistry, 84, 631–657.
- 842 https://doi.org/10.1146/annurev-biochem-060614-034124.
- 843 Kamiński, S., Koćwin-Podsiadła, M., Sieczkowska, H., Help, H., Zybert, A., Krzęcio, E., Antosik,
- K., Brym, P., Wójcik, E., & Adamczyk, G. (2010). Screening 52 single nucleotide
- polymorphisms for extreme value of glycolytic potential and drip loss in pigs. *Journal of Animal*
- 846 *Breeding and Genetics, 127,* 125-132. <u>https://doi.org/10.1111/j.1439-0388.2009.00820.x</u>
- Lorenz, D. R., Misra, V., & Gabuzda, D. (2019). Transcriptomic analysis of monocytes from HIV-
- positive men on antiretroviral therapy reveals effects of tobacco smoking on interferon and stress
- response systems associated with depressive symptoms. *Human genomics*, 13, 59. https://doi-
- 850 org/10.1186/s40246-019-0247-x
- Lunt, S. Y., & Vander Heiden, M. G. (2011). Aerobic Glycolysis: Meeting the Metabolic
- 852 Requirements of Cell Proliferation. Annual Review of Cell and Developmental Biology, 27, 441–
- 64. https://doi.org/10.1146/annurev-cellbio-092910-154237.
- 854 Ma, J., Yang., J., Zhou, L., Ren, J., Liu, X., Zhang, H., Yang, B., Zhang, Z., Ma, H., Xie, X., Xing,
- Y., Guo, Y., & Huang, L. (2014). A Splice Mutation in the PHKG1 Gene Causes High Glycogen
- 856 Content and Low Meat Quality in Pig Skeletal Muscle. *PLOS Genetics*, *10*, Article e1004710.
- 857 https://doi.org/10.1371/journal.pgen.1004710.
- 858 Martínez, J., Marmisolle, I., Tarallo, D., & Quijano, C. (2020). Mitochondrial Bioenergetics and
- 859 Dynamics in Secretion Processes. *Frontiers in Endocrinology*, *11*, Article 319.
- 860 https://doi.org/10.3389/fendo.2020.00319.

- Martinez-Reyes, I., & Chandel, N. S. (2017). Waste Not, Want Not: Lactate Oxidation Fuels the
 TCA Cycle. *Cell Metabolism*, 26, 803-804. https://doi.org/10.1016/j.cmet.2017.11.005.
- Matarneh, S. K., Beline, M., de Luz e Silva, S., Shia, H., & Gerrard, D. E. (2018). Mitochondrial
- F1-ATPase extends glycolysis and pH decline in an *in vitro* model. *Meat Science*, 13, 85–91.
- 865 https://doi.org/10.1016/j.meatsci.2017.11.009.
- 866 Matarneh, S. K., Yen, C. N., Bodmer, J., El-Kadi, S.W., & Gerrard, D.E. (2021). Mitochondria
- 867 influence glycolytic and tricarboxylic acid cycle metabolism under postmortem simulating
- conditions. *Meat Science*, *172*, article 108316. https://doi.org/10.1016/j.meatsci.2020.108316.
- 869 Matarneh, S. K., England, M. E., Scheffler, T. L., Yen, C., & Gerrard, D. E. (2017). A
- 870 mitochondrial protein increases glycolytic flux in muscle postmortem. *Meat Science*, 133, 119–
- 871 125. https://doi.org/10.1016/j.meatsci.2017.06.007.
- Matarneh, S. K., England, E. M., Scheffler, T. L., & Gerrard., D.E. (2017). The conversion of
- muscle to meat. In F. Toldrá (Ed.), *Lawrie's meat science* (pp. 159–185). Woodhead Publishing.
- Milan, D., Jeon, J. T., Looft. C., Amarger, V., Robic, A., Thelander, M., Rogel-Gaillard, C., Paul,
- S., Iannuccelli, N., Rask, L., Ronne, H., Lundström, K., Reinsch, N., Gellin, J., Kalm, E., Le
- 876 Roy, P., Chardon, P., & Andersson, L. (2000). A mutation in PRKAG3 associated with excess
- glycogen content in pig skeletal muscle. *Science*, 288, 1248–1251.
- 878 https://doi.org/10.1126/science.288.5469.1248
- Monin, G. (1988). Evolution post mortem du tissu musculaire et consequences sur les qualité de la
 viande de porc. *Journées de la Recherche Porcine* France, 20, 201–214.
- 881 Monin, G., & Sellier, P. (1985). Pork of low technological quality with a normal rate of muscle pH
- fall in the immediate post-mortem period: The case of the Hampshire breed. *Meat Science*, *13*,
- 49-63. https://doi.org/10.1016/S0309-1740(85)80004-8.

- 884 Monin, G., Mejenes-Quijano, A., & Talmant, A. (1987). Influence of breed and muscle metabolic
- type on muscle glycolytique potential and meat pH in pigs. *Meat Science*, 20, 149–

886 158.https://doi.org/10.1016/0309-1740(87)90034-9.

- 887 Mookerjee, S. A., Nicholls, D. G., & Brand, M. D. (2016). Determining maximum glycolytic
- capacity using extracellular flux measurements. *PLoS One*, 11, Article e0152016.

889 https://doi.org/10.1371/journal.pone.0152016.

- 890 Moreno, I., Lipová, P., Ladero, L., García, J. L. F., & Cava, R. (2020). Glycogen and lactate
- contents, pH and meat quality and gene expression in muscle *Longissimus dorsi* from iberian
- pigs under different rearing conditions. *Livestock Science*, 240, Article 104167.
- 893 https://doi.org/10.1016/j.livsci.2020.104167.
- Muoio, D. M., Way, J. M., Tanner, C. J., Winegar, D. A., Kliewer, S. A., Houmard, J. A., Kraus,
- 895 W. E., Dohm, G., L. (2002). Peroxisome proliferator-activated receptor-alpha regulates fatty acid
- utilization in primary human skeletal muscle cells. *Diabetes*, 51, 901-909.
- 897 https://doi.org/10.2337/diabetes.51.4.901.
- 898 Nanni Costa, L., Tassone, F., Davoli, R., Fontanesi, L., Colombo, M., Buttazzoni, L. & Russo, V.
- (2009). Glycolytic potential in Semimembranosus muscle of Italian Large White pigs. *Journal of muscle food*, 20, 392-400. https://doi.org/10.1111/j.1745-4573.2009.00155.x.
- 901 Nath, S. (2020). A Novel Conceptual Model for the Dual Role of FOF1- ATP Synthase in Cell Life
- 902 and Cell Death. *BioMolecular Concepts*, *11*, 143–152. https://doi.org/10.1515/bmc-2020-0014.
- 903 Nesci, S., Trombetti, F., Ventrella, V., & Pagliarani, A. (2018). From the Ca²⁺-activated F1FO-
- ATPase to the mitochondrial permeability transition pore: an overview. *Biochimie*, 152, 85-93.
- 905 https://doi.org/10.1016/j.biochi.2018.06.022.
- 906 Noh, S., Phorl, S., Naskar, R., Oeum, K., Seo, Y., Kim, E., Kweon, H. S., & Lee1, J. Y. (2020).
- 907 p32/C1QBP regulates OMA1-dependent proteolytic processing of OPA1 to maintain

- 908 mitochondrial connectivity related to mitochondrial dysfunction and apoptosis. *Scientific*
- 909 *Reports*, 10, Article 10618. https://doi.org/10.1038/s41598-020-67457-w.
- 910 Pfaffl, M. W., Tichopad, A., Prgomet, C., & Neuvians, T. P. (2004). Determination of stable
- 911 housekeeping genes, differentially regulated target genes and sample integrity: BestKeeper--
- 912 Excel-based tool using pair-wise correlations. *Biotechnology Letters*, *26*, 509–515.
- 913 https://doi.org/10.1023/b:bile.0000019559.84305.47.
- 914 Phipson, B., Lee, S., Majewski, I. J., Alexander, W. S., & Smyth, G. K. (2016). Robust
- 915 hyperparameter estimation protects against hypervariable genes and improves power to detect
- 916 differential expression. *The Annals of Applied Statistics*, *10*, 946–963.
- 917 https://doi.org/10.1214/16-AOAS920.
- 918 Phua, W. W. T., Wong, M. X. Y., Liao, Z., & Tan, N. S. (2018). An aPPARent Functional
- 919 Consequence in Skeletal Muscle Physiology via Peroxisome Proliferator-Activated Receptors.
- 920 *International Journal of Molecular Science*, *19*, Article 1425.
- 921 https://doi.org/10.3390/ijms19051425.
- 922 Pösö, A. R., & Puolanne, E. (2005). Carbohydrate metabolism in meat animals. *Meat Science*, 70,
- 923 423–434. https://doi.org/10.1016/j.meatsci.2004.12.017.
- R Core Team (2020). R: A language and environment for statistical computing. R Foundation for
 Statistical Computing, Vienna, Austria. Retrieved from https://www.R-project.org/. Accessed
- 926 July 10, 2020.
- 927 Rakhshandehroo, M., Knoch, B., Müller, M., & Kersten, S. (2010). Peroxisome Proliferator-
- Activated Receptor Alpha Target Genes. *PPAR Research*, 2010, Article 612089.
- 929 https://doi.org/10.1155/2010/612089.

- 930 Rasola, A., & Bernardi, P. (2007). The mitochondrial permeability transition pore and its
- 931 involvement in cell death and in disease pathogenesis. *Apoptosis*, *12*, 815–833.
- 932 https://doi.org/10.1007/s10495-007-0723-y.
- 933 Reynolds, T. H., Pak, Y., Harris, T. E., Manchester, B. E. J., & Lawrence, J. C. Jr. (2005). Effects
- of insulin and transgenic overexpression of UDP-glucose pyrophosphorylase on UDP-glucose
- and glycogen accumulation in skeletal muscle fibers. *Journal of Biological Chemistry*, 280,
- 936 5510- 5515. https://doi.org/10.1074/jbc.M413614200.
- 937 Ritchie, M. E., Phipson, B., Wu, D., Hu, Y., Law, C. W., Shi, W., & Smyth, G. K. (2015). Limma
- powers differential expression analyses for RNA-sequencing and microarray studies. *Nucleic*
- Acids Research, 43, Article e47. https://doi.org/10.1093/nar/gkv007.
- 940 Roach, P. J. (2002). Glycogen and its metabolism. *Current Molecular Medicine*, *2*, 101-120.
 941 https://doi.org/10.2174/1566524024605761.
- 942 Roach, P. J., Depaoli-Roach, A. A., Hurley, T. D., & Tagliabracci, V. S. (2012). Glycogen and its
- 943 metabolism: some new developments and old themes, *Biochemical Journal*, 441, 763–787.
- 944 https://doi.org/10.1042/BJ20111416.
- 945 Rosenvold, K. & Andersen, H. J. (2003a). The significance of pre-slaughter stress and diet on
- 946 colour and colour stability of pork. *Meat Science*, *63*, 199-209. https://doi.org/10.1016/s0309947 1740(02)00071-2.
- 948 Rosenvold, K., & Andersen, H. J. (2003b). Factors of significance for pork quality—A review.
- 949 *Meat Science*, 64, 219–237. https://doi.org/10.1016/S0309-1740(02)00186-9.
- 950 Rühle, T., & Leister, D. (2015). Assembly of F1F0-ATP synthases. *Biochimica et Biophysica Acta*,
- 951 *1847*, 849-60. https://doi.org/10.1016/j.bbabio.2015.02.005.

- 952 Russo, V., Fontanesi, L., Davoli, R., Chiofalo, L., Liotta, L., & Zumbo, A. (2004). Analysis of
- single nucleotide polymorphisms in major and candidate genes for production traits in Nero
- 954 Siciliano pig breed. *Italian Journal of Animal Science*, 3, 19-29.
- 955 <u>https://doi.org/10.4081/ijas.2004.19</u>.
- 956 Sambrook, J., & Russell, D. W. (2006). Purification of Nucleic Acids by Extraction with
- 957 Phenol: Chloroform. *Cold Spring Harbor Protocols*. doi:10.1101/pdb.prot4455
- 958 Scheffler, T. L., Matarneh, S. K., England, E. M., & Gerrard, D. E. (2015). Mitochondria influence
- postmortem metabolism and pH in an in vitro model. *Meat Science*, 110, 118–125.
- 960 https://doi.org/10.1016/10.1016/j.meatsci.2015.07.007.
- 961 Scheffler, T. L., Scheffler, J. M., Kasten, S. C., Sosnicki, A. A., & Gerrard, D.E. (2013). High
- glycolytic potential does not predict low ultimate pH in pork. *Meat science*, 95, 85-91.
- 963 https://doi.org/10.1016/j.meatsci.2013.04.013.
- 964 Scheffler, T. L., & Gerrard, D. E. (2007). Mechanisms controlling pork quality development: The
- biochemistry controlling postmortem energy metabolism. *Meat Science*, 77, 7–16.
- 966 https://doi.org/10.1016/j.meatsci.2007.04.024.
- 967 Scheffler, T. L., Park, S., & Gerrard, D.E. (2011). Lessons to learn about postmortem metabolism
- using the AMPKγ3 R200Q mutation in the pig. *Meat Science*, 89, 244-250.
- 969 https://doi.org/10.1016/j.meatsci.2011.04.030.
- 970 Senior, A. E., Nadanaciva, S., & Weber, J. (2002). The molecular mechanism of ATP synthesis by
- 971 F1F0-ATP synthase. *Biochimica et Biophysica Acta*, *1553*, 188–211.
- 972 https://doi.org/10.1016/s0005-2728(02)00185-8.
- 973 Shaikh, S. A., Sahoo, S., K. & Periasamy, M. (2016). Phospholamban and Sarcolipin: Are they
- 974 functionally redundant or distinct regulators of the Sarco(Endo)plasmic Reticulum Calcium

- 975 ATPase?. Journal of Molecular Cell Cardiology, 91, 81–91.
- 976 https://doi.org/10.1016/j.yjmcc.2015.12.030.
- 977 Shannon, P., Markiel, A., Ozier, O., Baliga, N. S., Wang, J. T., Ramage, D., Amin, N.,
- 978 Schwikowski, B., & Ideker, T. (2003). Cytoscape: A software environment for integrated models
- of biomolecular interaction networks. *Genome Research*, *13*, 2498–2504.
- 980 https://doi.org/10.1101/gr.1239303.
- 981 Shen, L. Y., Luo, J., Lei, H. G., Jiang, Y. Z., Bai, L., Li, M. Z., Tang, G. Q., Li, X. W., Zhang, S.
- H., & Zhu, L. (2015). Effects of muscle fiber type on glycolytic potential and meat quality traits
- in different Tibetan pig muscles and their association with glycolysis-related gene expression.
- 984 *Genetics and Molecular Research, 14*, 14366-14378.
- 985 https://doi.org/10.4238/2015.November.13.22.
- 986 Smolková, K., Bellance, N., Scandurra, F., Génot, E., Gnaiger, E., Plecitá-Hlavatá, L., Jezek, P., &
- 987 Rossignol, R. (2010). Mitochondrial bioenergetic adaptations of breast cancer cells to aglycemia
- and hypoxia. *Journal of Bioenergy and Biomembranes*, 42, 55-67.
- 989 https://doi.org/10.1007/s10863-009-9267-x.
- 990 Tait, S. W. G., & Green, D. R. (2010). Mitochondria and cell death: outer membrane
- permeabilization and beyond. *Nature Reviews. Molecular Cell Biology*, *11*, 621-632.
- 992 https://doi.org/10.1038/nrm2952.
- 993 Tanner, L. B., Goglia, A. G., Wei, M. H., Seghal, T., Parsons, L. R., Park, J. O., White, E.,
- 994 Toettcher, J. E., & Rabinowitz, J. D. (2018). Four Key Steps Control Glycolytic Flux in
- 995 Mammalian Cells. *Cell Systems*, 7, 49–62. https://doi.org/10.1016/j.cels.2018.06.003.
- 996 Teodoro, B. G., Sampaio, I. H., Bomfim, L. H., Queiroz, A. L., Silveira, L. R., Souza, A. O.,
- 997 Fernandes, A. M., Eberlin, M. N., Huang, T. Y., Zheng, D., Neufer, P. D., Cortright, R. N.,
- Alberici, L. C. (2017). Long-chain acyl-CoA synthetase 6 regulates lipid synthesis and

- 999 mitochondrial oxidative capacity in human and rat skeletal muscle. *The Journal of Physiology*,
- 1000 595.3, 677–693. https://doi.org/10.1113/JP272962.
- 1001 Vamecq, J., Colet, J. M., Vanden Eynde, J. J., Briand, G., Porchet, N., & Rocchi, S. (2012). PPARs:
- 1002 Interference with Warburg' Effect and Clinical Anticancer Trials. *PPAR Research*, 2012, Article
- 1003 304760. https://doi.org/10.1155/2012/304760.
- 1004 Vandesompele, J., De Preter, K., Pattyn, F., Poppe, B., Van Roy, N., De Paepe, A., & Speleman, F.
- 1005 (2002). Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of
- 1006 multiple internal control genes. *Genome Biology*, *3*, Article RESEARCH0034.
- 1007 <u>https://doi.org/10.1186/gb-2002-3-7-research0034</u>.
- 1008 Van Laack, R. L., & Kauffman, R. G. (1999). Glycolytic potential of red, soft, exudative pork
- 1009 longissimus muscle. *Journal of Animal Science*, 77, 2971–2973.
- 1010 Van Laack, R. L., Liu, C. H., Smith, M. O., & Loveday, H. D. (2000). Characteristics of pale, soft,
- 1011 exudative broiler breast meat. *Poultry Science*, 79(7), 1057-61. doi:
- 1012 https://doi.org/10.1093/ps/79.7.1057.
- 1013 Walker, J. E. (2013). The ATP synthase: the understood, the uncertain and the unknown.
- 1014 *Biochemical Society Transactions*, *41*, 1–16. https://doi.org/10.1042/BST20110773.
- 1015 Walker, J. E., Lutter, R., Dupuis, A., & Runswick M. J. (1991). Identification of the subunits of
- 1016 F1F0-ATPase from bovine heart mitochondria. *Biochemistry*, *30*, 5369–5378.
- 1017 https://doi.org/10.1021/bi00236a007.
- 1018 Wilson, D. F. (2017). Oxidative phosphorylation: unique regulatory mechanism and role in
- 1019 metabolic homeostasis. *Journal of Applied Physiology*, *122*, 611-619.
- 1020 https://doi.org/10.1152/japplphysiol.00715.2016.

- 1021 Xing, T., Gao, F., Tume, R. K., Zhou, G., & Xu, X. (2019). Stress Effects on Meat Quality: A
- 1022 Mechanistic Perspective. *Comprehensive Reviews in Food Science and Food Safety.* 18, 380-
- 1023 401. https://doi.org/10.1111/1541-4337.12417.
- 1024 Yan, W. L., Lerner, T. J., Haines, J. L., & Gusella, J. F. (1994). Sequence analysis and mapping of a
- novel human mitochondrial ATP synthase subunit 9 cDNA (ATP5G3). *Genomics*, 24, 375–377.
- 1026 https://doi.org/10.1006/geno.1994.1631.
- 1027 Yang, Y. H., Dudoit, S., Luu, P., Lin, D. M., Peng, V., Ngai, J., & Speed, T. P. (2002).
- 1028 Normalization for cDNA microarray data: a robust composite method addressing single and
- 1029 multiple slide systematic variation. *Nucleic Acids Research*, *30*, Article e15.
- 1030 https://doi.org/10.1093/nar/30.4.e15.
- 1031 Young, P. A., Senkal, C. E., Suchanek, A. L., Grevengoed, T. J., Lin, D. D., Zhao, L., Crunk, A. E.,
- 1032 Klett, E. L., Füllekrug, J., Obeid, L. M., & Coleman, R. A. (2018). Long-chain acyl-CoA
- synthetase 1 interacts with key proteins that activate and direct fatty acids into niche hepatic
- 1034 pathways. *Journal of Biological Chemistry*, 293, 16724–16740.
- 1035 https://doi.org/10.1074/jbc.RA118.004049.
- 1036 Zappaterra, M., Braglia, S., Bigi, M., Zambonelli, P., & Davoli, R. (2015). Comparison of
- expression levels of fourteen genes involved in the lipid and energy metabolism in two pig
- 1038 breeds. *Livestock Science*, *181*, 156-162. https://doi.org/10.1016/j.livsci.2015.09.007.
- 1039 Zappaterra, M., Sami, D., & Davoli, R. (2019). Association between the splice mutation
- 1040 g.8283C>A of the *PHKG1* gene and meat quality traits in Large White pigs. *Meat Science*, 148,
- 1041 38-40. https://doi.org/10.1016/j.meatsci.2018.10.003.
- 1042 Zhang, Z., Schwartz, S., Wagner, L., & Miller, W. (2000). A greedy algorithm for aligning DNA
- sequences. *Journal of Computer Biology*, 7, 203-214.
- 1044 https://doi.org/10.1089/10665270050081478.

- 1045 Zhou, A., Rohou, A., Schep, D. G., Bason, J. V., Montgomery, M. G., Walker, J. E., Grigorieff, N.,
- 1046 & Rubinstein, J. L. (2015). Structure and conformational states of the bovine mitochondrial ATP
- synthase by cryo-EM. *eLife.* 4, Article e10180. https://doi.org/10.7554/eLife.10180.