Transcriptome profile of skeletal muscle using different sources of dietary fatty acids in male pigs

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Abstract

Background

Pork is of great importance in world trade and represents the largest source of fatty acids in the human diet. Lipid sources such as soybean oil (SOY), canola (CO), and fish oil (FO) are used in pig diets and influence blood parameters and the ratio of deposited fatty acids. Scientific studies that improve our nutrigenomic knowledge about pig and human health through the consumption of pork are of great importance. In this study, the main objective was to evaluate changes in gene expression in porcine skeletal muscle tissue resulting from the dietary oil sources and to identify metabolic pathways and biological process networks through RNA-Seq.

Results

The addition of FO in the diet of pigs led to intramuscular lipid with a higher FA profile composition of C20:5 n-3, C22:6 n-3, and SFA (C16:0 and C18:0). Blood parameters for the FO group showed lower cholesterol and HDL content compared with CO and SOY groups. Skeletal muscle transcriptome analyses revealed 65 differentially expressed genes (DEG, FDR 10%) between CO *vs* SOY, and 531 DEG for SOY *vs* FO comparison, and 32 DEG for CO *vs* FO. The enrichment analysis of the DEG lists revealed lipid metabolism, metabolic diseases, and inflammation processes networks, such as "regulation of lipid metabolism: RXR-dependent regulation of lipid metabolism via PPAR, RAR and VDR", “transport HDL-mediated reverse cholesterol transport”, and "TNF- α , IL-1 β induce dyslipidemia and inflammation in obesity and type 2 diabetes in adipocytes".

Conclusion

The genes identified in this study provided relevant information on biological processes and lipid metabolism pathways. The enrichment of the basal diet with oil containing different FA profiles influenced fatty acid composition of intramuscular fat, blood parameters, and the gene differential expression in metabolic pathways and processes network in skeletal muscle of pigs. The results provide mechanisms to help us understand the behavior of genes according to fatty acids.

1. Background

Pigs (*Sus scrofa*) are monogastric animals, a widely used as scientific animal model, and one of the most globally important species in meat production [1]. Pigs produce the most consumed meat in the world [2]. Dietary lipids influence the quantity and quality of fatty acids (FA) present in pork products [3]. Soybean oil (SOY) is a primary source of lipids in pig diets and contains unsaturated fatty acids (UFA) ([4, 5]. However, other lipid sources such as canola (CO) and fish oil (FO) are also used in pig diets. Consumption of SOY compared to CO leads to significant differences between FA profiles, mainly for linoleic acid (AL), alpha-linolenic acid (ALA), and oleic acid (OA). Canola oil has approximately 7% of saturated fat (SFA) content and a high ALA content (11%), which is reflected in the ratio of UFA and SFA in pork carcass fat

[6, 7]. Fish oils contain a beneficial ratio of omega 3 (n-3) polyunsaturated fatty acids (PUFA) providing benefits in reduced inflammation, lipid metabolism, and reduced oxidative stress processes [8]. Feeding pigs different mixed lipid sources, such as SOY, FO or CO, results in changes in lipid profiles that can lead to high levels of UFA [9–11]. Although the use of different dietary lipid sources has been well known to result in changes in pig carcasses, the optimal lipid sources for pig growth and for support of human health is unclear.

The FA composition of foods affects human nutrition by altering risks for obesity, high plasma cholesterol, and cardiovascular disease [12, 13]. Monounsaturated (MUFA) and PUFA have appealing functional properties, providing beneficial effects on health, such as the prevention of chronic non-communicable diseases, regulation of the immune response, reduction of the risk of atherosclerosis, and reduced occurrence of type 2 diabetes [14, 15]. Some studies have demonstrated beneficial effects on the inflammatory system caused by the inclusion of lipids in the diet [16, 17].

Therefore, scientific studies that allow us to improve our nutrigenomic knowledge about pork products, and human health through their consumption, are of great importance. In this study, we evaluated changes in blood parameters, fatty acid profile in *Longissimus lumborum* intramuscular fat, and gene expression in the skeletal muscle tissue of immunecastrated male pigs fed diets enriched with different FA profiles, and identify affected metabolic pathways and biological process networks in which the identified genes are involved.

2. Results

In previous work of our group, Almeida et al. [18] observed that the inclusion of SOY or CO to pig diets reduced loin shear force but the diets did not alter growth performance from day 0 to 21, day 21 to 42, day 42 to 56, and day 70 to 98. Consumer overall liking score in loins were greater from pigs fed SOY. Thus, in the current study, we performed blood parameters, FA composition of intramuscular fat, and differential expression analysis of the animals fed with diets containing different types of oils (SOY, CO, FO).

2.1 Blood parameters and fatty acid profile

The three different oil dietary sources altered serum concentrations of total protein, albumin (*p*-value <0.01), cholesterol (*p*-value =0.04), and HDL (*p*-value =0.04). Total protein and albumin levels were lower in pigs fed SOY compared with CO or FO (*p*-value <0.01) (Table 1).

Table 1: Effects of dietary treatments on blood parameters of pigs

Variable	Dietary treatment			Pooled SEM[2]	*p*-value
	CO	FO	SOY		
Glucose (mg/dL)	86.11	89.54	83.40	5.07	0.48
Aspartate Aminotransferase (U/L)	42.72	42.88	38.13	3.01	0.21
Total Proteins (g/dL)	6.84[a]	6.82[a]	6.46[b]	0.14	0.01
Albumin (g/dL)	3.80[a]	3.87[a]	3.46[b]	0.09	<0.01
Globulin (g/dL)	3.04	2.94	3.00	0.13	0.73
Triglycerides (mg/dL)	45.67	39.78	35.70	4.89	0.13
Cholesterol (mg/dL)	99.60[a]	90.34[b]	96.49[a]	3.72	0.04
HDL (mg/dL)	45.59[a]	40.11[b]	43.66[a]	2.21	0.04
LDL (mg/dL)	44.89	42.28	45.71	2.53	0.38
VLDL (mg/dL)	9.11	7.94	7.13	1.00	0.14

[1]Pigs (n = 54) fed a corn-Soybean meal diet enriched with 3% Soybean oil (SOY), Canola oil (CO), or Fish oil (FO). Values represent the least square means from 18 pigs/treatment.

[2]SEM = standard error of the least square means.

[a–b]Within a row, values without a common superscript differ ($p \leq 0.05$) using Tukey's method.

The FA composition was different (*p*-value <0.05) in the *Longissimus lumborum* (LL) intramuscular fat. Concentrations of palmitic acid (*p*-value <0.01), stearic acid (*p*-value <0.01), oleic acid (OA) (*p*-value <0.01), ALA (*p*-value = 0.76), eicosapentaenoic acid (EPA) (*p*-value =0.52), docosahexaenoic acid (DHA) (*p*-value <0.01), SFA (*p*-value <0.01), MUFA (*p*-value <0.01), total n-3 PUFA (*p*-value <0.01), and n-6:n-3 PUFA ratio (*p*-value <0.01), were different across dietary treatments. Considering all FA, there were differences in the FO treatment compared to either SOY or CO. For palmitic acid (*p*-value <0.01), stearic acid (*p*-value <0.01), EPA (*p*-value <0.01), DHA (*p*-value <0.01), SFA (*p*-value <0.01) and total n-3 PUFA (*p*-value = <0.01) they were elevated in LL according to the FO dietary treatment. The FO dietary treatment decreased oleic acid (*p*-value <0.01), MUFA (*p*-value = <0.01), and n-6:n-3 PUFA ratio (*p*-value <0.01). The PUFA and the individual FA, myristic acid, palmitoleic acid, eicosenoic acid, LA, and ALA were similar among all diets. The highest atherogenic index was identified in FO group compared to other diets, between the CO and SOY diets the index was lower (*p*-value =0.02) (see Table 2).

Table 2: Effects of dietary treatments on the *Longissimus lumborum* intramuscular FA profile of pigs[1].

Fatty acid (%)	Dietary treatment			Pooled SEM[2]	*p*-value
	CO	FO	SOY		
Saturated fatty acid (SFA)					
Myristic acid (C14:0)	1.21	1.24	1.20	0.03	0.64
Palmitic acid (C16:0)	24.95[a]	26.43[b]	25.05[a]	0.35	<0.01
Stearic acid (C18:0)	11.28[a]	12.63[b]	11.83[ab]	0.30	<0.01
Monounsaturated fatty acid (MUFA)					
Palmitoleic acid (C16:1)	3.05	3.22	3.18	0.11	0.48
Oleic acid (C18:1 n-9)	44.95[a]	40.33[b]	44.28[a]	1.04	<0.01
Eicosenoic acid (C20:1 n-9)	0.58	0.56	0.55	0.02	0.52
Polyunsaturated fatty acid (PUFA)					
Linoleic acid (C18:2 n-6)	13.33	14.21	13.14	0.70	0.42
Alpha-linolenic acid (C18:3 n-3)	0.53	0.56	0.57	0.04	0.76
Eicosapentaenoic acid (C20:5 n-3)	0.09[a]	0.46[b]	0.15[a]	0.05	<0.01
Docosahexaenoic acid (C22:6 n-3)	0.11[a]	0.61[b]	0.15[a]	0.05	<0.01
Total SFA	37.44[a]	40.29[b]	38.08[a]	0.59	<0.01
Total MUFA	48.59[a]	44.11[b]	47.84[a]	1.11	<0.01
Total PUFA	14.24	16.61	14.63	1.05	0.15
Total n-3 PUFA[3]	0.68[a]	1.70[b]	0.83[a]	0.12	<0.01
Total n-6 PUFA[4]	13.33	14.21	13.14	0.70	0.42
PUFA:SFA ratio[5]	0.39	0.41	0.38	0.03	0.73
n-6:n-3 PUFA ratio[6]	22.48[a]	8.96[b]	17.19[c]	1.14	<0.01
Atherogenic index[7]	0.48[a]	0.53[b]	0.48[a]	0.01	0.02

[1]Pigs (*n* = 54) fed a corn-soybean meal diet enriched with 3% Canola oil or 3% Fish oil or 3% Soybean oil (SOY). Values represent the least square means from 18 pigs/treatment.

[2]SEM = standard error of the least square means.

[3]Total n-3 PUFA = {[C18:3 n-3] + [C20:5 n-3] + [C22:6 n-3]}.

[4]Total n-6 PUFA = C18:2 n-6.

[5]PUFA:SFA ratio = total PUFA/total SFA.

[6]Σ n-6/Σ n-3 PUFA ratio.

[7]Atherogenic index = (4 × [C14:0]) + (C16:0)/(total MUFA] + [total PUFA]), where brackets indicate concentrations [19].

[a,b,c]Within a row, values without a common superscript differ ($P \leq 0.05$) using Tukey's method (Adapted from ALMEIDA et al. [18].

2.2 Sequencing data and differential expression analysis

The average numbers of sequenced reads before and after filtering for samples from the skeletal muscle for the CO group were 33,568,010 and 33,085,594; for the SOY group were 31,955,613 and 31,491,236; and, for the FO group were 33,895,987 and 33,393,094 (Supplementary Table 1). A total of 65 DEG (log2-fold change ≥1; ≤−1; FDR-corrected *p*-value < 0.10) were identified between CO *vs* SOY groups, where 47 were down-regulated (log2-fold change ranging from -5.57 to -0.29) and 18 up-regulated (log2-fold change ranging 0.22 from to 3.07) in the CO group. A total of 32 DEG (log2-fold change ≥1; ≤−1; FDR-corrected *p*-value < 0.10) were identified between CO *vs* FO groups, where 21 were down-regulated (log2-fold change ranging from -4.84 to -0.47) and 11 up-regulated (log2-fold change ranging 0.50 from to 2.92) in the CO group. Finally, for the SOY *vs* FO groups, there were 406 down-regulated genes (log2-fold change ranging from -4.99 to -0.23) and 125 up-regulated genes (log2-fold change ranging 0.21 from to 3.52) in the SOY group (Supplementary Table 2). Table 3 displays a summary of the genes and the log2-fold changes between diet groups.

Table 3. Differentially expressed genes in the skeletal muscle of pigs

Diet group	DEG¹ Down-regulated	log2FC²	DEG¹ Up-regulated	log2FC²	Total DEG¹
CO *vs* SOY	47	-5.57 to -0.29	18	0.22 to 3.07	65
CO *vs* FO	21	-4.84 to -0.47	11	0.50 to 2.92	32
SOY *vs* FO	406	-4.99 to -0.23	125	0.21 to 3.53	531

¹Differentially Expressed Genes; ²log2-fold change. Comparisons between Canola Oil (CO); Soybean Oil (SOY) and Fish Oil (FO).

Supplementary Figure S1 shows the Volcano plot of log2-fold change (x-axis) *vs* -log10FDR-corrected *p*-value (y-axis) from the differential gene expression analysis for the skeletal muscle (A) CO *vs* SOY, (B) SOY *vs* FO, and (C) CO *vs* FO.

2.3 Functional enrichment analysis for skeletal muscle differential expression (CO *vs* SOY)

Ten different pathway maps (Figure 1) were detected (*p*-value <0.10), which are linked to the following DEG, Alcohol Dehydrogenase 7 (Class IV) (*ADH7*): “Fatty acid omega oxidation” pathway (Figure 2); Aldehyde Dehydrogenase 3 Family Member A2 (*AL3A2, ALDH3A2*): “FA omega oxidation” (Figure 2), “leukotriene 4 biosynthesis and metabolism”, “triacylglycerol metabolism p.1”, and “oxidative stress in adipocyte dysfunction in type 2 diabetes and metabolic syndrome X” pathways (Supplementary Figure 2, 3, 4); Angiopoietin 1 (*ANGPT1*): “role of adipose tissue hypoxia in obesity and type 2 diabetes” pathway (Supplementary Figure 5); Nuclear receptor subfamily 0 group B member 2 (*SHP, NR0B2*): “regulation of lipid metabolism RXR-dependent regulation of lipid metabolism via PPAR, RAR and VDR”, “regulation of lipid metabolism FXR-dependent negative-feedback regulation of bile acids concentration”, “selective Insulin resistance in type 2 diabetes in liver”, “regulation of metabolism: Bile acids regulation of glucose and lipid metabolism via FXR” pathways (Supplementary Figure 6, 7, 8, 9); and, T-Cell Surface Glycoprotein (*CD4*) DEG related to “breakdown of CD4+ T cell peripheral tolerance in type 1 diabetes mellitus” (Supplementary Figure 10).

In our study, several DEG were identified, including the *ADH7* in skeletal muscle of pigs fed with different oils (CO *vs* SOY), which was more expressed in the CO group (log2-fold change +2.34). Moreover, *AL3A2* (log2-fold change -0.79) DEG, which participates in the “fatty acid omega oxidation” (*p*-value <0.10) (Figure 4) and “leukotriene 4 biosynthesis and metabolism” pathways (Supplementary Figure 2), was less expressed in the CO group. The *AL3A2* DEG was further enriched (*p*- value <0.10) for the “triacylglycerol metabolism” pathway (Supplementary Figure 3) and identified in the “oxidative stress in adipocyte dysfunction in type 2 diabetes and metabolic syndrome X” pathway (Supplementary Figure 4), which catalyzes the oxidation of 4-Hydroxy-2(E)-nonenal to the nontoxic 1,4-Dihydroxynonene.

The DEG angiopoietin-1 *(Ang-1, ANGPT1)* was identified in our study as less expressed (log2-fold change -1.22) in the group of animals fed 3.0% CO. The enrichment analysis demonstrated its relation with the “role of adipose tissue hypoxia in obesity and type 2 diabetes” pathway (Supplementary Figure 5). Another DEG was the nuclear receptor subfamily gene 0 group B member 2 (*NR0B2*). Nuclear receptors (*NRs*) are a family of TF that play a critical role in different aspects in mammals as can be seen in the pathways enriched by MetaCore analysis (Supplementary Figure 7, 8 and 9). Lastly, the *SHP* DEG was more expressed in the CO group (log2-fold change +2.15).

The *CD4*, an essential gene in the immune response processes, was identified as DEG when comparing CO *vs* SOY diets, with lower expression (log2-fold change -1.55) in the CO group. This gene is involved in the "breakdown of *CD4+* T cell peripheral tolerance in type 1 diabetes mellitus" (Supplementary Figure 10) pathway map, enriched by MetaCore.

To observe the interactions of DEG in gene networks, the process networks analysis was performed using MetaCore software (Table 4) and allowed us to identify networks related (*p*-value <0.1) to immune response, metabolism regulation and signal transduction. The identified immune response networks "antigen presentation" was enriched for the *CD4* DEG (log2-fold change -1.55); the "transport bile acids transport and its regulation", "regulation of bile acid metabolism and negative *FXR*-dependent regulation of bile acids concentration", and "signal transduction_ESR2" pathways were enriched for the nuclear receptor subfamily 0 group B member 2 (*SHP, NR0B2*; log2-fold change +2.15) DEG.

Table 4. Processes networks by MetaCore software (*p*-value <0.10) from the list of differentially expressed genes (FDR 10%) in the skeletal muscle of pigs fed with different oil sources (Canola oil and Soybean oil CO *vs* SOY)

Process Networks	*p*-value	DEG[1]
Immune response_Antigen presentation	0.01	*CD4*
Transport_Bile acids transport and its regulation	0.07	*SHP*
Regulation of metabolism_Bile acid regulation of lipid metabolism and negative FXR-dependent regulation of bile acids concentration	0.07	*SHP*
Signal transduction_ESR2 pathway	0.08	*SHP*

[1] Differentially Expressed Genes

2.4 Functional enrichment analysis for skeletal muscle differential expression (CO *vs* FO)

Different pathway maps were detected using the gene list the CO *vs* FO comparison (*p*-value <0.10) (Figure 3), most of them presented the stearoyl-CoA desaturase (*SCD*) DEG, such as "adiponectin in pathogenesis of type 2 diabetes" (Figure 4); "regulation of lipid metabolism RXR-dependent regulation of lipid metabolism via PPAR, RAR and VDR" (Supplementary Figure 11); "putative pathways for stimulation of fat cell differentiation by Bisphenol A" (Supplementary Figure 12); "regulation of lipid metabolism via LXR, NF-Y, and SREBP" (Supplementary Figure 13); and, "regulation of metabolism: Bile acids regulation of glucose and lipid metabolism via FXR and Role of ER stress in obesity and type 2 diabetes" (Supplementary Figure 14). We also detected pathways presenting the *AL3A2* DEG, like the "fatty acid omega oxidation" (Supplementary Figure 15); "leukotriene 4 biosynthesis and metabolism" (Supplementary Figure 16); "triacylglycerol metabolism" (Supplementary Figure 17). As well as, the "breakdown of CD4+ T cell peripheral tolerance in type 1 diabetes mellitus" pathway with the *CD4* DEG (Supplementary Figure 18).

In our study, *SCD* expression was lower (log2-fold change -1.62) in the group of pigs fed CO diet. The *SCD*-enriched pathways were "adiponectin in pathogenesis of type 2 diabetes"; "regulation of lipid metabolism *RXR*-dependent regulation of lipid metabolism via PPAR, RAR and VDR"; "putative pathways for stimulation of fat cell differentiation by Bisphenol A"; "regulation of lipid metabolism regulation of lipid

metabolism via LXR, NF-Y and SREBP"; and, "regulation pathway of metabolism bile acids regulation of glucose and lipid metabolism via *FXR* and Role of ER stress in obesity and type 2 diabetes".

The *AL3A2* gene was identified as DEG between CO *vs* FO, the expression of *AL3A2* was lower (log2-fold change -0.60) in the CO group, as observed between CO *vs* FO in which *AL3A2* was less expressed (log2-fold change -0.79) in the CO group. This gene was enriched for "fatty acid omega oxidation", "leukotriene 4 biosynthesis and metabolism", and "triacylglycerol metabolism" pathways. Lastly, T-cell surface glycoprotein *CD4 (CD4)* was enriched in pathways related to the "breakdown of CD4+ T cell peripheral tolerance in type 1 diabetes mellitus".

A process networks analysis was performed for better understanding of the enriched pathways through interactions by gene networks, which are related to dietary oil manipulation. Emphasizing the pathways regulation of bile acid metabolism and negative *FXR* dependent regulation of bile acid concentration, signal transduction, inflammation amphoterin signaling, chemotaxis, development skeletal muscle with the genes *SCD, AKAP3, Myosin heavy chain (MyHC),* and *CD4* (Figure 5).

2.5 Functional enrichment analysis for skeletal muscle differential expression (SOY *vs* FO)

Some pathway maps detected (*p*-value <0.10) are shown in Table 5 with the corresponding *p*-value and DEG. The complete list of enriched pathway maps was showed in the Supplementary Table 3.

Table 5: Pathway maps enriched by MetaCore software (*p*-value <0.10) from the list of differentially expressed genes (FDR 10%) in the skeletal muscle of pigs fed with different oil sources

Pathway Maps	*p*-value	DEG
TNF-α, IL-1 β induce dyslipidemia and inflammation in obesity and type 2 diabetes in adipocytes	1.165E-04	*AZGP1, PDE3B, APOE, Perilipin, LIPS*
Putative pathways for stimulation of fat cell differentiation by Bisphenol A	4.803E-04	*PPAR-gamma, TCF7L2 (TCF4), SCD, C/EBPalpha*
Signal transduction_WNT/Beta-catenin signaling in tissue homeostasis	1.367E-03	*TCF7L2 (TCF4), Tcf(Lef), WNT, PPCKC*
Adiponectin in pathogenesis of type 2 diabetes	4.446E-03	*SCD, PPCKC, ACOX1*
Role of IL-6 in obesity and type 2 diabetes in adipocytes	5.884E-03	*PPAR-gamma, Perilipin, LIPS*
Dysregulation of Adiponectin secretion from adipocytes in obesity, type 2 diabetes and metabolic syndrome X	9.516E-03	*PPAR-gamma, IL-18, C/EBPalpha*
Regulation of metabolism_Bile acids regulation of glucose and lipid metabolism via FXR	1.173E-02	*APOE, SCD, PPCKC*
Transport_HDL-mediated reverse cholesterol transport	1.252E-02	*APOE, CES1, SR-BI*

The genes zinc α-2-glycoprotein 1 (*AZGP1*), phosphodiesterase 3B (*PDE3B*), apolipoprotein E (*APOE*), Perilipin 1 (*PLIN1*), and lipase E hormone sensitive type (*LIPS*) were identified as DEG. The *AZGP1* gene, a lipid-mobilizing adipokine, was identified with lower expression (log2-fold change -3.79) in pigs from the SOY group. The cited genes were enriched in the "*TNF-α* pathway, *IL-1* β induces dyslipidemia and inflammation in obesity and type 2 diabetes in adipocytes" pathway (Figure 6). In addition, *APOE* was enriched in "regulation of metabolism: Bile acids regulation of glucose and lipid metabolism via FXR" (Supplementary Figure 19) and "transport HDL-mediated reverse cholesterol transport" (Supplementary Figure 20) pathways.

Another DEG that may be inhibited by *TNF*-α is *Perilipin 1*, which presented lower expression (log2-fold change -2.99) in the SOY group. *APOE* showed lower expression (log2-fold change -2.10) in groups fed a diet containing SOY. Such as *Perillipin 1*, *LIPS* (log2-fold change -1.30) and *PPARG* (log2-fold change -1.92) were identified with lower expression in the SOY group. The second one, *PPARG* was enriched for the "putative pathways for stimulation of fat cell differentiation by Bisphenol A" (Supplementary Figure 21); the "role of IL-6 in obesity and type 2 diabetes in adipocytes" (Supplementary Figure 22); and the "dysregulation of adiponectin secretion from adipocytes in obesity, type 2 diabetes and metabolic syndrome X" (Supplementary Figure 23) pathways, the last two pathways together with the CCAAT enhancer binding protein alpha (*C/EBP-alpha,* log2-fold change -2.39).

The *SCD* gene was identified as a DEG with lower expression (log2-fold change -1.6) in the SOY group, and was enriched in the "putative pathways for stimulation of fat cell differentiation by Bisphenol A", "adiponectin in pathogenesis of type 2 diabetes", and "regulation of metabolism: Bile acids regulation of glucose and lipid metabolism via FXR" pathways. The transcription factor 7 like 2 (*TCF7L2*) was identified as the DEG with the lowest expression (log2-fold change -0.96) in the SOY group. In addition, *TCF7L2* participates in the "signal transduction_*WNT/Beta-catenin* signaling in tissue homeostasis" pathway (Supplementary Figure 24). *C/EBPalpha* was identified as DEG in our study with the smallest expression (log2-fold change -2.4) related to the SOY group. *C/EBPalpha* was enriched for the "putative pathways for stimulation of fat cell differentiation by Bisphenol A" pathway.

The analysis of process networks was performed using MetaCore software (Figure 7) to better understand the observed interactions of DEG in gene networks. Networks related (*p*-value <0.1) to muscle contraction (*p*-value 2.429E-06), "Regulation of angiogenesis" (*p*-value 7.863E-03), and "immune response" (*p*-value 1.878E-02) were identified herein. Some DEG were identified enriched in the networks like the *GPCR* in the "chemostaxis" network; *PPARG, APOE, MELC, PACAP* receptor 1 in the "development neurogenesis axonal guidance" network; *APOE, MELC, Actin, C/EBP, SR-BI,* among others, in the "immune response phagocytosis" network.

3. Discussion

Fatty acid profile and blood parameters: We observed with the analysis of the intramuscular lipid composition, that the addition of FO in the diet of growing and finishing pigs changed the FA profile

composition mainly for C20:5 n-3, C22:6 n-3, and SFA content, as C16:0 and C18:0. The total SFA also showed a higher content of pigs that were fed with FO. According to Jeromson et al. [20], diets rich in SFA may be associated with the onset of obesity and type 2 diabetes. In the other hand, pigs fed FO demonstrated the lowest n-6:n-3 PUFA ratio, thus showing the potential of the diet in modifying pork composition through FA modulation in the pig diet [21]. Our findings corroborate with Jeromson et al. [20], which reported that skeletal muscle may be manipulated according to changes in the animal diet.

In our study, we observed higher content of n-3 PUFA in the FO group. The n-3 PUFA has been linked to protection against chronic and metabolic diseases, whereas omega 6 (n-6) PUFA may be related to inflammation, blood vessel constriction, and aggregation. Therefore, the proportion of these PUFA and the dietary LA and ALA content may be relevant to the regulation of bodily homeostasis inflammation and anti-inflammatory effect [22]. The modification of pork FA can show us pathways and networks related to both inflammation and relevant diseases and metabolic processes. Moreover, the amount and type of fat play a key role in regulating the metabolic health of the whole body, FA are components of cell membranes, act as signaling molecules, and can change related to the muscle lipid pool, thus can modify both metabolic and physical function of the skeletal muscle [20].

According to Saini and Keum [22], n − 3 and n − 6 PUFA have opposite effects on the metabolic functions of the body, so they are important to be analyzed. Myristic acid, involved in the increase in plasma cholesterol concentration has a harmful cardiovascular effect in humans [23].

According to Corominas et al. [24] modifications related to the replacement of SFA by MUFA or PUFA decreases serum LDL-cholesterol and total cholesterol, in our study we did not observe a difference in serum LDL-cholesterol and total cholesterol in the groups fed with FO and SOY compared to the CO group. For FO diet, we observed higher total PUFA composition in the liver and in LL intramuscular FA, with higher deposition of total n-3 PUFA. The analysis of blood parameters is essential, mainly referring to serum lipids that are related to cardiovascular diseases and obesity-related metabolic diseases [25].

The CO diet showed the highest concentration of HDL, involved in the elimination of excess cholesterol, despite the greater deposition of total cholesterol and no statistical difference between LDL and the other oils used (SOY and FO). Evidence supporting the benefits of CO is related to the positive effects of MUFA compared to SFA effects. Moreover, in addition, this oil influences biological functions that affect several other disease risk biomarkers [26]. In our study we identified a greater amount of OA and consequently MUFA in animals of the CO group. The results of a systematic review and meta-analysis [27] on the effect of CO in humans showed that CO consumption could decrease serum LDL compared to sunflower oil and saturated fat, although it did not change significantly HDL, different from that the results obtained in our study. Despite these findings, the exact mechanisms of CO on serum lipids are still not well understood [27].

Canola oil has a high OA content, which leads to greater beneficial health effects compared to other oils, where a previous study showed that diets with higher oleic acid content increase HDL blood level [28]. Herein, we observed that our results corroborate with the previous study, the CO and SOY groups

presented higher OA content in the skeletal muscle and HDL blood level compared with FO group. The HDL regulates the level of transcription initiation by transcription factors, is related to post-transcriptional regulation through microRNAs and other non-coding RNAs, among other mechanisms in various processes [29]. Despite the CO group presenting a higher total blood cholesterol, an atherogenic factor, most of this was represented by HDL, which has an antiatherogenic effect and is involved in the decreased occurrence of inflammatory injury and oxidative stress [30].

The atherogenic index is related to predicting the risk of developing atherosclerosis and coronary heart disease. In addition, it is related to dyslipidemia, which is characterized by high plasma concentration of triglycerides and total cholesterol, HDL, LDL [31]. In our results the highest serum concentration of cholesterol was found in the CO group and the atherogenic index showed a low concentration in CO and SOY groups compared to FO. Previous studies have already linked CO to interference in cholesterol metabolism due to phytosterols that can inhibit cholesterol esterase enzymes reducing intracellular cholesterol esterification [27].

Transcriptome modulation in response to FA

The transcriptome analysis showed that the modification of FA source impacted gene expression between the comparisons. About the CO and SOY diets, led to similar deposited FA profiles, and both diets presented higher levels of cholesterol and HDL when compared to the FO group, in the transcriptomic comparison resulted in several DEG and important pathways related to lipid metabolism. Within the identified pathways there were "fatty acid omega oxidation", “leukotriene 4 biosynthesis and metabolism”, and “triacylglycerol metabolism”, with *AL3A2* DEG down-regulated in the CO group.

Among the DEG identified in the comparisons CO *vs* SOY and CO *vs* FO, the *AL3A2* gene was identified down-regulated in the CO group for both diets. The encoded enzyme AL3A2 participates in the oxidation of long-chain aliphatic aldehydes to FA [32], possibly being related to the oxidation of 12-oxo-dodecanoic acid to dodecanedioic acid. Furthermore, the aldehyde dehydrogenase family may play a relevant role in the function and induction of regulatory T cells, cellular detoxification, and amino acid metabolism [33]. Moreover, it is important to the detoxifying role in the ethanol-caused accumulation of free FA and triacylglycerol, ethanol inhibition of lipoprotein export, increasing FA uptake and lipid peroxidation [34]. Thus, the consumption of SOY and FO may be related to the improvement of lipid oxidation rate when compared to the CO group. In our previous study, similar results were observed in the liver with comparison of different proportions of SOY, in which the increase of SOY in the pig diet (3.0% of SOY) resulted in a possible improvement of the lipid oxidation rate [35]. Studies are still needed to understand the specific functions of *AL3A2* [35]. Also, in the CO *vs* SOY comparison, the *AL3A2* was enriched in the “leukotriene 4 biosynthesis and metabolism” pathway. This pathway is important because leukotrienes are pro-inflammatory mediators. Leukotriene B4 (LBT4) is a lipid mediator derived from arachidonic acid and plays a relevant role in chronic inflammatory diseases such as arthritis, cardiovascular diseases, cancer, and metabolic disorders. In studies using fibroblasts, the LBT4 was increased in obese adipose tissue, contributing to obesity [36–38]. Leukotriene B4 (20-Carboxy-LTB4) can undergo more beta

oxidation, which can be directly impacted by the enzyme *AL3A2*, which in the CO group has lower expression. In addition, *AL3A2* was enriched in the “triacylglycerol metabolism” pathway. In muscle, FA are a substrate for oxidation, producing triacylglycerol that will later be secreted as very low-density lipoprotein. There is a cooperation among different tissues, mainly the adipose, liver, and skeletal muscle tissues, so if there is an accumulation of triacylglycerol in skeletal muscle and liver it will probably result in insulin resistance [39]. Furthermore, *AL3A2* has been linked to Sjögren-Larsson syndrome, as an interruption of *AL3A2* function causes fat accumulation in cells, underscoring the importance of this enzyme in detoxification in various lipid degradation pathways [35]. The *AL3A2* was also identified in the “oxidative stress in adipocyte dysfunction in type 2 diabetes and metabolic syndrome X” pathway. Lower expression levels of *AL3A2* and *GSTA4* may result in attenuation of the lipid peroxide elimination system in obese and insulin-resistant humans, increasing the levels of 4-Hydroxy-2(E)-nonenal, which, when binding xanthine oxidase, increases oxidative activity, and consequently, the additional generation of intracellular reactive oxygen species (ROS) [40–42]. When pigs were fed SOY, there was a higher expression of *AL3A2*, which may be involved in the increase of the lipid peroxide elimination system. *AL3A2* have cell-specific functions associated with inflammation, differentiation, or oxidative stress responses in which the CO group may be targeting a lower incidence of the inflammatory process [33, 43].

In this study, we observed that the enriched pathways in the CO *vs* SOY and CO *vs* FO comparisons corroborate the relationship of SHP transcription factor (CO *vs* SOY) and DEG *SCD* (CO *vs* FO) to HDL in "regulation of lipid metabolism: RXR-dependent regulation of lipid metabolism via PPAR, RAR, and VDR" pathway. As well as between the comparison SOY *vs* FO with the enrichment of the pathways “regulation of metabolism: Bile acids regulation of glucose and lipid metabolism via FXR”, and “transport_HDL-mediated reverse cholesterol transport”. Due to the negative regulation of some identified DEG that participate in these pathways and are directly related to HDL functions, they may have caused an increase in cholesterol as observed.

In the study performed by Puig-Oliveras et al., [44], *SCD* was enriched in FA divergent pigs related to oxidation, accumulation, concentration, and lipid homeostasis. In this study, SCD was identified as down-regulated in the adipose tissue of animals with higher PUFA content in Iberian pigs x Landrace, and with greater expression in animals that showed greater accumulation of intramuscular fat [45]. In addition, *SCD* affected meat quality traits in the population of pigs analyzed by Piórkowska [45], in a work evaluating mutations in candidate genes. The *SCD* DEG is also present in the “putative pathways for stimulation of fat cell differentiation by Bisphenol A” pathway (SOY *vs* FO) was down-regulated in the SOY group. We hypothesize that it is related to the biosynthesis of FA, mainly the synthesis of oleic acid, fundamental in the regulation of the expression of genes that are involved in lipogenesis [32]. In a study evaluating some candidate genes from commercial crossbred pigs (Shanzhu × Duroc), *SCD* expression levels showed a correlation with intramuscular fat content [46]. Our findings indicate that even with a higher expression of *SCD*, the diet with the addition of FO did not present a higher amount of OA when compared to the other diets analyzed.

In the “putative pathways for stimulation of fat cell differentiation by Bisphenol A” other DEG (*TCF7L2 and C/EBPalpha*) were enriched for SOY *vs* FO and may be related to a direct effect or mechanism on *PPARG.* In addition, *PPARG* (down-regulated in SOY group) was enriched in "role of IL-6 in obesity and type 2 diabetes in adipocytes", and "dysregulation of adiponectin secretion from adipocytes in obesity, type 2 diabetes and metabolic syndrome X". Studies have identified the relationship of *PPAR* to nutrition and involvement in skeletal muscle, such as the study from Yu et al. [47] evaluating a diet high in saturated fat (beef tallow) or high in unsaturated fat (fish oil) in transgenic mice, which resulted in the decreased deposition of lipids in the liver by PUFA, and the ability to stimulate the expression of adipogenic genes and glucose metabolism genes in *PPARG* transgenic mice when fed FO. Another study in mice identified that *PPARG* knockout in muscle affected insulin sensitivity in skeletal muscle [48]. Furthermore, there is an important involvement of *PPARG* in the direct regulation of lipid metabolism in immune cells [49] and may be related to adipogenesis [50]. The *PPAR* form heterodimers with retinoid X receptors (RXRs) and these heterodimers regulate the transcription of several genes [32]. Thus, in the FO group there seems to be a direction to be implicated in inflammatory processes due to the PPARG functions related to the pathology of diseases, such as obesity, diabetes, and also atherosclerosis and cancer.

Another group of enriched DEG were related to pathways involved in obesity-associated metabolic diseases, in which a higher expression of these genes was identified in the FO group. *AZGP1, APOE, Perilipin, LIPS*, and *PDE3B* (down-regulated in SOY group) were enriched in the “TNF-α pathway, IL-1 β induce dyslipidemia and inflammation in obesity and type 2 diabetes in adipocytes”. This signaling pathway is related to the overexpression of TNF-α and IL-1 β, which in obese adipose tissue activate *NF-KB* and *EKR*, impairing genes with important functions. Obesity predisposes to insulin resistance, type 2 diabetes, and cardiovascular problems [51]. The cytokines TNF-α, and IL1 β are considered the main regulators of inflammation involved in the pathogenesis of type 2 diabetes mellitus [52]. TNF-α inhibits *PDE3B* leading to the accumulation of Cyclic AMP [53]. In this some pathway, TNF-α also inhibits both *LIPS* inducing lipolysis, as well as *AZGP1* and *APOE* that are underexpressed in obese tissue and may promote the development of dyslipidemia [51, 54]. Thus, in the TNF-α pathway, the diet with FO leads to a greater expression of important genes in normal functions in the body that, if inhibited by TNF-α, can be relate to inflammation.

The DEG *AZGP1* (SOY *vs* FO) was identified as DEG and is related to the stimulation of lipid degradation in adipocytes, in addition to be involved in extensive fat loss in some advanced cancers [32]. The *PLIN1* belongs to the *Perilipin* protein family and was differentially expressed in *Longissimus* muscle regulating the deposition of the intramuscular fat content of commercial hybrids (Pietrain × Duroc) × (Landrace × Yorkshire) [55]. In the study performed by Li et al. [55], the authors suggested that a *PLIN1* knockdown can decrease the level of triglycerides and lipid droplet size in adipocytes. In Gandolfi et al. [56] study, *Perilipin1* and *Perilipin2* were expressed in the semimembranosus muscle of commercial crossbred male castrated pigs and the specificity of the antibodies was confirmed by Western blot analysis [56].

Another DEG identified was *APOE* (SOY *vs* FO), a protein associated with lipid particles that is essential for the normal catabolism of the constituents of triglyceride-rich lipoproteins, and is involved in the immune innate adaptive responses. In addition, it participates in the biosynthesis of VLDL by the liver [32]. Dysfunctions in APOE protein can result in familial dysbetalipoproteinemia (type III hyperlipoproteinemia), and consequently, atherosclerosis [57]. Using the CRISPR/Cas9 technique, Fang et al. [57] interrupted *APOE* expression in miniature pigs fed an induction diet high in fat and cholesterol, revealing that the animals had severe hypercholesterolemia and developed progressive atherosclerotic lesions. Additionally, *APOE* may be involved in the "transport HDL-mediated reverse cholesterol transport" pathway, in which *APOE* increases HDL binding to SRBI and selective cholesteryl ester uptake [58]. In FO group it was up-regulated, *APOE* helps in the normal function of parameters such as VLDL even though no statistical difference was observed in our results. Finally, in a study performed by Song et al. [59], in which the animals were fed a diet with an n-6:n-3 PUFA ratio of 4:1 or 2:1, *LIPS/HSL* showed a higher relative gene expression compared to the control group with an 18:1 ratio. In our study, the pattern obtained was similar, in which the FO diet showed a lower n-6:n-3 PUFA ratio and consecutively higher expression of *LIPS/HSL* when compared to SOY diet.

The importance of enriched DEG that are involved in regulatory pathways and networks related to bile acids that regulate glucose and lipid metabolism is noted. In relation to the processes networks enriched, we identified networks related to muscle contraction with DEG down-regulated from the SOY group, demonstrating that SOY negatively regulated muscle contraction genes compared to animals from the FO group.

We identified (FDR 10%) 65 DEG between CO *vs* SOY, with genes involved in the improving of lipid peroxide elimination system in SOY group; 531 DEG in the comparison SOY *vs* FO; and 32 DEG for CO *vs* FO with genes related to lipogenesis regulation in FO. In general, the main processes involved are related to lipid metabolism, metabolic diseases, and inflammation. In addition, the different oils modified the cholesterol and HDL levels, with a lower amount in the FO group. The CO and SOY have a high OA content, which leads to a greater beneficial health effect, in addition to having a higher amount of HDL when compared to FO group. More studies are needed to understand the genes directly related to each of the fatty acids and their association with blood parameters.

4. Conclusion

The enrichment of the basal diet with oil containing different FA profiles influenced FA profile, blood parameters, and gene expression in the skeletal muscle of pigs. Diets containing CO and SOY showed higher OA content, and higher amount of HDL when compared to FO group. Conversely, they also had a higher amount of cholesterol. The FO showed a higher amount of EPA, DHA, total n-3 PUFA, and genes related to the regulation of lipogenesis. The current study provided important details and relationships between relevant genes in lipid metabolism, contributing to human health as the pig is an excellent animal model. The results provide mechanisms to help us understand the behavior of concerning different FA profile.

5. Methods

All animal procedures were conducted in accordance with the Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching [60] and were approved by the Animal Care and Use Committee of Luiz de Queiroz College of Agriculture (University of São Paulo, Piracicaba, Brazil, protocol: 2018.5.1787.11.6 and number CEUA 2018-28).

5.1 Animals and diets

Fifty-four genetically purebred male pigs (offspring of Large White sires x Large White dams) with 71 ± 1.8 days of age and initial body weight (BW) of 28.44 ± 2.95 kg were allocated into one of three dietary treatments in a randomized complete block design with six replicate pens per treatment and three pigs per pen. Pigs were housed in an all-in/all-out double-curtain-sided building. Each pen was equipped with a three-hole dry self-feeder and a nipple drinker which allowed *ad libitum* access to feed and water throughout the experimental period (98 days). All pigs were halothane *(RYR1* gene) homozygous-free (NN) by molecular test and immunecastrated through administration of two 2-mL dose of Vivax® (Pfizer Animal Health, Parkville, Australia) on fatting day 56 (127 days of age) and fatting day 70 (141 days of age), in accordance with the manufacturer's recommendations.

The experimental diets were modified according to growing and finishing phases, where day 0 represents the start of the trial when pigs averaged 71 days of age: day 0 to 21 for grower I diets, day 21 to 42 for grower II, day 42 to 56 for finisher I, day 56 to 63 for finisher II, day 63 to 70 for finisher III, and day 70 to 98 for finisher IV diets [18]. Dietary treatments consisted of corn-soybean meal growing-finishing diets I, II, III and IV, supplemented with 3% canola oil (CO) or 3% fish oil (FO) or 3% soybean oil (SOY). Diets were formulated to meet or exceed Rostagno [61] recommendations for growing-finishing pigs. No antibiotics were used, and all diets were provided in a mash meal form.

5.2 Fatty acid profile of samples

5.2.1 Sample collection and FA profile

After 98 days on trial, all pigs were slaughtered (average final body weight of 133.9 ± 9.4 kg, and 155 days of age on average), and *Longissimus lumborum* muscle samples were collected. The tissue samples were quickly excised, snap-frozen in liquid nitrogen, and then stored at -80^{o}C until further analyses.

The FA profile determination was performed from the total lipid isolated from 100 g of the *Longissimus lumborum* samples using the cold extraction method by Bligh and Dyer (1959) and methylated according to the procedure outlined by AOCS (2004; Method AM 5 – 04). A full description of the analyses can be found in our previous study [18].

5.2.2 Blood parameters

Four days before slaughter, blood was sampled from the jugular vein and immediately transferred into serum separating vacuum tubes (Becton Dickinson Vacutainer Systems, Franklin Lakes, NJ, USA) for the blood parameters analysis. Then, the samples were stored at room temperature for 2 hours to allow for coagulation then centrifuged at 3,000 × g for 10 min to obtain serum that was stored in duplicated 1.5-mL tubes at − 80°C. Serum lipid and others parameters were analyzed by using the Mindray BS-120 chemistry analyzer (Guangdong, China) in the Pathology Laboratory at the University of São Paulo, Pirassununga, SP, Brazil. Blood serum glucose content was quantified by the colorimetric enzymatic method according to Trinder [62] using the commercial kits (VIDA Biotecnologia S/A, Minas Gerais, Brazil), following the manufacturer's protocol. The quantification of total cholesterol and fractions was performed by enzymatic-colorimetric method, by selective precipitation using commercial kits (Gold Analisa Diagnóstica Ltda, Belo Horizonte, Minas Gerais, Brazil), according to the manufacturer's instructions. The analysis for the determination of total proteins was performed using commercial kits (VIDA Biotecnologia S/A, Lagoa Santa, Minas Gerais, Brazil), following manufacturer's protocol with modifications described by Gornall; Bardawill, and David [63].

5.2.3 Statistical analyses

Statistical analyzes to verify the differences in the FA profile of skeletal muscle between the diets were performed using the MIXED procedure of the SAS statistical software (SAS Inst. Inc., Cary, NC, v. 9.4), where a mixed model was fitted using restricted maximum likelihood (REML) methodology. In the model, the block effects were assumed as random effects and the dietary treatments as fixed effects. The UNIVARIATE procedure (v. 9.4) was used to test for divergence from a normal distribution with homogeneity of residuals for each of the variables. Diagnostics of the density distribution of the Studentized Residual of the model were made with the Shapiro-Wilk test (SAS v.9.4). Means were adjusted by using the LSMEANS statement. Differences were declared significant when p-value ≤ 0.05 based on the Tukey-test.

5.3 RNA extraction, library preparation and sequencing

Total RNA was extracted from muscle samples using the RNeasy® Mini Kit (Qiagen Hilden, Germany), according to the manufacturer's instructions. Total RNA quantification, purity, and integrity were evaluated by Nanodrop 1000 and Bioanalyzer. All samples presented an RNA Integrity Number (RIN) greater or equal to seven. From the total RNA of each sample, 2µg were used for library preparation according to the protocol described in the TruSeq RNA Sample Preparation kit v2 guide (Illumina, San Diego, CA). The Agilent Bioanalyzer 2100 (Agilent, Santa Clara, CA, USA) was used to calculate libraries average size, and the libraries were quantified using quantitative PCR with the KAPA Library Quantification kit (KAPA Biosystems, Foster City, CA, USA). Quantified samples were diluted and labeled by barcoding and pooled to be run in different lanes (five pools of all 36 samples each), using the TruSeq DNA CD Index Plate (96 indexes, 96 samples, Illumina, San Diego, CA, USA). All samples were sequenced across five lanes of a sequencing flow cell, using the TruSeq PE Cluster kit v4-cBot-HS kit (Illumina, San Diego, CA, USA), and were clustered and sequenced using the HiSeq2500 equipment (Illumina, San Diego, CA, USA) with a TruSeq SBS Kit v4-HS (200 cycles), according to manufacturer's instructions. All the

sequencing analyses were performed at the Genomics Center at ESALQ, localized in the Animal Biotechnology Laboratory at ESALQ – USP, Piracicaba, São Paulo, Brazil.

5.4 RNA-sequencing, quality control, and alignment

The quality control and alignment processing was previously described [64]. Briefly, sequencing adaptors and low complexity reads were removed in an initial data filtering step by Trim Galore 0.6.5 software. Reads with Phred score higher than 33 and a length higher than 70 nucleotides were kept after trimming. Quality control and reads statistics were estimated with FASTQC version 0.11.8 software [http://www.bioinformatics.bbsrc.ac.uk/projects/fastqc/]. *Sus Scrofa* 11.1 available at Ensembl [http://www.ensembl.org/Sus_scrofa/Info/Index] was used as the reference assembly. The abundance (*read counts*) of mRNAs for all annotated genes was calculated using STAR-2.7.6a [65].

5.5 Differentially expressed genes

Differentially expressed genes (DEG) between the pairwise comparisons of different diets (CO *vs* SOY, SOY *vs* FO and CO *vs* FO) were identified using DESeq2, available at Bioconductor open-source software for bioinformatics, using a multi-factor design [66]. Prior to statistical analysis, the read count data was filtered as follows: i) unexpressed genes were genes with zero counts for all samples, ii) very lowly expressed were genes with less than one read per sample on average; iii) rarely expressed genes that were not present in at least 50% of the samples. Unexpressed, very lowly expressed and rarely expressed genes were all removed from the analysis. Sire was fit as a factor in the multi-factor model. The cut-off approach performed to identify the DEG was Benjamini; Hochberg, [67] methodology, used to control false discovery rate (FDR) at 10% [68] according to previous studies and DESeq2 recommendations [66, 68].

5.6 Functional enrichment analysis

Between CO *vs* SOY the functional enrichment analysis by MetaCore software (Clarivate analytics) [69] was applied to identify the pathway maps from the list of 65 DEG, and SOY vs FO to identify the pathway maps from the list of 531 DEG (FDR 10%), and for CO *vs* FO the pathway maps from the list of 32 DEG.

The functional enrichment analysis of DEG (FDR < 0.10) was performed to obtain comparative networks by “analysis of a single experiment” using *Homo sapiens* genome annotation as background reference and a standard parameter of MetaCore software v.21.4 build 70700, filtering for the metabolic maps: energy metabolism, lipid metabolism, steroid metabolism; cardiovascular diseases: atherosclerosis; regulation of metabolism; nutritional and metabolic diseases, and nervous system diseases.

Declarations

Ethics approval

All animal procedures were conducted in accordance with the Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching [60] and were approved by the Animal Care and Use

Committee of Luiz de Queiroz College of Agriculture (University of São Paulo, Piracicaba, Brazil, protocol: 2018.5.1787.11.6 and number CEUA 2018-28). All procedures were carried in accordance with the ARRIVE guidelines.

Consent for publication

Not applicable.

Data Availability Statement

The dataset supporting the conclusions of this article is available in the in the European Nucleotide Archive (ENA) repository (EMBL-EBI), under accession PRJEB52629 [http://www.ebi.ac.uk/ena/data/view/PRJEB52629]. The original contributions presented in the study are included in the article/SupplementaryMaterial, further inquiries can be directed to the corresponding author.

Competing interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Author Contributions

All authors contributed to this study. Conceptualization, S.L.F., G.C.M.M., J.R., J.K., D.K., H.F., J.C.C.B., M.D.P., L.L.C., and A.S.M.C.; writing-original draft preparation, S.L.F. and A.S.M.C.; performed data analysis: S.L.F., V.V.A., F.A.O.F., and A.S.M.C.; interpretation and discussion of the results: S.L.F., G.C.M.M., V.V.A., L.L.C., H.F., L.C.A.R., A.L.F., A.S.M.C. critically reviewed and editing, B.P.M.S., J.D.G., V.V.A., B.S.V, J.A., G.C.M.M., J.R., J.K., D.K., D.G., H.F., L.C.A.R., G.B.M., L.L.C., S.M.A., A.L.F, and A.S.M.C.; supervision, A.S.M.C.; funding acquisition, A.S.M.C. Corresponding author: correspondence to Aline Silva Mello Cesar, alinecesar@usp.br.

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Figures

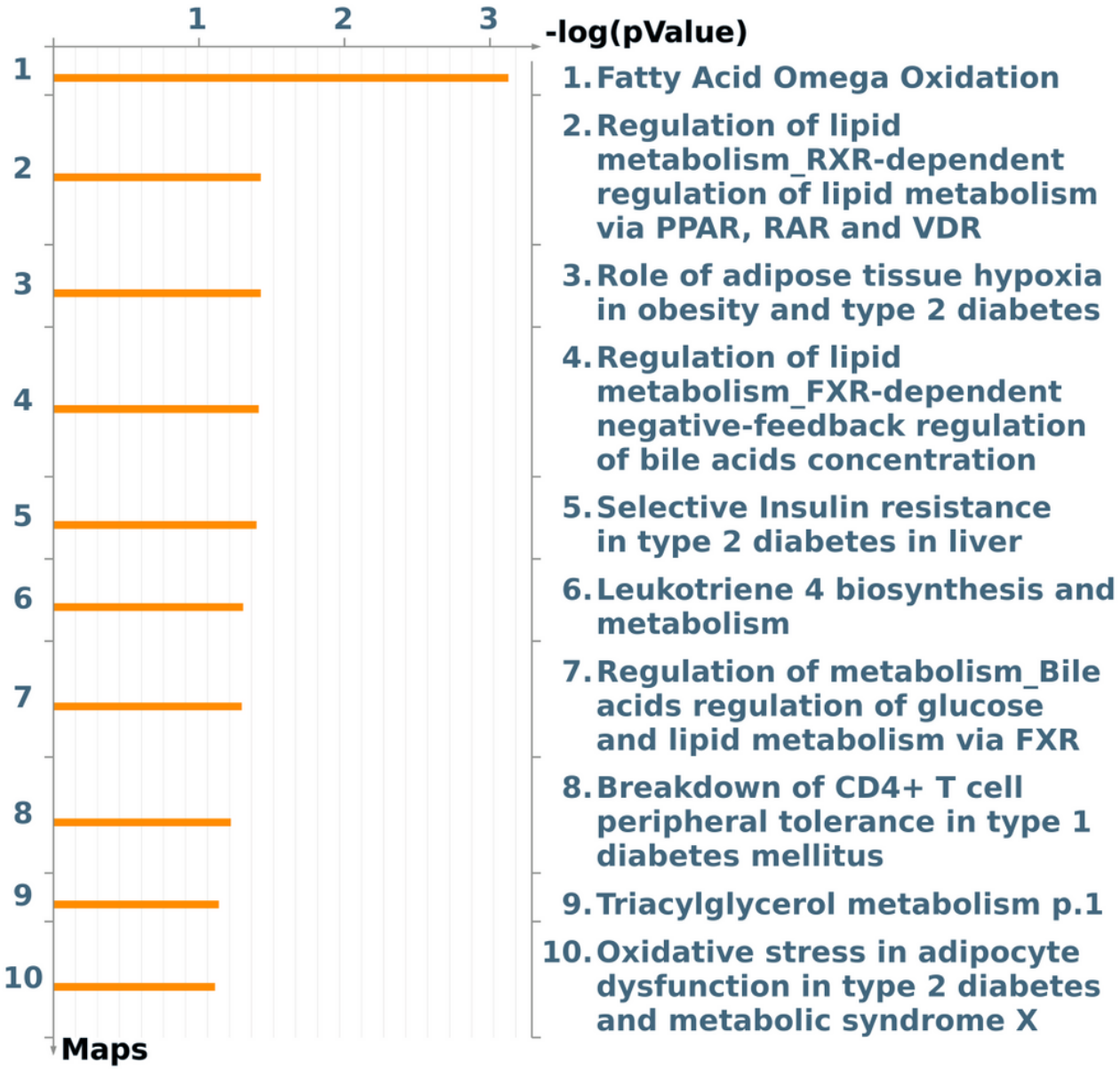

Figure 1

Pathway maps by MetaCore software (*p*-value <0.10) from the list of differentially expressed genes (FDR 10%) in the skeletal muscle of pigs fed with different oils (3.0 % canola oil *vs* 3.0% soybean oil).

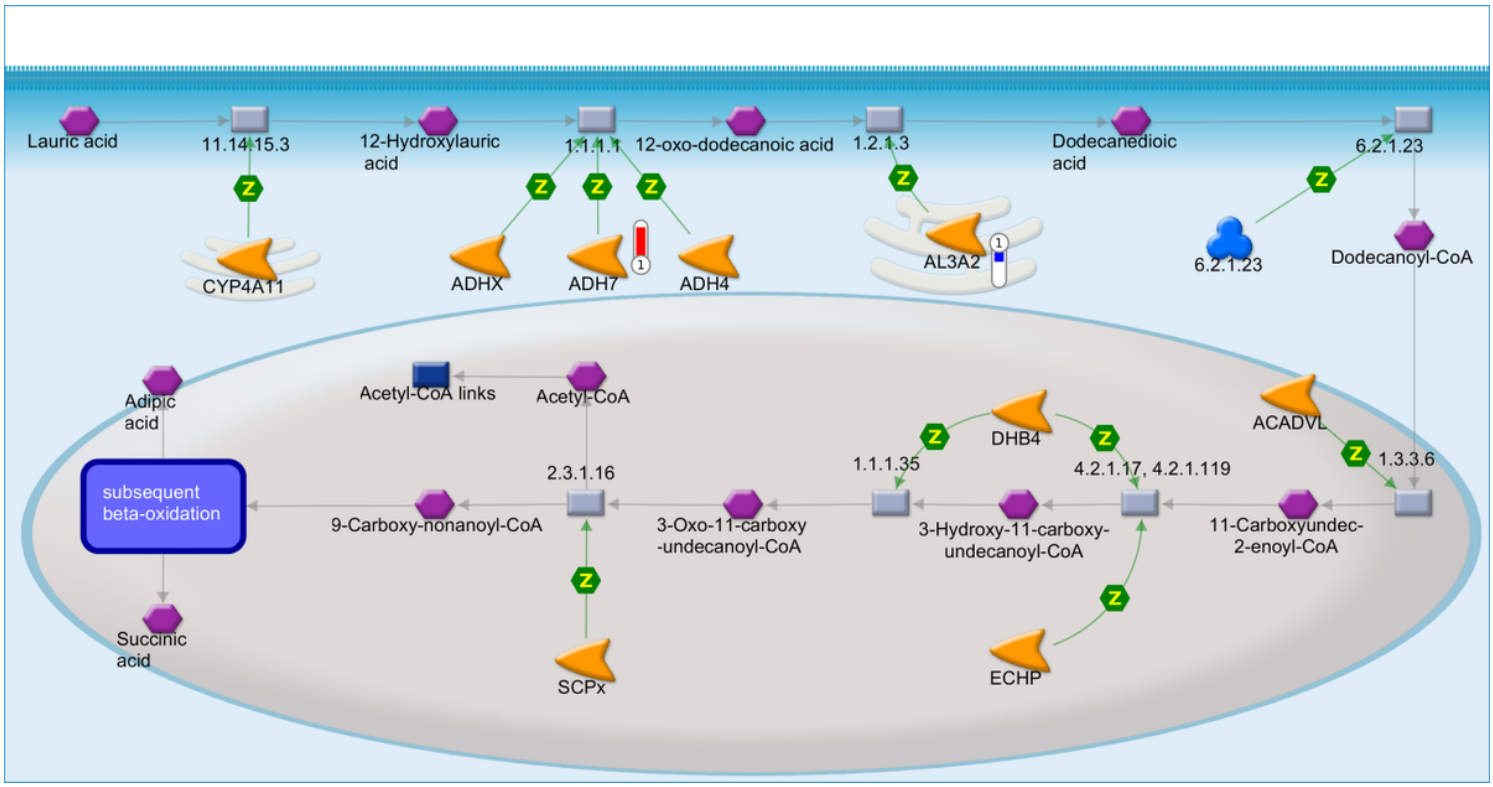

Figure 2

Fatty Acid Omega Oxidation pathway map by MetaCore software (*p*-value <0.10) from the list of differentially expressed genes (FDR 10%) in the skeletal muscle of pigs fed different oil sources in the diet (3.0 % canola oil and 3.0 % soybean oil). The blue thermometer indicates down-regulation of DEG (log2-fold change -0.79), and the red thermometer indicates up-regulation of DEG (log2-fold change +2.37) in the diet with 3.0 % of canola oil (CO). Green arrows indicate positive interaction and gray arrows indicate unspecified interaction. For a detailed definition, see

https://portal.genego.com/legends/MetaCoreQuickReferenceGuide.pdf.

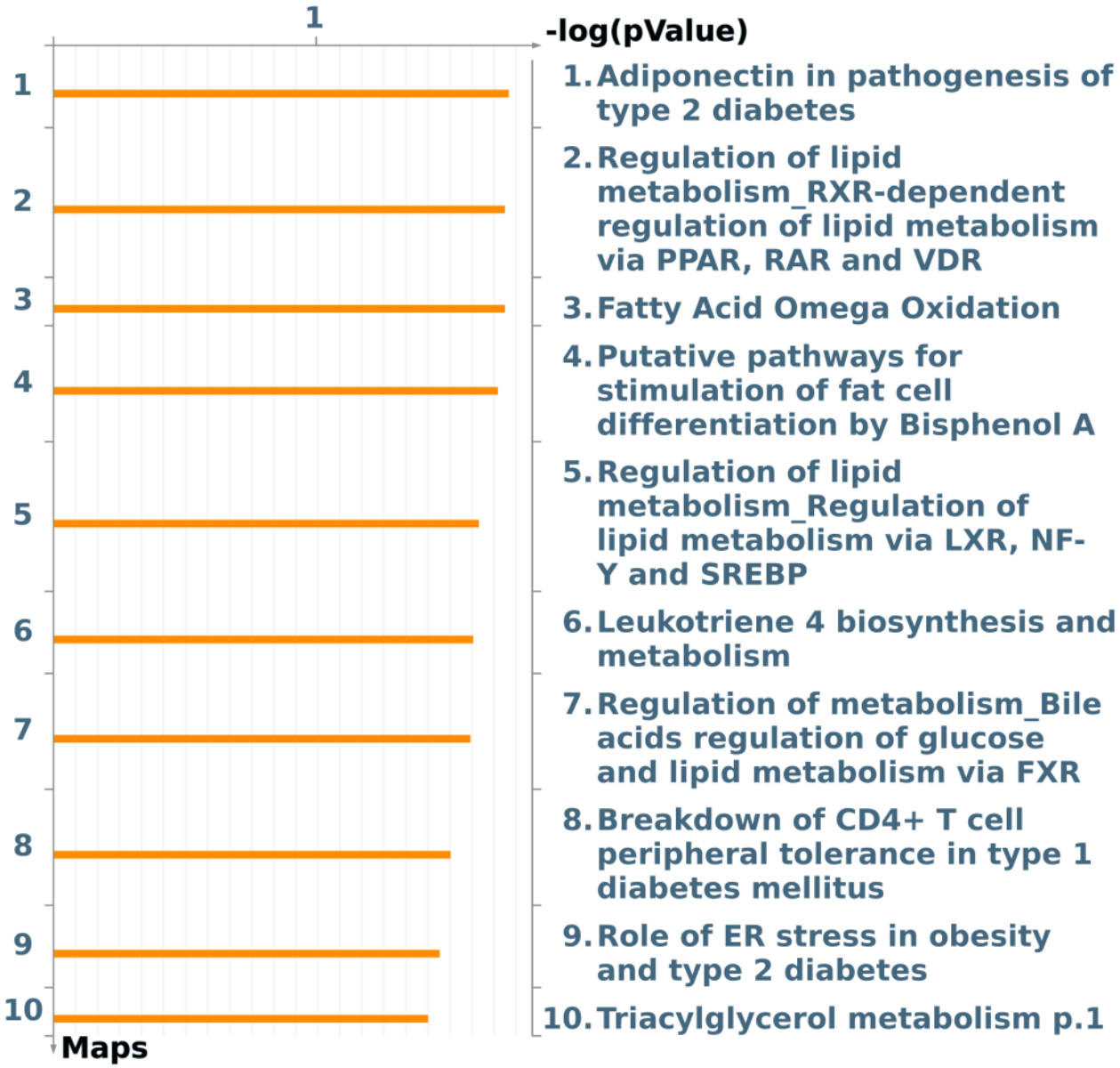

Figure 3

Pathway maps by MetaCore software (*p*-value <0.10) from the list of differentially expressed genes (FDR 10%) in the skeletal muscle of pigs fed with different oil (3.0 % canola oil *vs* 3.0% fish oil)

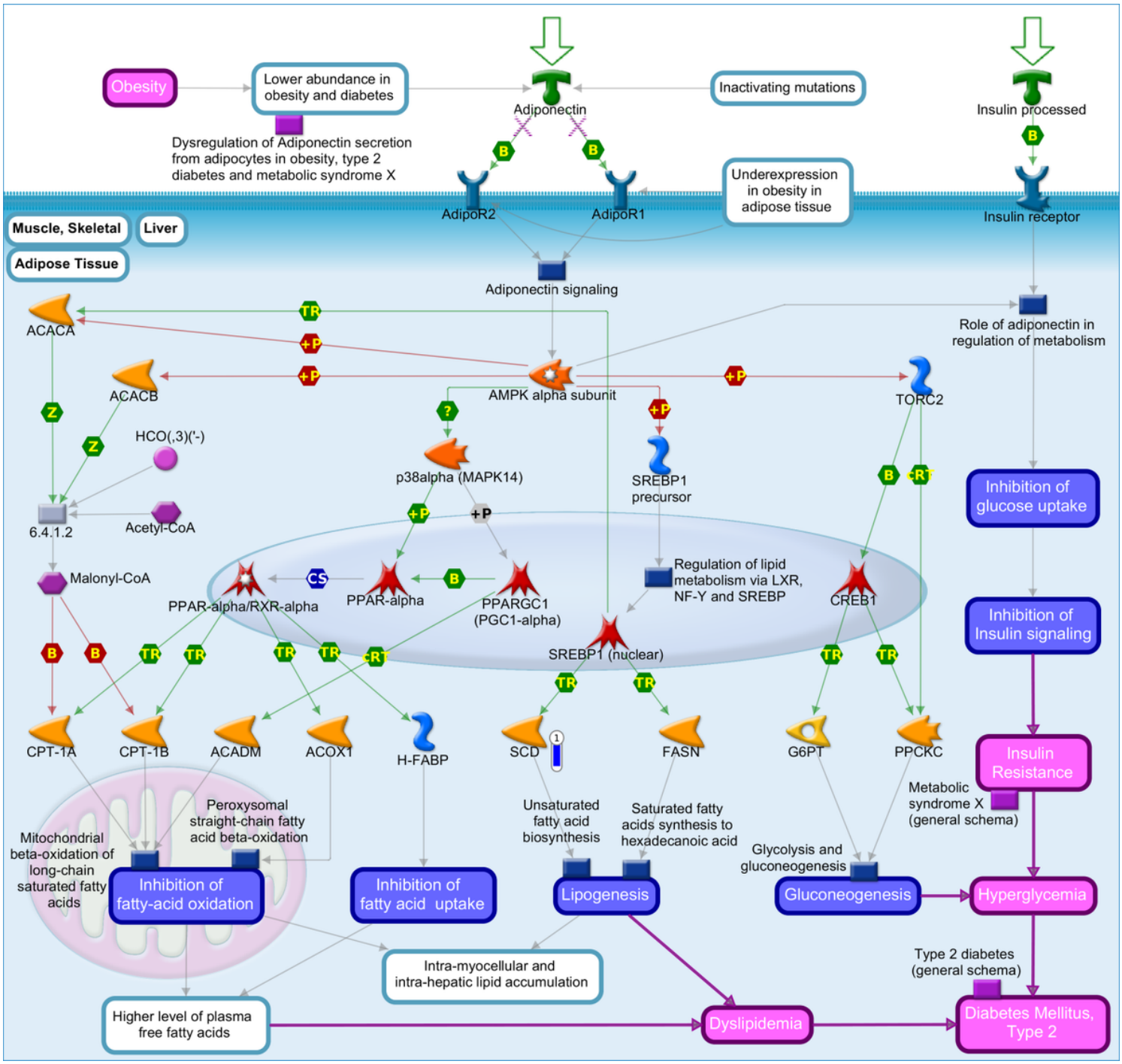

Figure 4

Adiponectin in pathogenesis of type 2 diabetes pathway map by MetaCore software (*p*-value <0.10) from the list of differentially expressed genes (FDR 10%) in the skeletal muscle of pigs fed different oil sources in the diet (3.0 % canola oil and 3.0 % fish oil). The large green arrows indicate path to start, blue thermometer indicates down-regulation of DEG in the diet with 3.0 % of canola oil (CO). Green arrows indicate positive interaction, red arrows indicate negative interaction (inhibition), gray arrows indicate unspecified interaction, and smaller blue boxes indicate normal process. For a detailed definition, see

https://portal.genego.com/legends/MetaCoreQuickReferenceGuide.pdf.

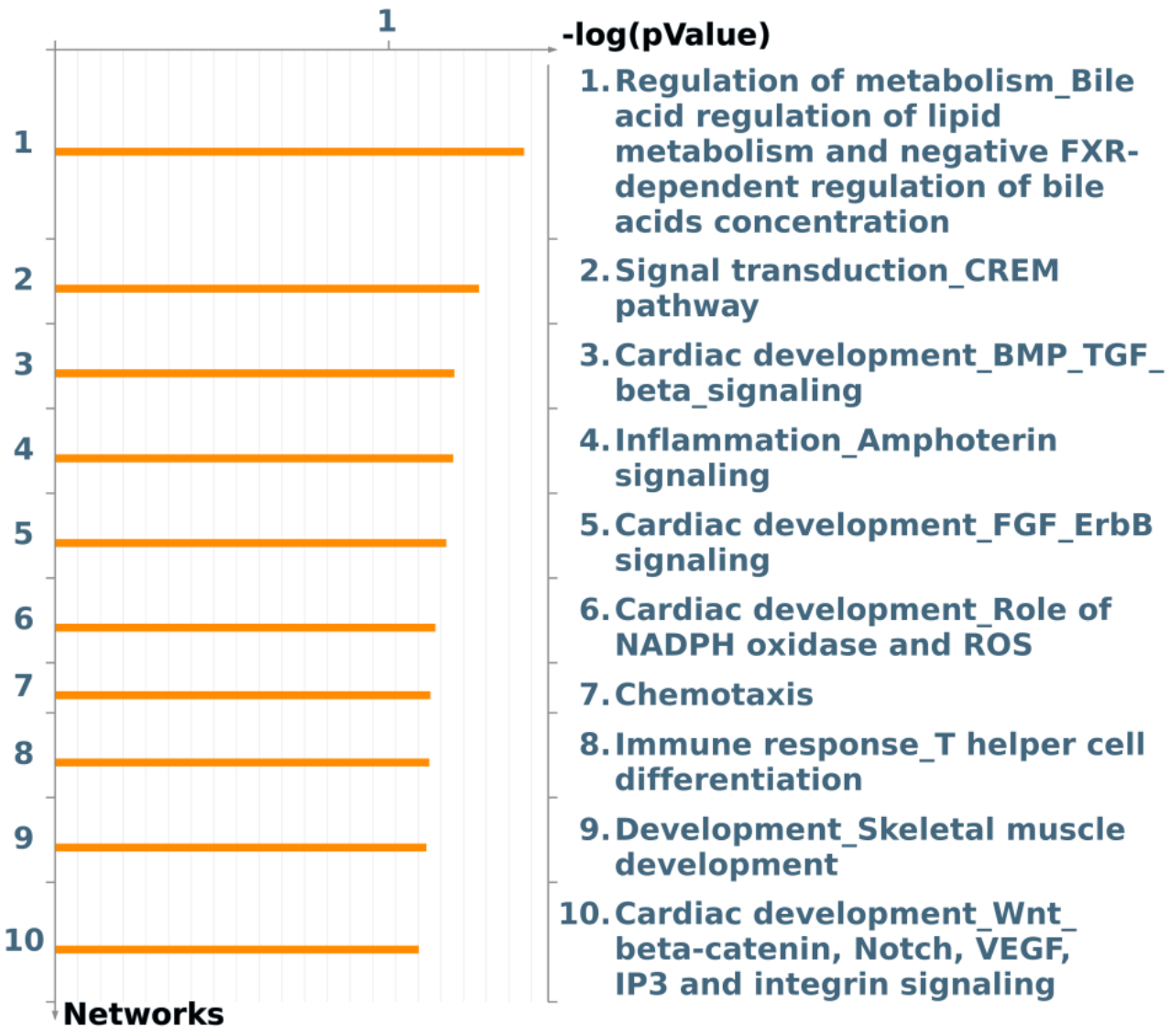

Figure 5

Top 10 enriched networks identified by MetaCore software from the list of differentially expressed genes (FDR 10%) in the skeletal muscle of pigs fed with different oils (3.0 % canola oil and 3.0 % of fish oil).

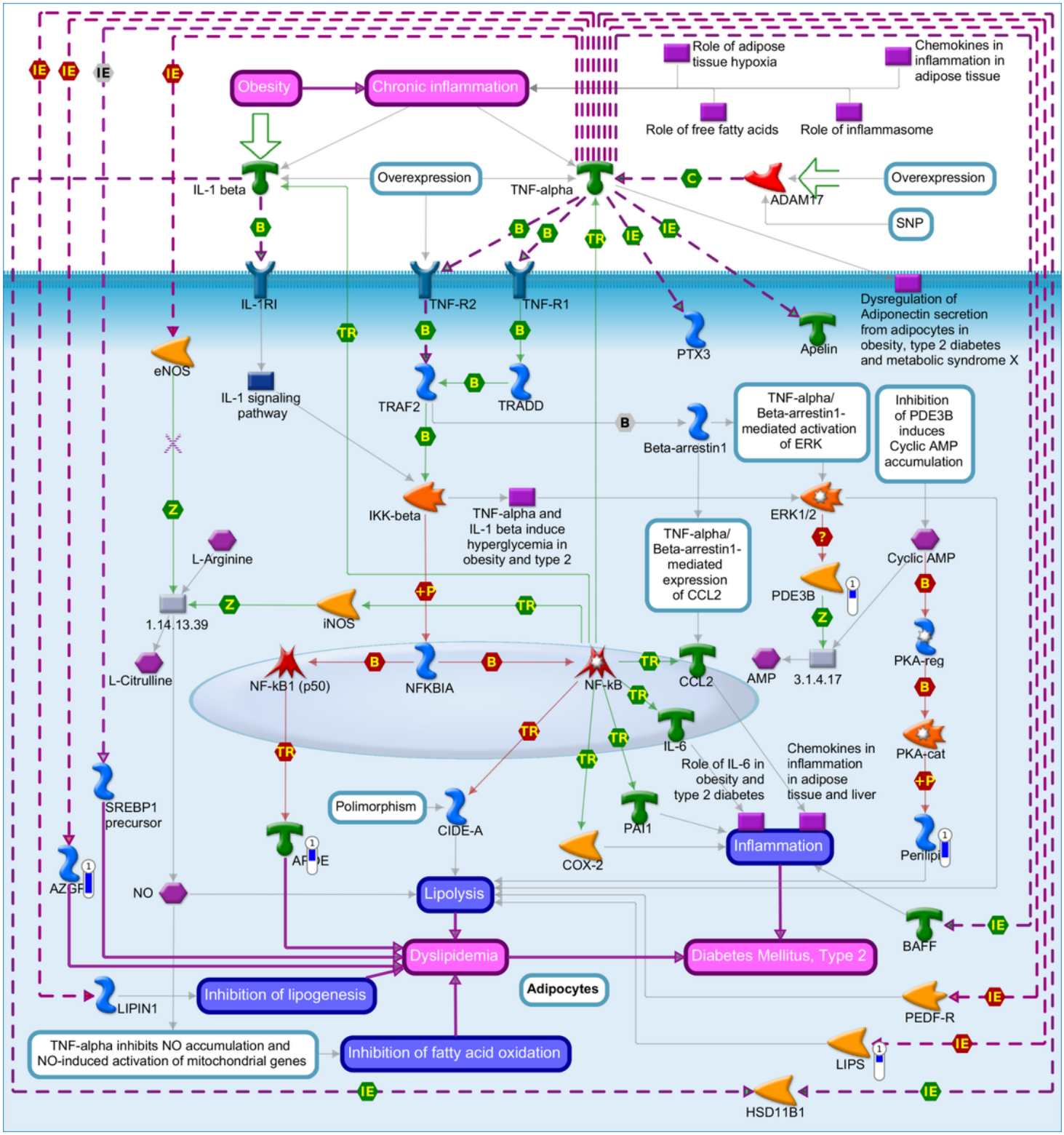

Figure 6

TNF-α, IL-1 β induces dyslipidemia and inflammation in obesity and type 2 diabetes in adipocytes pathway map by MetaCore software (*p*-value <0.10) from the list of differentially expressed genes (FDR 10%) in the skeletal muscle of pigs fed different oil sources in the diet (3.0 % soybean oil and 3.0% fish oil). The large green arrows indicate path to start, blue thermometer indicates down-regulation of DEG in the diet with 3.0 % of soybean oil (SOY). Purple lines indicate enhancement in diseases and purple dotted

line emerges in diseases. Green arrows indicate positive interaction, red arrows indicate negative interaction (inhibition), and gray arrows indicate unspecified interaction. For a detailed definition, see https://portal.genego.com/legends/MetaCoreQuickReferenceGuide.pdf.

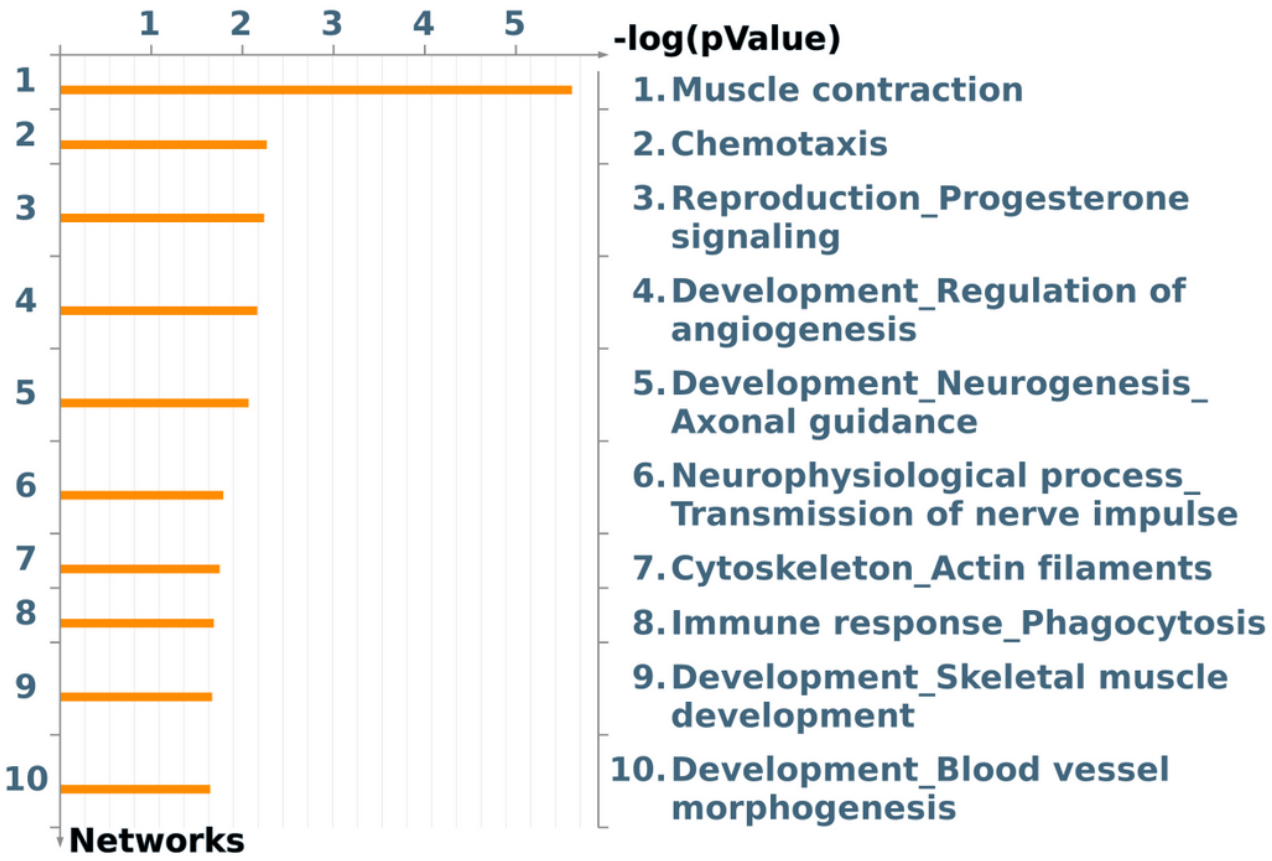

Figure 7

Top 10 enriched networks identified by MetaCore software from the list of differentially expressed genes (FDR 10%) in the skeletal muscle tissue of pigs fed with different oils (3.0 % soybean oil and 3.0 % of fish oil).

Supplementary Files

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- AdditionalfileTableS3figures.docx
- AdditionalfiletablesS1S2.xlsx