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A gene and protein expression study on four porcine genes related to intramuscular fat deposition

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1 A gene and protein expression study on four porcine genes related  
2 to intramuscular fat deposition

3

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## 22 Abstract

23 Intramuscular fat (IMF) content has a prominent role in meat quality, affecting sensory  
24 attributes such as flavour and texture. In the present research, we studied in samples of  
25 porcine *Semimembranosus* muscle four genes related to lipid metabolism and whose gene  
26 expressions have been associated to IMF deposition: *FASN*, *SCD*, *LIPE* and *LPL*. We  
27 analysed both mRNA and protein expressions in two groups of Italian Large White pigs  
28 divergent for *Semimembranosus* IMF deposition, with the aim of comparing the levels of four  
29 genes and enzymes between the two groups and identifying possible coexpression links.  
30 The obtained results suggest a prominent role of LIPE enzyme in IMF hydrolysis, as the  
31 samples with low IMF deposition show a significantly higher amount of this lipase. Finally, a  
32 poorly known correlation was found between LIPE and FASN enzymes only in female  
33 individuals. These results provide new information for the understanding of IMF deposition.

34

## 35 1 Introduction

36 During the last decades, pig selection has aimed at satisfying the request of the pork industry  
37 mainly focused on the increase of muscle deposition and carcass lean cut amount, resulted  
38 in a reduction in fat storage and adipogenesis (Wood, 1990). This selective pressure has  
39 also led to a progressive lowering in the total lipid content of muscle, in particular in some  
40 breeds selected for their predisposition to lean mass deposition (Wood and Warriss, 1992).  
41 In Large White breed the selection lowered noticeably the marbling percentage, passing from  
42 an average of 2-4% of intramuscular fat (IMF) in Large White pigs bred in 1960's (Wood,  
43 1990) to less than 1% in *Longissimus* muscle of the modern Large White pigs (Wood *et al.*,  
44 2008). IMF is composed of lipid droplets stored within myofibers cytoplasm and adipocytes  
45 located between the fiber *fasciculi*, consequently IMF amount is strongly related to the  
46 number of intramuscular adipocytes (Zheng and Mei, 2009). It is known that sensory  
47 attributes such as flavour and juiciness are influenced by IMF content (Wood, 1990;  
48 Fernandez *et al.*, 1999).

49 The number of Genome Wide Association studies aimed at identifying SNPs and genes  
50 affecting IMF deposition and composition in different pig breeds (Ma et al., 2013; Muñoz et  
51 al., 2013; Nonneman et al., 2013; Kim et al., 2015; Davoli et al., 2016) is increasing, however  
52 little is known about the relative enzyme quantity of the putative genes involved in IMF  
53 deposition in pig muscle tissue. Therefore, in the present study we considered four genes  
54 involved in lipid metabolism and whose mRNA levels in literature are reported to be linked to  
55 IMF deposition in different pig breeds (Zhao et al., 2009; Wang et al., 2012). Two of the  
56 selected enzymes are involved in synthesis and desaturation of fatty acids (*fatty acid*  
57 *synthase, FASN; stearoyl-CoA desaturase (delta 9 desaturase), SCD*) and the two remaining  
58 enzymes are involved in fatty acids catabolism (*hormone sensitive lipase, LIPE; lipoprotein*  
59 *lipase, LPL*). *FASN* gene is located on *Sus scrofa* chromosome 12 (Muñoz et al., 2003), and  
60 the coded protein plays an essential role in long-chain fatty acid synthesis, starting from  
61 acetyl CoA and using malonyl-CoA as a 2 carbon donor and NADPH as reducing equivalent  
62 (Wakil, 1989; Menendez et al., 2009). In pigs both *SCD* and *LPL* genes are localised on  
63 chromosome 14 (Gu et al., 1992; Ren et al., 2003). *SCD* catalyses the desaturation of  
64 palmitoyl-CoA and stearoyl-CoA at the position  $\Delta 9$  producing de novo palmitoleoyl-CoA and  
65 oleoyl-CoA, while *LPL* has the dual function of hydrolysing the circulating chylomicron  
66 triglycerides to diglycerides and of ligand/bridging factor for receptor mediated lipoprotein  
67 uptake. On the other hand, *LIPE* hydrolyses the triglycerides stored in muscle to diglycerides,  
68 then to monoglycerides and at the end to free fatty acids. *LIPE* gene has been assigned to  
69 porcine chromosome 6, and its position coincides with a Quantitative Trait Locus (QTL)  
70 region linked to sensory quality in porcine meat (Pena et al., 2013). In the present study, we  
71 analysed both the protein quantifications and the transcription profiles of these four genes in  
72 the *Semimembranosus* muscle of two groups of Italian Large White (ILW) pigs divergent for  
73 IMF deposition, with the aims: i) of testing whether the mRNA and enzyme levels of *FASN*,  
74 *LIPE*, *SCD* and *LPL* differed between two groups of pigs divergent for IMF (LOW IMF group  
75 vs. HIGH IMF group), ii) of identifying common trends in the expression levels of the four  
76 studied genes and proteins, suggesting coexpression links.

77 The focus both on gene and protein levels of the analysed enzymes could be useful to  
78 highlight a possible involvement of FASN, LIPE, SCD and LPL proteins on IMF deposition in  
79 pig meat.

## 80 2 Materials and methods

### 81 2.1 Protein expression study

#### 82 2.1.1 Sampling

83 For the present study, a set of 155 pigs was selected among a population of 950 Italian  
84 Large White (ILW) pigs. As reported in Davoli et al. (2016), using the Soxhlet extraction  
85 method the whole sample of 950 ILW pigs has been characterised for IMF content, reported  
86 as percentage (grams of IMF on 100 grams of pig *Semimembranosus* muscle). The 155  
87 individuals used for the quantitation of FASN, SCD, LIPE and LPL enzymes in  
88 *Semimembranosus* muscle have been selected among the 950 ILW pigs based on their  
89 extreme and divergent IMF phenotype (Table 1) and divided in two groups differing for IMF  
90 content (LOW IMF group and HIGH IMF group). The LOW IMF group consists of 77 pigs  
91 presenting IMF values lower than the average IMF level of the 950 pigs (total population)  
92 minus one standard deviation unit. Furthermore, the HIGH IMF group consists of individuals  
93 with IMF contents higher than the average IMF value of the total population plus 3.5 standard  
94 deviation units. The two IMF divergent groups have been chosen avoiding as much as  
95 possible full and half sibs, in order to prevent the family effect on protein and gene  
96 quantitation results. Additionally, since sex and batch may influence protein and gene  
97 expressions the two groups were balanced for these two factors. The pigs are pure breed  
98 animals included in the Italian sib test genetic evaluation scheme performed by ANAS  
99 (Associazione Nazionale Allevatori Suini, ANAS; [www.anas.it](http://www.anas.it)), reared in the same  
100 environmental conditions at the genetic test station with a *quasi ad libitum* feeding level (60%  
101 of the pigs was able to ingest the entire supplied ration). The sib test program calculates the  
102 estimated genetic value of each candidate boar testing three of its full sibs, two females and  
103 one castrated male. For this reason, the considered sample of 155 individuals is composed

104 of two thirds of sows and one third of castrated males (Table 1), with the purpose of  
105 maintaining the proportions of the total population and of using a representative sample of  
106 the 950 pigs population. At the end of the test, the animals were transported to a commercial  
107 abattoir located about 25 km from the test station in accordance with Council Rule (EC) No.  
108 1/2005 regarding the protection of animals during transport and related operations and,  
109 amending Directives 64/432/EEC and 93/119/EC and Regulation (EC) No. 1255/97. At the  
110 slaughterhouse, the pigs were electrically stunned and bled in a supine position in agreement  
111 with Council Regulation (EC) No. 1099/2009 regarding the protection of animals at the time  
112 of slaughter. All slaughter procedures were monitored by the veterinary team appointed by  
113 the Italian Ministry of Health. Moreover, for all the pigs, ANAS provided us with the Estimated  
114 Breeding Values (EBVs): Average Daily Gain (ADG, calculated from 30 to 155 kg of live  
115 weight with *quasi ad libitum* feeding level, expressed in grams), Backfat Thickness (BFT,  
116 recorded *post mortem* at the level of *Gluteus medius* muscle, expressed in mm), Lean Cuts  
117 (LC, the sum of neck and loin weight, expressed in kg), Feed Conversion Ratio (FCR,  
118 obtained from feed intake recorded daily and body weight measured bimonthly, expressed in  
119 units), and Ham Weight (HW, expressed in kg). The listed EBVs have been calculated by  
120 ANAS according to the statistical model reported by Russo et al. (2000). Moreover, the  
121 slaughterhouse technicians provided us with the phenotypic measures of the carcass weight  
122 expressed in kg (carcass weight), the percentage of lean cuts over the total carcass weight  
123 (%lean), and the backfat and loin thickness measured at 8 cm off the midline of the carcass  
124 at the level placed between the third and fourth last ribs measured with Fat-O-Meter (FOM).

#### 125 2.1.2 Protein extraction and total protein quantitation

126 At the slaughterhouse, *Semimembranosus* muscle tissue was quickly frozen in Liquid  
127 Nitrogen (LN<sub>2</sub>) and then stored at -80°C for subsequent total protein extraction.  
128 Approximately 300 mg of tissue sample were homogenized on ice in 900 µl of T-PER Tissue  
129 Protein Extraction (Thermo Fisher Scientific Inc., Waltham, Massachusetts, USA), to which  
130 1x protease and phosphatase inhibitors (PhosSTOP™, Roche, Hoffmann-La Roche, Basel,

131 Switzerland) were added. Tissue lysates were then centrifuged at 15,000 x g for 20 min at  
132 4°C to remove lipids and insoluble debris. For LPL, before the quantitation of the total  
133 proteins and the quantification of LPL enzyme, the extracted proteins were concentrated  
134 through a filtering step, using Amicon® Ultra-4 filters (Merck Millipore, Darmstadt, Germany).  
135 This additional step was needed to obtain LPL quantitation within the sensitivity range of the  
136 commercial ELISA kit used for the quantitation of this protein.

137 The total extracted proteins were quantified using the BCA reagent (Thermo Fisher Scientific  
138 Inc., Waltham, Massachusetts, USA) and the optical density of each sample was determined  
139 using a microplate optical reader.

#### 140 2.1.3 Quantification of intramuscular FASN, LIPE, SCD and LPL enzymes

141 FASN, LIPE, SCD and LPL were quantitatively detected using ELISA kits produced by  
142 Cusabio (Cusabio Biotech, Wuhan, China), according to manufacturer's instructions. Briefly,  
143 100 µl of blank, standards or samples were added to well, covered, incubated for 2 hours at  
144 37°C and the liquid of each well was removed without washing. Then 100 µl of biotin  
145 antibody working solution was added to each well, incubated for 1 hour at 37°C and then  
146 washed three times with wash buffer (200 µl). At each well, 100 µl of HRP avidin working  
147 solution were added, the well covered, incubated for 1 hour at 37°C and washed three times  
148 with wash buffer (200 µl). Finally, TMB substrate (90 µl) was then added to each well,  
149 covered, dark incubated for 30 minutes at 37°C and stopped with 50 µl of stop solution. The  
150 optical density of each well was determined within 5 minutes using a microplate reader at  
151 450 nm.

152 For all the samples, the relative quantification of each enzyme was calculated, through the  
153 ratio between the enzyme absolute quantitation and the total extracted protein amount.

## 154 2.2 Gene expression study

### 155 2.2.1 Sampling

156 The gene expression study was carried out on a smaller sample, composed of 47 ILW pigs  
157 chosen among the overall group of 155 animals. These selected pigs, which were extreme  
158 and divergent for IMF phenotype (Table 1), were the progeny of 36 boars and 44 sows. Both  
159 Subset 1 (for the low IMF pigs) and Subset 2 (for the high IMF individuals) have been chosen  
160 avoiding as much as possible full and half sibs.

#### 161 2.2.2 RNA source, total RNA extraction and cDNA preparation

162 *Semimembranosus* muscle samples were withdrawn at the slaughterhouse and immediately  
163 frozen at -80°C. Total RNA was then extracted using TRIZOL reagent (Invitrogen  
164 Corporation, Carlsbad, California), the quality and integrity of the RNAs were both checked  
165 and RNA was retrotranscribed to cDNA as described in Davoli et al. (2011).

#### 166 2.2.3 Gene expression quantitation

167 The quantitative Real-Time PCR (qRT-PCR) standard curve method (Pfaffl, 2004) was used  
168 to analyse the four genes expression. qRT-PCR was performed on Rotor Gene™ 6000  
169 (Corbett Life Science, Concorde, New South Wales) using 5 µl of SYBR® Premix Ex Taq™  
170 (TAKARA Bio INC, Olsu, Shiga, Japan), 5 pmol of each primer, 2 µl of cDNA template diluted  
171 1:10 and make up to the total volume of 10 µl with water. Rotor Gene™ 6000 protocol was  
172 optimised using specific annealing temperatures for each primer couple (Supplementary  
173 Table 1). Six genes were tested to be used as normalising genes (*polymerase (RNA) II (DNA*  
174 *directed) polypeptide A, 220kDa, POLR2A; beta-2-microglobulin, B2M; tyrosine 3-*  
175 *monooxygenase/tryptophan 5-monooxygenase activation protein, zeta, YWHAZ;*  
176 *hypoxanthine phosphoribosyltransferase 1, HPRT1; TATA box binding protein, TBP;*  
177 *peptidylprolyl isomerase A (cyclophilin A), PPIA*) and using NormFinder (Andersen et al.,  
178 2004) were identified the three most stable ones to be used as normalising genes (*B2M,*  
179 *YWHAZ,* and *HPRT1*). For the normalising and the 4 studied genes a specific standard curve  
180 was obtained, amplifying 11 serial dilutions (from 10<sup>9</sup> to 50 molecules/µl) of a known  
181 concentration sample of cDNA amplicon, obtained with a PCR with the external primer pairs  
182 (Supplementary Table 1). The PCR efficiency was calculated as  $E = 10 \exp(-1/\text{slope})$ , with a

183 range between -2.7 and -4.3, indicating a good PCR efficiency results. All the PCR products  
184 were checked on a polyacrylamide gel and washed from primer dimers with the QIAquick®  
185 PCR Purification Kit (QIAGEN, Venlo, Netherlands). The absence of unspecific amplicons  
186 during qRT-PCR on Rotor Gene™ 6000 was tested using the melt step after the cycling. The  
187 variation coefficient (CV = Standard Deviation of the Crossing Points/Average of the  
188 Crossing Points) of the replicated analysis for each samples (three in two different qRT  
189 PCRs) was accepted for CV < 0.2.

### 190 2.3 Statistical analysis

191 All the statistical analyses were carried out using SAS software, version 9.4 (SAS Inst. Inc.,  
192 Cary, NC). To decide what statistical approach to use, we performed a general linear model  
193 (GLM) where we tested the fixed effects of sex, batch and group (LOW and HIGH IMF  
194 groups) on the studied gene and protein levels. As sex and batch did not affect the gene and  
195 protein levels, we decided to take into account only the group effect, comparing the protein  
196 and gene levels between the divergent groups of pigs. PROC NPAR1WAY command of SAS  
197 (the Wilcoxon test) was used to compare the protein levels between the groups of pigs  
198 divergent for IMF deposition (as protein values were not normally distributed), and the PROC  
199 TTEST of SAS (Student's t test) was utilised to compare the gene expression data between  
200 groups (as gene expression values were normally distributed). The PROC CORR SAS  
201 command was considered to calculate the correlations between the different gene and  
202 protein expressions.

## 203 3 Results

204 The main results are reported in Figure 1, Figure 2 and in Table 2, where for the two IMF  
205 divergent groups the protein and gene expression quantifications are showed. In particular  
206 we investigated both the mRNA and protein levels of *FASN*, *LIPE*, *SCD* and *LPL* genes,  
207 which have been already described in literature to be likely involved in IMF deposition.  
208 However, to date, the association of these genes with IMF deposition was reported mainly on  
209 the basis of their mRNA levels while in the present research both the mRNA and protein

210 amounts were considered. For the quantification of these four enzymes in the two groups of  
211 ILW pigs extreme for IMF, we used commercially available ELISA kits. Owing to some  
212 problems in SCD and LPL protein quantitation and in order to avoid uncertain data, we  
213 decided to present and compare only the most reliable quantification values, maintaining  
214 however the divergence between the two groups for IMF value and balancing the samples  
215 for sex (Supplementary Table 2).

216 The means  $\pm$  the standard errors of the quantified enzymes in the LOW IMF and HIGH IMF  
217 groups are represented in Figure 1. It is worth noting that LIPE is the only enzyme showing a  
218 higher amount in samples with LOW IMF deposition ( $P < 0.05$ ) (Figure 1 C), while SCD protein  
219 presents a trend ( $P < 0.1$ ) and on average a higher enzyme expression in the HIGH IMF group  
220 (Figure 1 A). The transcription levels obtained for the four considered genes are reported in  
221 Figure 2: the two groups (LOW and HIGH IMF) show differences in the mRNA levels for  
222 *FASN*, *LPL* and *SCD* genes. In Table 2 are indicated the means  $\pm$  the standard deviations of  
223 the gene and enzyme expressions in the LOW and HIGH IMF groups, in the LOW and HIGH  
224 IMF sows and castrated males, and in the two sexes. In the same table are reported the  
225 significant comparisons between groups. In order to identify links between gene transcription  
226 levels and enzyme expressions, the correlations between the mRNA and the protein levels  
227 were performed, and the results reported in Table 3. The lack of correlation between the  
228 mRNA level of each gene and the expression of its coded protein clearly stands out.  
229 Furthermore, we calculated the correlations between each one of the analysed mRNA and  
230 protein expressions and all the other available quantitation data, in order to look for the  
231 presence of some possible coexpressions (Table 3). This approach revealed the presence of  
232 two significant correlations between the mRNA of *LIPE* gene and the amount of LPL and  
233 SCD proteins. Furthermore, *FASN* gene resulted highly correlated to *SCD* and *LPL* gene  
234 expressions, while for the enzymes a single positive correlation between LIPE and FASN  
235 was identified (Table 3). In particular, this positive and high correlation between LIPE and  
236 FASN enzymes showed to be strongly linked to sex, as only sows presented a highly  
237 significant correlation coefficient, not observed in castrated males (Table 4). Furthermore, in

238 order to find some possible links between gene and protein expressions and carcass traits, a  
239 correlation analysis considering gene and enzymes expression levels towards EBVs and  
240 phenotypic measures of carcass traits was carried out (Supplementary Table 3). The results  
241 in Supplementary Table 3 show low correlations between the gene and protein expression  
242 data and the EBVs or phenotypic measures, but all the associations we found are consistent  
243 with the roles of the studied genes and enzymes. For example, *FASN* is negatively  
244 correlated to ADG and HW EBVs, and to carcass weight measure, in agreement with the  
245 lipogenic role of this enzyme. Moreover, IMF content resulted to be positively associated with  
246 *FASN*, *SCD* and *LPL* gene expression levels, and among the tested enzymes only LIPE  
247 showed a negative correlation with IMF deposition (Supplementary Table 3).

#### 248 4 Discussion

249 The primary aim of the study was to investigate the expression levels of *FASN*, *LIPE*, *SCD*  
250 and *LPL* enzymes between two groups of ILW pigs divergent for IMF deposition, in order to  
251 look for the involvement of these proteins in porcine IMF deposition. The results suggest a  
252 prominent role of *LIPE* enzyme in IMF hydrolysis: indeed, low IMF pigs have a significantly  
253 higher amount of *LIPE* enzyme as compared to the high IMF group (Figure 1 C and Table 2).  
254 This result agrees with the role of *LIPE*, which hydrolyses to free fatty acids the triglycerides  
255 stored in adipose tissue. According to this result, we also found a negative correlation  
256 between IMF deposition and *LIPE* enzyme amount (Supplementary Table 3). The different  
257 *LIPE* relative quantitation between high and low IMF individuals is also maintained between  
258 IMF divergent pigs of the same sex, indicating the importance of *LIPE* activity in IMF  
259 deposition for both castrated male and female individuals (Table 2). To date, in different  
260 animal species several authors have found associations between *LIPE* gene or enzyme  
261 expressions and the carcass traits (Zhao et al., 2010 in pigs; Ying et al., 2013 in mice; Zhang  
262 et al., 2014 in yaks). In particular, Zhao et al. (2009) found a divergent expression of *LIPE*  
263 gene and protein between 12 Wujin pigs with high IMF deposition and 12 Landrace pigs with  
264 low IMF deposition. Due to the different diet compositions and the use of two samples

265 coming from distinct pig breeds, the diverging *LIPE* mRNA levels found by the authors  
266 between the two breeds may be affected by the different characteristics of the diets.  
267 However, on the basis of the results of the present study, it is possible to suggest a diet  
268 independent role of LIPE on IMF deposition, as LIPE enzyme showed different expressions  
269 between IMF divergent pigs, although they were reared in the same environmental  
270 conditions.

271 On the other hand, in pigs with a more pronounced IMF deposition a higher level of SCD  
272 protein was found, but the difference between the SCD enzyme expressions in LOW IMF  
273 and HIGH IMF groups showed only a trend towards significance ( $P < 0.1$ ) (Figure 1). This  
274 result suggests that SCD enzyme could have an important role in IMF metabolism and is in  
275 agreement with the data reported by Wu et al. (2013), who detected an overexpression of  
276 *SCD* gene in high IMF Jinhua pigs as compared to Landrace pigs.

277 In addition, we tested also the linear correlation between the genes and the relative protein  
278 expressions (Table 3), finding that no one of the enzyme amounts significantly covaried with  
279 the relative gene transcription level. This result indicates that post-transcriptional regulation  
280 for these genes may play an essential role in the modulation of their enzyme synthesis and  
281 activity. However, despite this result, it is worth noting that *FASN*, *LPL* and *SCD* maintained  
282 on average a higher expression of both mRNA and protein levels in high IMF pigs as  
283 compared to the low IMF group (Figure 1 and 2) suggesting the existence of a conserved  
284 trend between the transcription and translation levels of these three genes.

285 Furthermore, the strong correlation found between LIPE and *FASN* (Table 3) was further  
286 investigated through an additional correlation analysis performed in castrated males and in  
287 sows (Table 4). Based on the results obtained, in muscle tissue we can suppose the  
288 activation of distinct biological pathways controlling IMF adipogenesis between female and  
289 male individuals. Mukherjee et al. (2014) found that in rat white adipose tissue the quantity of  
290 LIPE enzyme varies together with the expression of Caveolin1 (*CAV1*): in particular, *CAV1*  
291 showed to be stimulated by estrogen (the primary female sex hormone) and suppressed by

292 androgen (typically the male sex hormone). On the other side, CAV1 is coexpressed with  
293 FASN and interacts with this enzyme in human prostate cancer (Di Vizio et al., 2008). CAV1  
294 is an important target in sex hormone dependent regulation of several metabolic pathways,  
295 particularly in cancer and diabetes (Mukherjee et al., 2014). Considering the results reported  
296 in literature, it is possible to hypothesise that the strong correlation we found in sows  
297 between LIPE and FASN proteins may be the effect of an estrogen activated pathway,  
298 possibly linking these two enzymes through the mediation of CAV1. Anyway, further studies  
299 are needed to prove in pigs this hypothesis.

300 In Table 3, also the correlation between *FASN* and *SCD* gene expressions stands out. The  
301 expressions of these two genes have already been found to covary in *Longissimus dorsi*  
302 muscle between pigs with divergent muscle lipid deposition (Wang et al., 2015).  
303 Nevertheless this coexpression was not detected at the protein level (Table 3).

304 Moreover, the correlations found between the EBVs or carcass traits and the gene and  
305 protein expression levels agree with the known roles of the studied genes: FASN and SCD  
306 are both enzymes involved in the synthesis and desaturation of fatty acids, and in fact  
307 showed negative correlations with the EBV of Average Daily Gain and more generally to lean  
308 mass deposition, causing a worsening in Feed Conversion Ratio EBV (Supplementary Table  
309 3).

## 310 5 Conclusions

311 The study of FASN, LIPE, LPL and SCD gene and protein expression levels allowed  
312 obtaining a more comprehensive view of their involvement in pig intramuscular fat deposition.  
313 The results suggest for LIPE and SCD enzyme a role in muscle fat deposition and indicate  
314 that these two genes may be involved in similar regulatory pathways and respond to similar  
315 transcription cues. Anyway, future studies are needed to better understand the role of *LIPE*  
316 gene and its coded protein in pork quality. Finally, in female individuals a poorly known  
317 correlation between LIPE and FASN enzymes was found, suggesting the need of dedicated  
318 studies aimed at identifying and elucidating the pathways involved.

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412

413 **Figure captions**

414 Figure 1. Relative quantitation of the studied enzymes in the LOW and HIGH IMF groups.

415 Mean values are shown and error bars represent the standard errors.

416 Section A: relative quantitation of FASN and SCD enzymes in the low IMF and high IMF

417 groups; section B: relative quantitation of LPL enzyme in the low IMF and high IMF groups;

418 section C: relative quantitation of LPL enzyme in the low IMF and high IMF groups.

419 Significant differences between the two divergent groups are expressed with \*\*\* for  $P < 0.001$ .

420 § is used to indicate differences with  $P$  value  $< 0.10$ .

421 Figure 2. Relative quantitation of the gene expressions in Subset 1 (LOW IMF) and Subset 2

422 (HIGH IMF). Mean values are shown and error bars represent the standard errors.

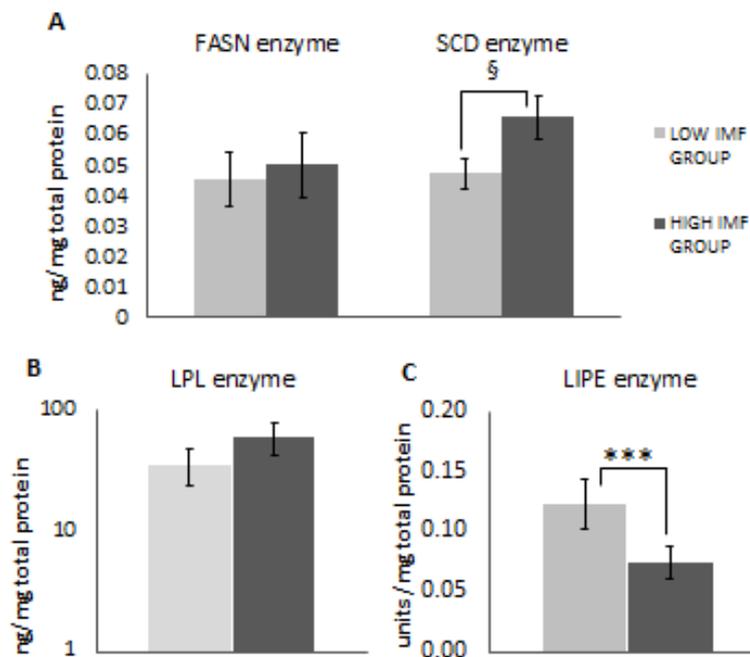
423 Significant differences between the two divergent groups are expressed with \* for  $P < 0.05$

424 and \*\* for  $P < 0.01$ . § is used to indicate differences with  $P$  value  $< 0.1$ .

425

426 **Figure 1.** Relative quantitation of the studied enzymes in the LOW and HIGH IMF groups.

427 Mean values are shown and error bars represent the standard errors.



428

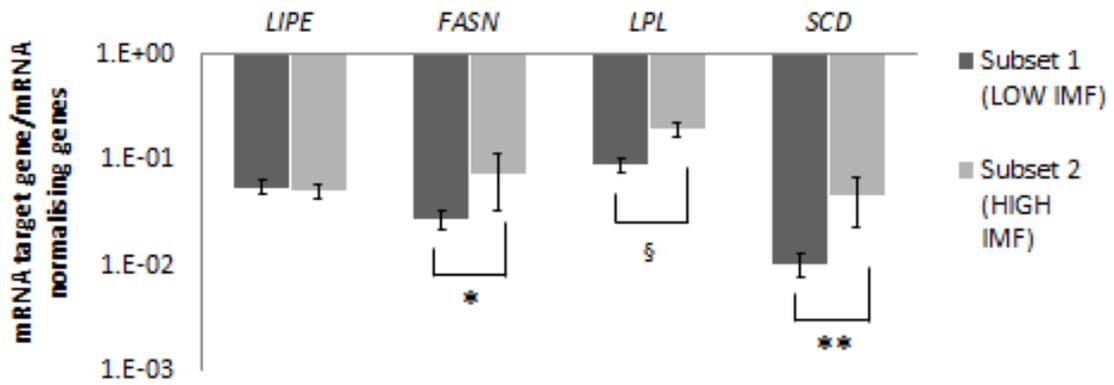
429 Section A: relative quantitation of FASN and SCD enzymes in the low IMF and high IMF  
430 groups; section B: relative quantitation of LPL enzyme in the low IMF and high IMF groups;  
431 section C: relative quantitation of LPL enzyme in the low IMF and high IMF groups.

432 The significant comparison between the two divergent groups is expressed with \*\*\* for  
433  $P < 0.001$ . § is used to indicate differences with  $P$  value  $< 0.10$ .

434

435

436 **Figure 2.** Relative quantitation of the gene expressions in Subset 1 (LOW IMF) and Subset 2  
437 (HIGH IMF). Mean values are shown and error bars represent the standard errors.



438

439 The significant comparisons between the two divergent groups are expressed with \* for  
440  $P < 0.05$  and \*\* for  $P < 0.01$ . § is used to indicate differences with  $P$  value  $< 0.10$ .