The Efffect of Gender Differences to Fat Deposition In Yaks Based on an Integrated Transcriptomic and Metabonomics Analysis

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Abstract

Background: The study of fat deposition in yaks of different gender plays a positive role in improving the meat quality and breeding of yaks. In this work, the characteristic of fat deposition in male and female yaks, and the regulations of gender difference to fat deposition in yaks were explored.

Result: Compared with male yaks, the female yaks possessed higher or more body fat rate (BFR) of visceral except liver, fat content in muscle and liver and subcutaneous fat ($p < 0.05$); the cholesterol (CH), triglyceride (TG), very low-density lipoprotein (VLDL), leptin (LEP) and cortisol (COR) in serum of female yaks were higher ($p < 0.05$); meanwhile, the fatty acid synthase (FAS), acetyl-CoA carboxylase (ACC), stearoyl-CoA desaturase (SCD) and diacylglycerol acyltransferase 1 (DGAT-1) in livers of female yaks were higher ($p < 0.05$). The metabonomics analysis showed 13(S)-HODE, linoleic acid and linolenic acid and myo-inositol in subcutaneous fat of female yaks increased ($p < 0.05$); meanwhile, the transcriptomic analysis the gene expressions of prenylcysteine oxidase 1 (PCYOX1), acyl-coenzyme a-cholesterol acyltransferase (ACAT), protein farnesyltransferase subunit beta (FNTB), apolipoprotein E (ApoE), DGAT2, very long chain fatty acids protein 6 (ELOVL6), NADP-dependent malic enzyme (ME1), SCD, acyl-CoA-binding protein (ACBP) and perilipin-5 (PLIN5), LEP, sterol regulatory element-binding protein 1 (SREBF1) upregulated in subcutaneous fat of female yaks ($p_{FDR} < 0.05$), whereas the gene expressions of CPT1, acetyl-CoA acyltransferase 1(ACAA1) and adipose triglyceride lipase (ATGL) downregulated ($p_{FDR} < 0.01$).

Conclusion: The fat and cholesterol synthesis increased in liver of female yaks, and the fat transport in blood of female yaks increased too. In subcutaneous fat of female yaks, the high levels of myo-inositol activated the calcium signaling, following the increase of secretion in fat cell. Then, the high levels of LEP and 13(S)-HODE strengthened PPAR signal, and the fat synthesis, cholesterol synthesis, fatty acid biosynthesis of the linoleic and linolenic acid being the precursor increased, whereas lipolysis and fatty acid oxidation decreased by the regulation of downstream genes in PPAR signal. Meanwhile, SREBF1 also played an important part in the regulation of fat metabolism in yaks of different gender.

Background

Fat plays an important part in the life activities of livestock [1], including confronting starvation, lactation and parturition [2, 3]. Meanwhile, as the core of the nutritional value of meat, the type and amount of fat are among those key factors which influence meat quality like appearance, flavor and tenderness [4–6], and is also essential to protect the carcass during cooling, avoiding cold shortening, drip loss and dark cutting [7–10]. As we all know, liver and adipose tissue are the major site of fat metabolism in livestock, whereas abundant fat only deposited in adipose tissue of livestock under normal physiologic condition at last [11].

Yak (*Bos grunniens*) is one of unique rare livestock in Tibetan Plateau and its adjacent areas, and the yield of yak meat is approximately 300 thousand tons every year [12]. Yak meat is the major source of animal protein in local human diet, and has been increasingly becoming popular among the consumers

beyond the Tibetan Plateau in recent years [13, 14]; whereas yak meat is very low in fat, which leads to some serious problems on meat quality, including bad tenderness, flavor and processability. Therefore, the improvement of fat content is one of the main aim for yak beef production. On the other hand, fat possessed important physiology meaning in metabolism and propagation of yak. As a classic grazing livestock, yaks suffer long-term starvation during cold season, and maintain their life by fat mobilization; the pregnant and parturition of female yaks are during cold season too, therefore the amount of fat in female yaks is closely related to reproduction.

Many factors such as genetics, diet, gender and breed identity, can influence the fat deposition in livestock [15, 16]. Data obtained from studies conducted in livestock demonstrates that gender influences the metabolic responses to energy intake. The significant directly factors affecting gender are hormones in livestock, and the effects of gender to fat deposition in livestock are realized by the regulation of hormones [17, 18]. The finding shows that androgenic hormone can promote energy expenditure and increase lipolysis by inhibiting the activity of lipoprotein lipase (LPL); estrogen is thought to be the main factor of regulating the fat deposition in female livestock [19]. As far as we know, there are very few reports on fat deposition in yaks, and the studies on fat deposition in yaks especially the differences in fat deposition between male and female yaks possess greatly positive influence on the development of industrialization, healthful aquaculture and breeding and genetics of yaks.

Fat deposition is a very complex process [20], and it is difficult to expound the effect of gender difference to fat deposition in yaks by the single technique. Transcriptome enables us to simultaneously and globally examine the complete responses at a transcriptional level [21], and the newest RNA-Sequencing (RNA-Seq) analysis can interrogate the level of mRNA in adipose tissue of yaks to estimate its gene expression profile under different gender; meanwhile, the high throughput metabolomics mass spectrum (MS) technologies can definitely help to identify metabolic networks of fat deposition in adipose tissue of yaks possessing different gender. The approach utilizing integrated metabolomics and transcriptomics can reveal fat phenotype-related gene functions and pathways of fat metabolism based on a series of gene actions and their final products, metabolites in adipose tissue of yaks possessing different gender.

In the present study, we carried out a relatively comprehensive study to observe the characteristics of fat deposition in male and female yaks and explore the effect of gender difference to fat depossition in yaks by an integrated transcriptomic and metabonomics analysis for the first time. Differentially expressed genes (DEGs)and metabolites (DEMs) in subcutaneous fat of male and female yaks were identified and then annotated by gene ontology (GO) and kyoto encyclopedia of genes and genome (KEGG). Additional investigations including fat metabolism in liver, serum profiles, reverse transcription-quantitative PCR (qPCR) and absolute quantification of metabolites using gas chromatography (GC) were performed to support or supplement the sequencing results. This study could establish theoretical basis for improving yak meat quality, yak breed improvement and yak healthful aquaculture.

Result

Characteristics of fat deposition in female yaks by contrast with male yaks

The fat amounts in female and male yaks were showed in Table 1. The body fat rate (BFR) of perirenal fat in female yaks was 2.15%, which was higher than 1.38% in male yaks ($p < 0.01$); the BFR of omental fat in female yaks was 2.30%, which was higher than 1.35% in male yaks ($p < 0.01$); the BFR of mesentery fat in female yaks was 2.33%, which was higher than 1.33% in male yaks ($p < 0.01$); whereas there was no difference in the BFR of fat around liver between female and male yaks. The subcutaneous fat thickness in back of female yaks was 12.34 mm, which was thicker than 8.16 mm in male yaks ($p < 0.01$); the subcutaneous fat thickness in waist of female yaks was 13.78 mm, which was thicker than 12.14 mm in male yaks ($p < 0.01$). The fat content in liver of female yak was 2.62%, which was higher than 2.21% in liver of male yak ($p < 0.01$), and the intramuscular fat in female yaks was 2.50%, which was higher than 1.84% in female yaks ($p < 0.01$). Therefore, the amounts of fat in female yaks were significantly more than male yaks.

Table 1
Fat amount in female and male yaks

Fat amount		Female yaks (n = 10)	Male yaks (n = 10)
BFR (%)	perirenal fat	2.15 ± 0.17^{A}	1.38 ± 0.10^{B}
	fat around liver	0.12 ± 0.01	0.12 ± 0.01
	omental fat	2.30 ± 0.13^{A}	1.35 ± 0.07^{B}
	mesentery fat	2.33 ± 0.17^{A}	1.33 ± 0.06^{B}
Subcutaneous fat thickness (mm)	back	12.34 ± 1.41^{A}	8.16 ± 0.45^{B}
	waist	13.78 ± 1.38^{A}	12.14 ± 0.86^{B}
Content (%)	liver	2.62 ± 0.11^{A}	2.21 ± 0.32^{A}
	muscle	2.50 ± 0.08^{A}	1.84 ± 0.07^{B}

Note: BFR, body fat rate. Values in the same row with different lowercase superscripts show $p < 0.05$, different capital superscripts show $p < 0.01$.

Levels of metabolites in serum and enzymes in livers of female yaks by contrast with male yaks

The levels of metabolites in serum were shown in Table 2. The high-density lipoprotein cholesterol (HDL), low-density lipoprotein cholesterol (LDL), cholesterol (CH), triglyceride (TG), very low-density lipoprotein (VLDL), leptine (LEP) and cortisol (COR) in serum of female yaks were higher than male yaks ($p < 0.05$), whereas insulin like growth factor-1 (IGF-1) in serum of female yaks was lower than male yaks ($p < 0.01$);

there were no differences in nonesterified fatty acid (NEFA), glucose (GLU), total protein (TP) and albumin (ALB) between female and male yaks. Meanwhile, fatty acid synthase (FAS), acetyl-CoA carboxylase (ACC), stearoyl-CoA desaturase (SCD), diacylglycerol acyltransferase 1 (DGAT-1) and carnitine palmitoyl transferase 1 (CPT-1) in livers of female yaks were higher than male yaks ($p < 0.05$), whereas there were no differences in hormone-sensitive lipase (HSL), LPL and adipose triglyceride lipase (ATGL) levels in liver between female and male yaks.

Table 2
The levels of metabolites in serum and enzyme activity in livers of female and male yaks

Item		Female yaks (n = 10)	Male yaks (n = 10)
Serum	HDL (mmol/L)	1.92 ± 0.09^{A}	1.77 ± 0.06^{B}
	LDL (mmol/L)	0.83 ± 0.13^{A}	0.65 ± 0.03^{B}
	CH (mmol/L)	2.98 ± 0.15^{A}	2.66 ± 0.06^{B}
	TG (mmol/L)	0.29 ± 0.02^{a}	0.25 ± 0.01^{b}
	NEFA (mmol/L)	0.18 ± 0.00	0.18 ± 0.00
	GLU (mmol/L)	4.68 ± 0.25	4.93 ± 0.10
	VLDL (mmol/L)	0.28 ± 0.02^{a}	0.25 ± 0.01^{b}
	TP (g/L)	72.17 ± 1.34	73.63 ± 2.24
	ALB (g/L)	29.53 ± 2.52	31.23 ± 1.77
	LEP (μg/L)	0.92 ± 0.05^{A}	0.75 ± 0.04^{B}
	COR (μg/L)	24.17 ± 1.48^{a}	20.68 ± 1.70^{b}
	IGF-1 (μg/mL)	0.28 ± 0.02^{A}	0.34 ± 0.04^{B}
Liver	FAS(U/mg tissue)	6.90 ± 0.17^{A}	6.15 ± 0.15^{B}
	ACC(U/mg tissue)	2.40 ± 0.12^{A}	2.17 ± 0.12^{B}
	SCD(U/mg tissue)	6.58 ± 0.15^{A}	5.99 ± 0.20^{B}
	DGAT-1(U/mg tissue)	6.17 ± 0.15^{A}	5.82 ± 0.14^{B}
	HSL(U/mg tissue)	7.41 ± 0.17	7.43 ± 0.18
	LPL(U/mg tissue)	0.19 ± 0.02	0.21 ± 0.03
	ATGL(U/mg tissue)	20.68 ± 1.74	21.11 ± 1.28
	CPT-1 (U/mg tissue)	27.51 ± 1.83^{a}	30.26 ± 2.35^{b}

Note: Values in the same row with different lowercase superscripts show $p < 0.05$, different capital superscripts show $p < 0.01$.

Transcriptome analysis for subcutaneous fat of female yaks by contrast with male yaks

In this study, $p < 0.05$ and FC > 2 or FC < 0.5 were set as the threshold for significantly differential expression. Compared with male yaks, there were 1027 differentially expressed genes (DEGs) in subcutaneous fat of female yaks (Supplementary Table S1). Of these, the expression of 416 genes downregulated, whereas the expression of 611 genes upregulated. The volcano plot of total expression genes and hierarchical clustering analysis for the transcriptome profiles were showed in Fig. 1A and 1B, respectively.

Gene ontology (GO) and kyoto encyclopedia of genes and genomes (KEGG) enrichment analyses were carried out to gain insights into the biological significance of DEGs (Supplementary Table S2 and Table S3). The top GO terms were showed in Fig. 2A. The biological process mainly included biological adhesion, biological regulation, cell killing, cellular component organization or biogenesis, cellular process, developmental process and so on. The unique DEGs in subcutaneous fat of female by contrast with male yaks were enriched in 66 KEGG pathways. The most enriched 20 KEGG pathways of DEGs were shown in a scatter diagram (Fig. 2B). Of these, biosynthesis of unsaturated fatty acids, p53 signaling pathway, peroxisome proliferators-activated receptor (PPAR) signaling pathway, fatty acid elongation, glutathione metabolism, primary bile acid, extracellular matrix (ECM)-receptor interaction, adenosine 5’-mohanophospte-activated protein kinase (AMPK) signaling pathway, fructose and mannose metabolism, focal adhesion, peroxisome and regulation of lipolysis in adipocytes were mostly involved in fat metabolism.

Untargeted metabolome analyses for subcutaneous fat of female yaks by contrast with male yaks

Unsupervised multivariate analyses of the metabolomics data were applied to provide an initial evaluation of metabolic perturbations in subcutaneous fat of yaks that had been caused by gender difference. Principal components analysis (PCA) score plots in the positive and negative ionization mode showed the distribution between subcutaneous fat of male and female yaks as well as quality control (QC) samples in Fig. 3A. The QC group samples were congregated tightly in a small area, indicating that the instrument was stable and the analysis was reliable. Further analysis using orthogonal partial least-squares-discriminant analysis (OPLS-DA) yielded a better understanding of the variables responsible for differentiation in subcutaneous fat of male and female yaks in Fig. 3B. Permutation testing using 200 random was used to validate the OPLS-DA models, and R2 Y and the vertical intercept were 0.923 and –0.497, respectively, therefore the model was effective and stable. There was a distinct difference between female and male yaks groups, demonstrating that gender differences induced a marked perturbation of metabolites in subcutaneous fat of yaks. The differential metabolites (DEMs) were selected on the basis of the combination of a statistically significant threshold of variable important in projection (VIP) values obtained from OPLS-DA model and p values from a two-tailed Student's t test on the normalized peak areas (Supplementary Table S4). These DEMs with VIP > 1.0 and $p < 0.05$ were screened (Supplementary

Table S5), and total 237 DEMs were obtained. Of these, 161 metabolites upregulated and 76 metabolites downregulated in subcutaneous fat of female yaks.

These DEMs were enriched in 27 KEGG pathways ($p < 0.05$) (Supplementary Table S6), and the bubble chart of top 20 was showed in Fig. 4. Of them, the metabolic pathway related to fat deposition were primary bile acid biosynthesis, linoleic acid metabolism, cholesterol metabolism, biosynthesis of unsaturated fatty acids (UFAs), tricarboxylic acid cycle (TCA) cycle, carbon metabolism, mammalian target of rapamycin (mTOR) signaling pathway and PPAR signaling pathway. Total 30 DEMs were enriched in KEGG pathways, and they were malic acid, citric acid, L-Glutamate, glycocholic acid, chenodeoxycholic acid glycine conjugate, cholic acid, 9(S)-HpODE, 8–13(S)-8-HODE, PC(15:0/14:0), lysoPC(18:2(9Z,12Z)), creatine, deoxycorticosterone, inosine, myo-inositol, L-Arginine, D-Lactic acid, α-linolenic acid, eicosapentaenoic acid (EPA), imidazolepropionic acid, γ-linolenic acid, 7-dehydrodesmosterol,9-7-(9xi,10xi,12xi)-9,10-dihydroxy-12-octadecenoicacid,D-2,3-dihydroxypropanoic acid,2-trans,6- trans-farnesal, thromboxane, (+/-)14,15-diHETr-E, docosapentaenoic acid (DPA), di-trans, poly-cis-undecap-renyl diphosphate, 25-hydroxyvitamin D3/25-hydroxycholecalciferol/calcidiol and chenodeoxycholic acid. The correlations of DEGs and DEMs were carried out by the network analysis of the KEGG Markup Language (KGML), and the results were showed in Fig. 5.

Quantitative real-time PCR validation

LEP, SREBF1, SCD, SLC27A4, APOE, LPL, LDLR, ACOT7, ELOVL6 and *ACAA1* genes were discussed in this paper and play an important part in fat metabolism. Therefore, above genes were selected for validation. Previous research indicated that the expression levels of *β-actin* gene in different tissues of yaks are partially variable. However, a good linear relationship between the expression level of *β-actin* and the amount of total RNA in the specific tissues, especially adipose tissue, was obtained in the confirmatory experiments. Therefore, *β-actin* gene was selected as a reference gene for qPCR in subcutaneous fat of yaks. All selected DEGs showed concordant expression patterns between RNA-seq and qPCR analysis.

Quantitation of differential metabolites (DEMs)

The absolute concentrations of α-linolenic acid, γ-linolenic acid and EPA were showed in Fig. 6, and all increased in the subcutaneous fat of female yaks ($p < 0.05$). Meanwhile, these three metabolites showed significant upregulation in subcutaneous fat of female yaks in metabolomics analyses. The trends in the absolute concentration variations for three metabolites in subcutaneous fat of female and male yak were similar with the trends in the untargeted metabolomics. Therefore, the untargeted metabolomics analyses was reliable and accurate.

Discusson

Fat metabolism in liver of female yaks by contrast with male yaks

Liver is one of main site in which fat is synthesized. FAS catalyzes the formation of long-chain fatty acids from acetyl-CoA and malonyl-CoA [22, 23]; ACC catalyzes the carboxylation of acetyl-CoA to malonyl-CoA, the first and rate-limiting step of de novo fatty acid biosynthesis [24]; SCD catalyzes the insertion of a double bond at the delta-9 position into fatty acyl-CoA substrates including palmitoyl-CoA and stearoyl-CoA [25]; DGAT-1 catalyze the last and the only committed step of triglyceride biosynthesis [26]. All of above enzymes are the promoters of fat synthesis, and their levels in livers of female yaks were higher than male yaks ($p < 0.01$). Therefore, the fat synthesis in livers of female yaks increased. CPT1 catalyzes the transfer of long- and medium-chain fatty acids from cytoplasm into mitochondria and is the rate-limiting enzyme in fatty acid oxidation [27], and its level in livers of female yaks was lower than male yaks ($p < 0.05$). This result showed the fatty acid oxidation in livers of female yaks decreased. HSL catalyzes the release of fatty acids from storage triglycerides in adipocytes, liver and muscle; LPL is a key enzyme in adipolysis, and catalyzes the hydrolysis of triglycerides from circulating chylomicrons and VLDL [28]; ATGL is the rate-limiting enzyme in triacylglycerol hydrolysis. All of above enzymes are the promoter of lipolysis, and there were on differences in their levels in livers of female and male yaks. Therefore, the lipolysis in liver of female yaks was similar with male yaks.

Fat transport in blood of female yaks by contrast with male yaks

Livestock in a normal physiological state can not store fat in liver, and synthetic fat in liver was transferred into blood. CH level in serum of female yaks was higher than male yaks ($p < 0.01$), and CH synthesis in female yaks increased. VLDL transports endogenous products [29], and its level in serum of female yaks was higher than male yaks ($p < 0.05$). Meanwhile, HDL and LDL levels in serum of female yaks were also higher than male yaks ($p < 0.01$). These results were corresponding with the results of enzyme activity in liver of yaks and TG level. IGF-1 has much higher growth-promoting activity than insulin, and lowers total triglyceride, low density lipoprotein triglycerides and LDL cholesterol levels in blood [30], and its level in serum of female yaks was lower than male yaks ($p < 0.01$); COR promotes the fat synthesis and fat deposition in *vivo* [31], and its level in female yaks was higher than male yaks ($p < 0.01$). IGF-1 and COR levels in serum of female yaks were verified by the high levels of TG, VLD, HDL and LDL. These results indicated more fat was transferred in blood of female yaks.

Cholesterol metabolisms in female yaks by contrast with male yaks

CH strongly associates with the fat metabolism especially fat transport in livestock, and many steroid hormones effecting the fat metabolism, such as cortisol, aldosterone, and testosterone, are derived from CH. Most of CH is synthesized in liver, whereas a small part is synthesized in adipocyte, and CH is the ingredient of LDL, VLDL and HDL [32]. The LDL, VLDL and HDL levels in serum of female yaks were higher than male yaks ($p < 0.05$), and CH level in serum of female yaks was higher than male yaks ($p < 0.05$) too. Therefore, the cholesterol synthesis in liver of female yak increased.

Prenylcysteine oxidase 1 (PCYOX1) hydrolyzes the thioether bond of prenylcysteines in the final step in the degradation of prenylated proteins [33]; acyl-coenzyme a-cholesterol acyltransferase (ACAT) catalyzes cholesterol and long chain fatty acyl-CoA to produce cholesteryl ester [34]; protein farnesyltransferase subunit beta (FNTB) catalyzes the transfer of farnesyl moiety from farnesyl diphosphate to a cysteine [35]. They involved in terpenoid backbone biosynthesis and the expression levels of their genes all upregulated in subcutaneous fat of female yaks ($p_{FDR} < 0.05$). Meanwhile, 2-trans, 6-trans-farnesal levels in the pathsway of terpenoid backbone biosynthesis increased in subcutaneous fat of female yaks. Therefore, it can be inferred that terpenoid backbone biosynthesis in subcutaneous fat of female yaks increased. The terpenoid backbone biosynthesis is one of links in the cholesterol and steroid synthesis.

Apolipoprotein E (ApoE) transports cholesterol, and its gene expression downregulated in subcutaneous fat of female yak ($p_{FDR} < 0.05$), so less cholesterol was transfered out of fat cell in female yaks. The low-density lipoprotein receptor (LDLR) is important for the clearance of VLDL, intermediate density lipoprotein (IDL) and LDL, and *LDLR* gene expression upregulated in subcutaneous fat of female yaks ($p_{FDR} < 0.05$); cholesteryl ester hydrolase (LIPA) catalyzes the deacylation of cholesteryl ester core lipids of LDL to generate cholesterol [36], and *LIPA* gene expression upregulated in subcutaneous fat of female yaks ($p_{FDR} < 0.05$); LPL positively regulates cholesterol storage, and *LPL* gene expression upregulated in subcutaneous fat of female yaks ($p_{FDR} <0.05$); steroidogenic acute regulatory protein (STAR) controls the rate-limiting step in steroidogenesis, and *STAR* gene expression upregulated in subcutaneous fat of female yaks ($p_{FDR}< 0.05$). Meanwhile, glycocholic acid (VIP4.38) involved in the emulsification of fats, and chenodeoxycholic acid glycine conjugate (VIP 1.17) is a bile acid-glycine conugate. They can be generated by cholesterol metabolism, and their levels increased in subcutaneous fat of female yak ($p < 0.05$). Therefore, the cholesterol synthesis and storage increased in the subcutaneous fat of female yaks.

Steroid metebolisms in female yaks by contrast with male yaks

Delta (24)-sterol reductase (DHCR24) catalyzes sterol ring modifications [37]; squalene monooxygenase (SQLE) is a vital sterol synthesis enzyme across eukaryotic life [38]. It can be found that *DEGs LIPA, DHCR24* and *SQLE* gene expressions in steroid biosynthesis all upregulated in subcutaneous fat of female yaks ($p_{FDR} < 0.05$). Meanwhile, 25-hydroxyvitamin D3 (VIP3.53) in steroid biosynthesis decreased in subcutaneous fat of female yaks ($p < 0.01$); 7-dehydrodesmosterol (VIP2.20) is a substrate of 24-dehydrocholesterol reductase which is an important enzyme in the steroid biosynthesis, and its level increased in subcutaneous fat of female yaks ($p < 0.01$). The existing researches indicate that androgens can be translated into estrogen in fat cell, and both androgens and estrogen belong to steroid. Therefore, it can be inferred that steroid biosynthesis was promoted in subcutaneous fat of of female yak.

Fat synthesis in subcutaneous fat of female yaks by contrast with male yaks

The fat can be synthesized in adipose tissue too, and not only synthetic fat in *vivo* but also fat from food only deposite in adipose tissue finally. The fat is mainly component of triglyceride, and triglyceride synthesis includes pathways of monoglyceride and 1,2-diglyceride-3-pho-s-3-phate. 1-acyl-sn-glycerol-3-phosphate acyltransferase beta (AGPAT2) is the key catalyst in two pathways; DGAT2 and glycerol-3-phosphateac-yltransferase (GPAT) are two key enzymes promoting the triglyceride synthesis in the pathway of 1,2-diglyceride-3-phosphate [39]. *AGPAT2, DGAT2* and *GPAT* gene expressions upregulated in subcutaneous fat of female yak ($p_{FDR} < 0.01$). Meanwhile, glutamate (VIP1.68), malic acid (VIP1.45), citric acid (VIP1.99) and D-2,3-dihydroxypropanoic acid (VIP1.62) all increased in subcutaneous fat of female yaks ($p < 0.05$), and these metabolisms involved in the carbon metabolism. D-2,3-dihydroxypropanoic acid can translate into glycerate-3P which can be used to synthesize triglyceride; malic and citric acid are the intermediates of TCA cycle, and can translate into the acetyl-CoA which is initiator in the process of fat acid biosynthesis. Therefore, it was inferred that the triglyceride synthesis in subcutaneous fat of female yak increased.

Lipolysis and fatty acid oxidation in subcutaneous fat of female yaks by contrast with male yaks

ATGL being the rate-limited enzymes in fat catabolism, and its gene *PNPLA1* expression downregulated in subcutaneous fat of female yak ($p_{FDR} < 0.05$). Therefore, fat catabolism in subcutaneous fat of female yak decrease. Protocadherin fat 2 (FAT2) transfers fatty acis into the adipocyte, and *FAT2* gene expression upregulated in subcutaneous fat of female yak ($p_{FDR} < 0.01$), so the fat acid transport was promoted in subcutaneous fat of female yaks. *CPT1C* gene expression downregulated in subcutaneous fat of female yak ($p_{FDR} < 0.01$), so the fatty acids oxidation decreased in subcutaneous fat of female yaks.

Fatty acid synthesis in subcutaneous fat of female yaks by contrast with male yaks

Estradiol 17-beta-dehydrogenase 8 (HSD17B8) [40], trifunctional enzyme subunit beta (HADHB) [41], hydroxyacyl-coenzyme A dehydrogenase (HADH) [42], elongation of very long chain fatty acids protein 6 (ELOVL6) [43], very-long-chain (3R)-3-hydroxyacyl-CoA dehydratase 2 (HACD2) [44] and cytosolic acyl coenzyme A thioester hydrolase (ACOT7) [45] were the positive regulation of fatty acid elongation. Their gene *HADHB*, *HADH*, *ELOVL6*, *HSD17B8, HACD2* and *ACOT7* expressions all upregulated in subcutaneous fat of female yak ($p_{FDR} < 0.05$). Therefore, the fatty acid synthesis increased in subcutaneous fat of female yak.

Polyunsaturated fatty acids (PUFAs) in livestock can not directly be synthesized *in vivo*, and must be derived from the precursor compounds, like linoleic acid and linolenic acid from grass or feed [46]. The ω-6 PUFAs like arachidonic acid are synthesized by linoleic acid and ω-3 PUFAs like DHA and EPA are synthesized by linolenic acid [47]. SCD, ACOT7, HACD2, non-specific lipid-transfer protein (SCP2) [48], ELOVL6 [49] and acetyl coenzyme-A acyl-transferase-1 (ACAA1) [50] are all the promoter of unsaturated

fatty acid biosynthesis, and their gene expression all upregulated in the subcutaneous fat of female yaks (p_{FDR}<0.05). Meanwhile, α-linolenic acid (VIP3.10), γ-linolenic acid (VIP4.83), EPA (VIP1.63) and DPA (VIP1.43) in unsaturated fatty acids biosynthesis increased in subcutaneous fat of female yak ($p < 0.05$). γ-linolenic acid is can convert into dihomo-gamma-linolenic acid which is a biosynthetic precursor of monoenoic prostaglandins such as PGE1; α-linolenic acid is an important structural components of cell membranes; EPA and DPA serve as the precursor for the prostaglandin-3 and thromboxane-3 families. Therefore, it was inferred that the unsaturated fatty acids biosynthesis especially EPA, γ-linolenic acid and DPA increased in subcutaneous fat of female yaks.

Five DEMs including PC(15:0/14:0) (VIP1.12), γ-linolenic acid, (9R,10S,12Z)-9, 10-dihydroxy-12-octadecenoic acid (VIP1.53), 13(S)-HODE (VIP1.19) and 9(S)-HpODE (VIP1.21) involved in linoleic acid metabolism, and all increased in subcutaneous fat of female yaks ($p < 0.05$). γ-linolenic acid is an intermediate in biosynthesis of ω-6 PUFAs like arachidonic acid. Meanwhile, PC(15:0/14:0), thromboxane (VIP2.04) and (+/-)14,15-DiHETrE (VIP1.15) involved in arachidonic acid metabolis, and PC(15:0/14:0) and (+/-) 14,15-DiHETrE increased in subcutaneous fat of female yaks, wheres thromboxane decreased. PC(15:0/14:0) and α-linolenic acid involved in α-linolenic acid metabolism, and increased in subcutaneous fat of female yaks ($p < 0.05$). Stearidonic acid, conelenic acid, heptadecatrienoic acid and etherolenic acid are all downstream products in alpha-Linolenic acid metabolism. Therefore, many active substances deriving from ω-3 and ω-6 PUFAs increased in subcutaneous fat of female yak.

Summary, as showed in Fig. 7, the fat synthesis and cholesterol synthesis in liver of female yaks increased, whereas the fatty acid oxygen in liver of female yaks decreased; the fat transport in blood of female yaks increased; the fatty acid synthesis, fat synthesis, cholesterol synthesis in subcutaneous fat of female yaks increased, whereas lipolysis in subcutaneous fat of female yaks decreased .

Secretion of adipocytokines in female yaks by contrast with male yaks

Metabolic hormones such as insulin, cortisol or thyroid hormones play an important role in fat metabolism [51]. Calcium ion is one of the most abundant ions in livestock and many studies have shown calcium signaling plays a major role in increasing exocytosis secretion like hormones, autacoids and neuotransmitters. COR level in serum of female yaks was higher than male yaks ($p < 0.01$); meanwhile, myo-inositol (VIP 3.87) increased in subcutaneous fat of female yaks ($p < 0.01$). Above compounds can strengthen calcium signaling in fat cell of female yaks, and the secretion of adipocytokinein female yaks in fat cell was promoted by the calcium signaling.

LEP is a key player in the regulation of energy balance, and acts as an appetite-regulating factor [52], and has central and peripheral effects by binding leptin receptor (LEPR), and activate several major signaling pathways like PPARs signaling. In this study, *LEP* gene expression upregulated in subcutaneous fat of female yaks ($p_{FDR} < 0.05$), and LEP level in serum of female yaks was also higher than male yaks ($p <$

0.01); adiponectin(ADPN) is another essential hormone secreted by adipocytes and regulates glucose and lipid metabolism. Adiponectin receptor 2 protein (ADIPOQ2) is the receptor for ADPN, and *ADIPOQ2* gene expression upregulated in subcutaneous fat of female yaks ($p_{FDR} < 0.01$). ADIPOQ-binding activates a signaling cascade that leads to increase PPAR activity, and ultimately to regulate fatty acid metabolism, increase glucose uptake and decrease gluconeogenesis in subcutaneous fat of female yaks.

Regulation of fat deposition in female yaks by the PPAR and SREBF1 signaling by contrast with male yaks (Fig. 8)

PPAR is essentially a class of transcription regulators relying on ligand, and can be activated by fatty acids especially unsaturated fatty acid and their derivatives [53]. Long-chain fatty acids are transferred through adipocyte plasma membrane by long-chain fatty acid transport protein 4 (SLC27A4), and *SLC27A4* gene expression upregulated in subcutaneous fat of female yaks ($p_{FDR} < 0.05$); meanwhile, ω-3 fatty acids can modulate the expression of many genes involved in fatty acid metabolism, by interacting with transcription factors PPARs [54]. 13(S)-HODE is a eicosanoid belong to ω-3 fatty acids, and increased in subcutaneous fat of female yaks ($p < 0.01$). Therefore, the subcutaneous fat in female yaks can obtain more long-chain fatty acids especially 13(S)-HODE from blood than male yaks, and these acids combined with PPARs in fat cell. On the other hand, LEP and ADIPOQ-binding also activated PPAR, and *PPARD* gene expression upregulated in female yaks (FC = 1.43, $p_{FDR} < 0.01$). Therefore, it can be inferred that the fat metabolism in yaks of different gender was mainly regulated by downstream genes in PPAR signaling, and downstream genes included the gene of NADP-dependent malic enzyme (ME1) [55], SCD, Acyl-CoA-binding protein (ACBP) [56], LPL, CPT1, SLC27A4, ACAA1 and perilipin-5 (PLIN5). ME1 and SCD promote fat synthesis, and *ME1* and *SCD* gene expressions upregulated in the subcutaneous fat of female yaks ($p_{FDR} < 0.01$); ACBP, LPL and SLC27A4 positively regulates the fatty acid transport, and therie genes *DBI, LPL* and *SLC27A4* expression upregulated in the subcutaneous fat of female yaks ($p_{FDR} < 0.01$); ACAA1 catalyzes the last step of the mitochondrial beta-oxidation pathway, and *CPT1C* and *ACAA1 gene* expression downregulated in the subcutaneous fat of female yaks ($p_{FDR} < 0.01$); PLIN5 possesses positive regulation of lipid storage, and *PLIN5* gene expression upregulated in the subcutaneous fat of female yaks ($p_{FDR} < 0.05$). On the other hand, sterol regulatory element-binding protein 1 (SREBF1) is a transcriptional activator required for fat homeostasis, and regulates transcription of the lipogenic genes [57], and the higher levels of LEP can lead to the activation of the SREBF1 activity, and *SREBF1* gene expression upregulated in subcutaneous fat of female yaks ($p_{FDR} < 0.01$). *SCD1* gene expression is regulated by SREBF1 [58, 59], and upregulated in subcutaneous fat of female yaks ($p_{FDR} < 0.05$). In a word, the fatty acid synthesis, the fatty acid transport and fat synthesis increased by the regulation of PPAR and SREBF1 signal in subcutaneous fat of female yaks, whereas the fatty acid oxidation decreased, and they resulted in that the amount of fat deposition in female yaks was more than male yaks.

Conclusion

In this study we have investigated the characteristic of fat depossition in female and male yaks in natural state, and the regulatory mechanisms of gender differences to fat deposition in yaks were explored. The amount of intramuscular fat, visceral fat and subcutaneous fat in female yaks were higher than male yaks. The fat and cholesterol synthesis in liver of female yaks increased, and the fat transport in blood of female yaks in creased too. In subcutaneous fat of female yaks, the high levels of COR and autacoids myo-inositol strengthened the calcium signaling in fat cell, and then the secretions in fat cell increased. The high level of adipokines LEP and eicosanoid 13(S)-HODE strengthened the PPAR signal in subcutaneous fat of female yaks; meanwhile, the high level of LEP strengthened SREBF1 signal in subcutaneous fat of female yaks too. By the regulation of ME1, DBI, LPL, CPT1C, ACAA1, PLIN5 and SCD in PPAR and SREBF1 signal pathway, the fat synthesis, fat acid biosynthesis especially unsaturated fatty acids biosynthesis in which the linoleic acid and linolenic acid are the precursor in subcutaneous fat of female yaks increased, whereas lipolysis and fatty acid oxidation decreased. Moreover, the cholesterol and steroid synthesis in subcutaneous fat of female yaks increased too. Therefore, more fat was deposited in female yaks, and the amounts of fat in female yaks were greater than male yaks.

Methods

Animals and samples collection

The procedures on the animals were approved by the Ethics Committee of Lanzhou Institute of Husbandry and Pharmaceutical Sciences,Chinese Academy of Agricultural Sciences. The experimental location was set at a pasture in Haiyan County in Qinghai Province, China. Four years old female (n = 10) and male (n = 10) yaks were bought from Qinghai Xiahua meat food Co. LTD (Haiyan, China) on May. All yaks were kept in grazing conditions from May to September, and were given free-choice access to diet and water. In late september, the day before the yaks were sacrificed, all yaks were deprived of feed overnight and they were sacrificed the following morning through electrical stunning. Before being sacrificed, yaks were weighed, and 20 mL blood were collected from the jugular vein of each yak under limosis. The serum samples were obtained by the centrifugation. At last, the longissimus dorsi (12th-13th rib level), liver and subcutaneous fat samples were collected and kept in liquid nitrogen or refrigerator at − 20 °C.

Measurement of fat amount in female and male yaks

BFT was measured using a vernier caliper (Hengliang Inc., Shanghai) within 10 min after slaughter; the subcutaneous fat on back (on both sides of midline of the dorsal at the 5–6 thoracic vertebra) and on waist (on both sides of midline at the cruciate region) were involved; BFR of visceral fat was evaluated by the weighing method, and the perirenal fat, omentum majus, mesentery fat and fat around liver were involved; the fat contents in longissimus dorsi and liver were detected according to soxhlet extraction principle, using a Soxtec 2050 soxhlet apparatus (FOSSInc., Hillerød, Denmark).

Determination of metabolites in serum and enzyme activity in livers of female and male yaks

The levels of GLU, CH, TG, HDL, LDL, NEFA, TP and ALB in serum of yaks were determined by the colorimetric method on a BS-420 automatic biochemical analyser (Mindry Inc., Shenzhen, China); the levels of VLDL (kit number: XY-SJH-N1069), LEP (kit number: XY-SJH-N1144), IGF-1 (kit number: XY-SJH-N1144), COR (kit number: XY-SJH-N1103), FAS (kit number:XY-SJHN-1481), ACC (kit number: XY-SJH-N1144), SCD (kit number: XY-SJH-N1147) and DGAT-1(kit number: XY-SJHN1140), HSL (kit number: XY-SJH-N1210), LPL (kit number: XY-SJH-N1320), ATGL (kit number: XY-SJH-N1454), CPT-1 (kit number: XY-SJH-N1663) were measured using commercial bovine enzyme-linked immunosorbent assay (ELISA) kits from Xuanya biotechnology Co., Ltd (Shanghai, China).

RNA extraction, sequencing, and bioinformatics in subcutaneous fat of famale and male yaks

Three subcutaneous fat samples were randomly chosen in each group, respectively. Total RNA was extracted using the mirVana™ miRNA Isolation Kit (Ambion Inc., Foster City, CA, USA) following the manufacturer's protocol. The mRNA libraries for sequencing were prepared using TruSeq Stranded mRNA LTSample Prep Kit (Illumina, San Diego, CA, USA). Then these libraries were sequenced on the Illumina sequencing platform (HiSeqTM 2500) and 125 bp paired-end reads were generated.

Raw data (raw reads) were processed using Trimmomatic. Then the clean reads were mapped to reference genome using hisat2. Meanwhile, the Q30 and GC-content of clean reads were calculated. Fragments Per Kilobase per Million (FPKM) value of each gene was calculated using cufflinks, and the read counts of each gene were obtained by htseq-count. DEGs were identified using the DESeq (2012) R package functions estimate SizeFactors and nbinomTest. GO enrichment and KEGG pathway enrichment analysis of DEGs were respectively performed using R based on the hypergeometric distribution.

Metabolites extraction, MS data and bioinformatics in subcutaneous fat of famale and male yaks

Fat sample was extracted with the solution of 2-chloro-l-phenylalanine in methanol and methanol-water (4:1, v:v). After centrifuging, 300 mL supernatant was dried in a freeze concentration centrifugal dryer. The residue was dissoloved with 400 μL solution of methanol-water (1:4, v:v) and vortexed for 30 s. The solution was centrifuged and 150 μL supernatant was collected, and filtered through 0.22 μm microfilters.UHPLC-TOP-MS with ACQUITY UPLC BEH C_{18} column (100 mm × 2.1 mm, 1.7 μm) was used. The elution solution was consisted of (A) water containing 0.1% formic acid (v:v) and (B) the mixed solution of acetonitrile and methanol (2:3, v:v) containing 0.1% formic acid. The elution program was as follows: 5–20% B over 0–2 min, 20–25% B over 2–4 min, 25–60% B over 4–9 min, 60–100% B over 9–17 min, holding at 100% B for 2 min, 100–5% B over 19-19.1 min and holding at 5% B from 19.1 to 20.1 min. The flow rate, column temperature and injection volume was 0.4 mL/min, 45 °C and 5 μL,

respectively. MS system was operated using the ESI + and ESI- mode and the mass range was set at 70-1000 m/z in the full scan mode combined with IDA mode. Parameters of MS were as follows: ion source temperature, 550 °C (+) and 550 °C (−); ion spray voltage, 5500 V (+) and 4500 V (−); curtain gas, 35 PSI; declustering potential, 100 V (+) and − 100 V (−); collision energy, 10 eV (+) and − 10 eV (−); interface heater temperature, 550 °C(+) and 600 °C (−). The acquired raw data of MS were preprocessed using the software Progenesis QI v2.3 (Nonlinear Dynamics, Newcastle, UK). The combined date of positive and negative icon was dealed with R ropls package. PCA, OPLS-DA, KEGG pathway enrichment analysis were performed using R based.

Quantitative reverse-transcription PCR (qPCR)

A total of 6 samples from the male and female yaks group, respectively, were selected for qPCR analysis. The qPCR analysis was performed using ABI Prism 7500 instrument (Applied Biosystems, Carlsbad, CA). Relative gene expression levels were determined were using the $2^{-\Delta\Delta Ct}$ method using GAPDH gene for normalization [60].

Gas chromatography (GC) analysis

To confirm the metabolic changes observed in the untargeted metabolomics study, DEMs concentrations were investigated using GC according the method described in Folch et al [61] and Song et al [62]. Finally, α-linolenic acid, γ-linolenic acid and EPA were identified by external standard method. First, the fat in subcutaneous fat tissue was extracted using methylene chloride-methanol solution, and then the fat in organic phase was obtained by salting out using 0.88% potassium chloride and vacuum evaporation. Next, 50 mg extracted fat sample was dissolved in the solution of acetone and methanol (2:1, v:v) and cleaned with anion exchange resin, and then these free fatty acids were derived using boron fluoride-methanol solution. At last, the derivatives fatty acid methyl esters (FAMEs) were determinated using GC (7890A, Agilent Corp., Santa Clara, US) coupled with a flame ionization detector (FID). The analytes were determined based on their retention times, and the concenstrations of fatty acids were calculated by FAMEs.

Abbreviations

BFR: Body fat rate; LEP: Leptin; PPAR: Peroxisome proliferators-activated receptor; SREBF1: Sterol regulatory element-binding transcription factor 1;IGF-1:Insulin-like growth factor 1; MS: Mass spectrum; DEGs:Differentially expressed genes; DEMs: Differentially metabolites; GO:Gene ontology; KEGG: Kyoto encyclopedia of genes and genome; qPCR: Reverse transcription-quantitative PCR; GC:Gas chromatography; HDL: High-density lipoprotein cholesterol; LDL:Low-density lipoprotein cholesterol; VLDL: Very low-density lipoprotein; NEFA: Nonesterified fatty acid; PCA: Principal components analysis; QC: Quailty control OPLS-DA:Orthogonal partial least-squares-discriminant analysis; VIP: Variable important in projection.

Declarations

Ethics approval and consent to participate

The animal study was approved by the Ethics Committee of the Lanzhou Institute of Husbandry and Pharmaceutical Sciences, Chinese Academy of Agricultural Sciences. The methods used in this study were carried out in accordance with the Laboratory Animal Management Principles of China.

Consent for publication

Not applicable.

Availability of data and materials

Not applicable

Competing interests

The authors have declared that no competing interests exist.

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Authors' contributions

P. Y. and L. X. designed the study project and write manuscript, L. X., X. G. and J. P. analyzed the data and prepared the manuscript, X. W., X. G. and X. Y. provided helps for collection of the samples,L. X., X. G., Q. K and J. P. contributed to result discussion and data interpretation. All authors read and approved the final manuscript.

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Figures

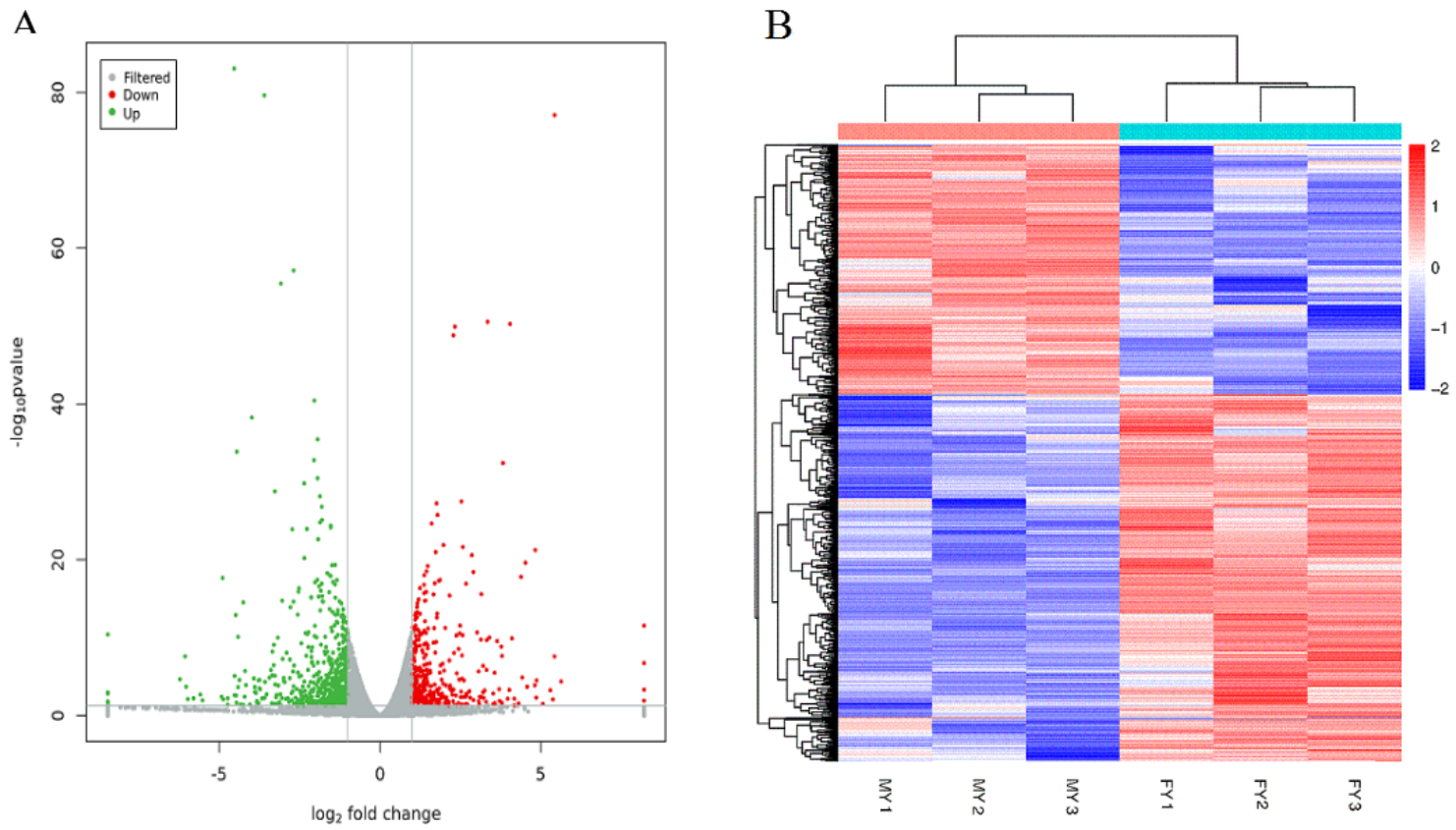

Figure 1

(A) The volcano plot of total expression genes in subcutaneous fat of male and female yaks; Red dots indicate downregulated differentially expressed genes (DEGs), green dots indicate upregulated DEGs and grey dots indicated non-differentially expressed genes; (B) Clustering of the DEGs in the subcutaneous of male and female yaks. Columns indicate individual samples, rows represent each DEG, and the color scale represents the relative expression level of the DEGs.

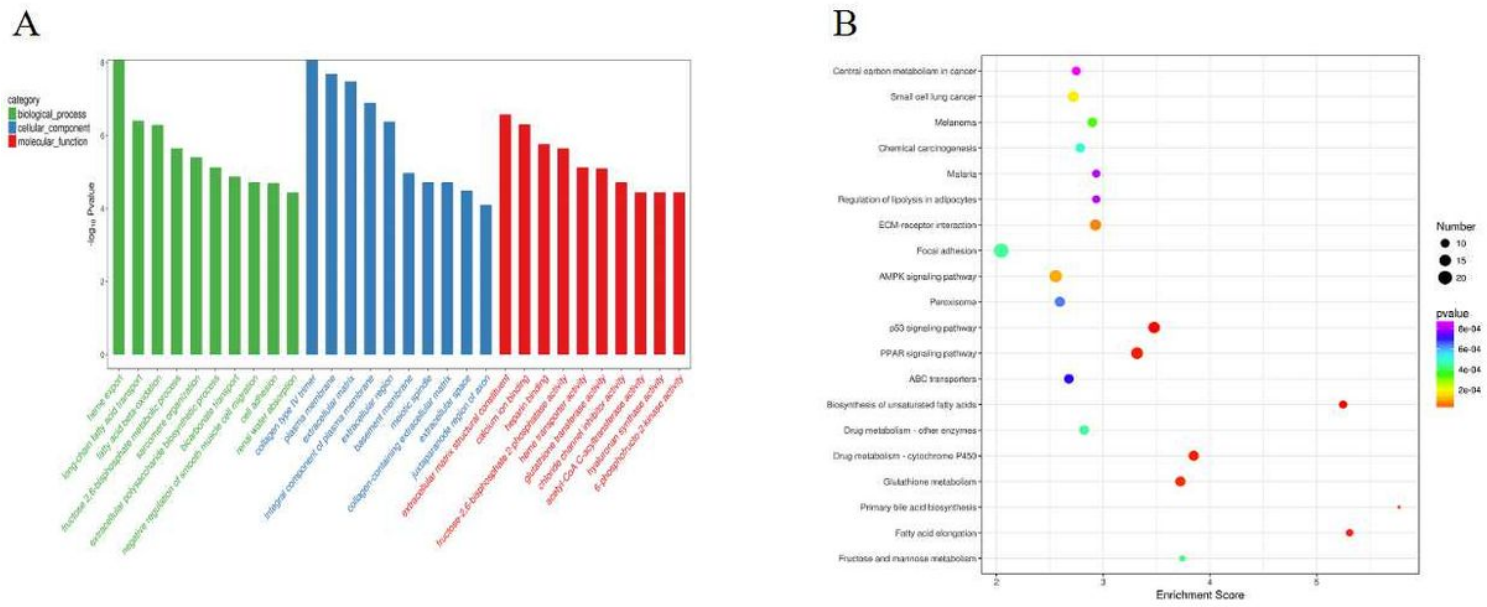

Figure 2

(A) Gene Ontology (GO) analysis in level 2 of DEGs in the subcutaneous fat of female and male yaks. The top 10 GO terms with lowest p-values in molecular function, cellular component and biological process were shown, respectively; (B) Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway terms ($p < 0.05$) enriched by DEGs of subcutaneous fat of female and male yaks. X-axis means rich factor (Rich factor = DEGs enriched in the pathway ÷ background genes in the pathway). Y-axis represents the KEGG pathway terms. The color of roundness represents p-value.The area of roundness represents number of DEGs enriched in this pathway.

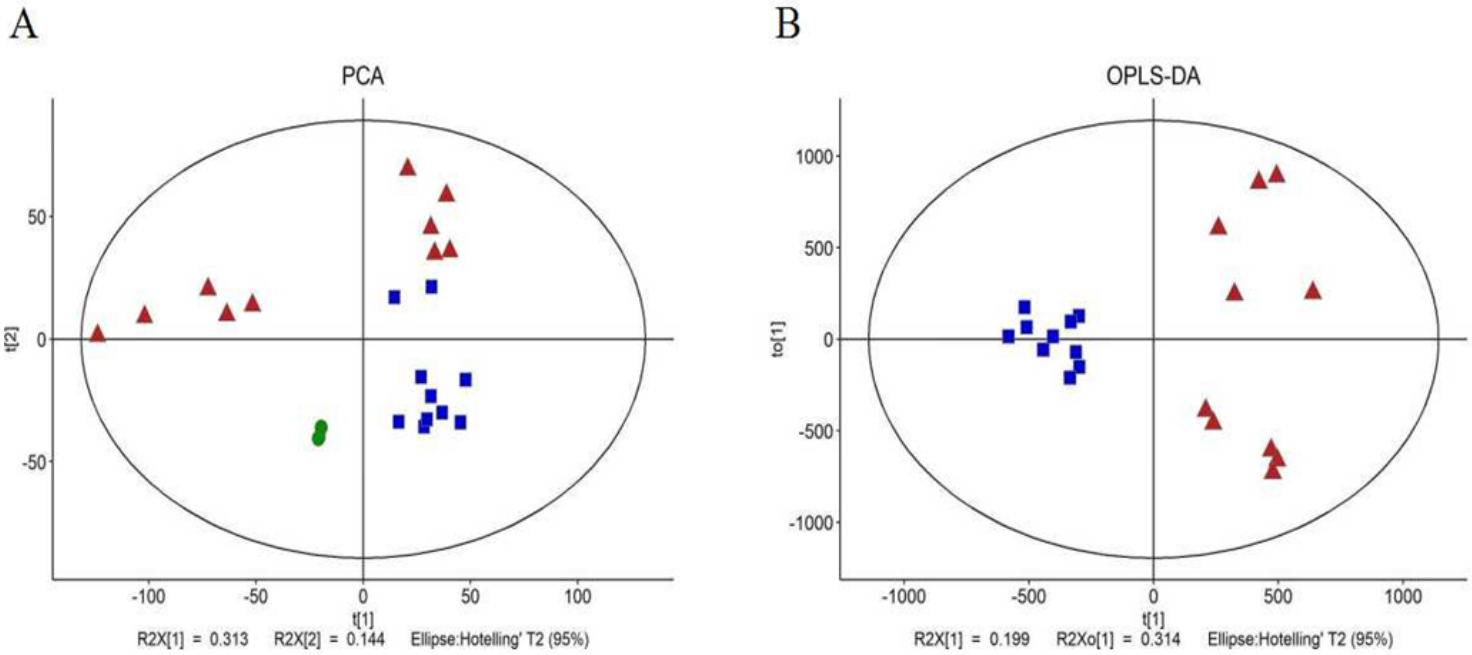

Figure 3

(A) Principle component analysis (PCA) of metebolites in subcutaneous fat of famale yak and male yak; (B) Orthogonal partial least squares discrimination analysis (OPLS-DA) of metebolites in subcutaneous fat of famale yaks and male yaks. , male yaks; , female yaks; , quality control.

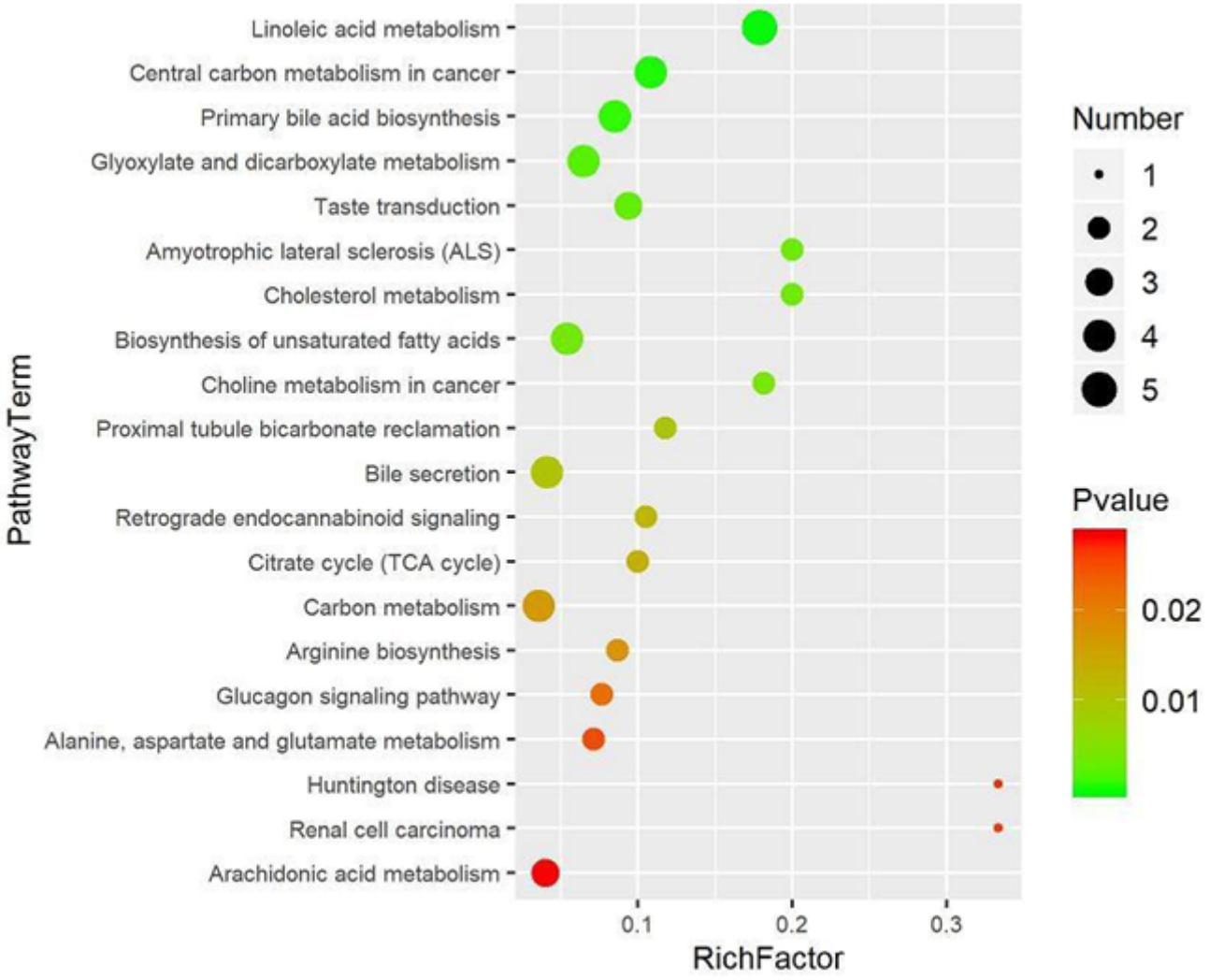

Figure 4

KEGG pathway terms ($p < 0.05$) enriched by differential metabolites (DEMs) in subcutaneous fat of female and male yaks. X-axis means rich factor (Rich factor = DEGs enriched in the pathway ÷ background genes in the pathway). Y-axis represents the KEGG pathway terms. The color of roundness represents p-value.The area of roundness represents number of DEGs enriched in this pathway.

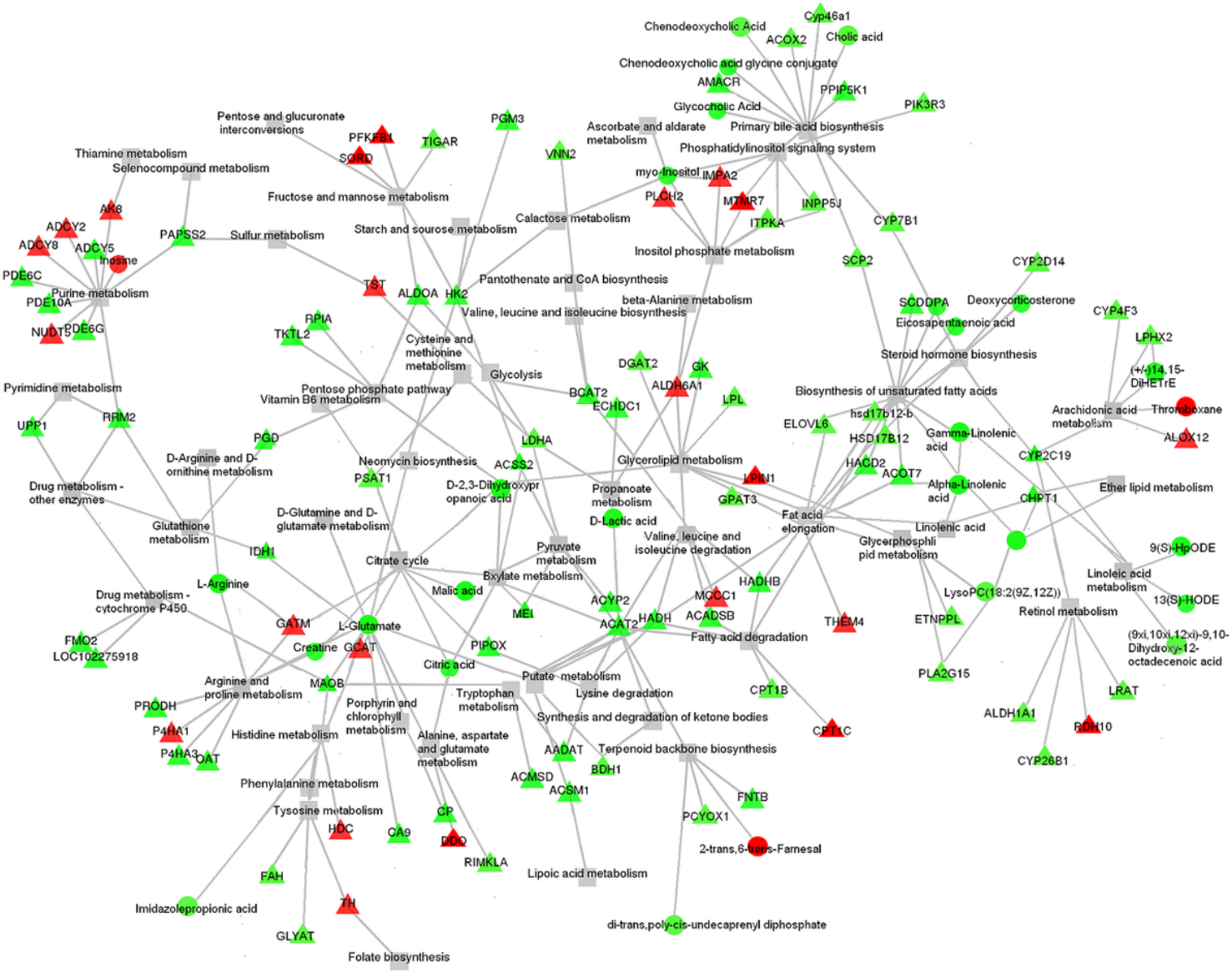

Figure 5

The KEGG Markup Language (KGML) of DEGs and DEMs in subcutaneous fat of female yaks by contrast with male yaks. ▲, gene; ■, map; ●, metabolites. Green, upregulate or increase; red, downregulate or decrease

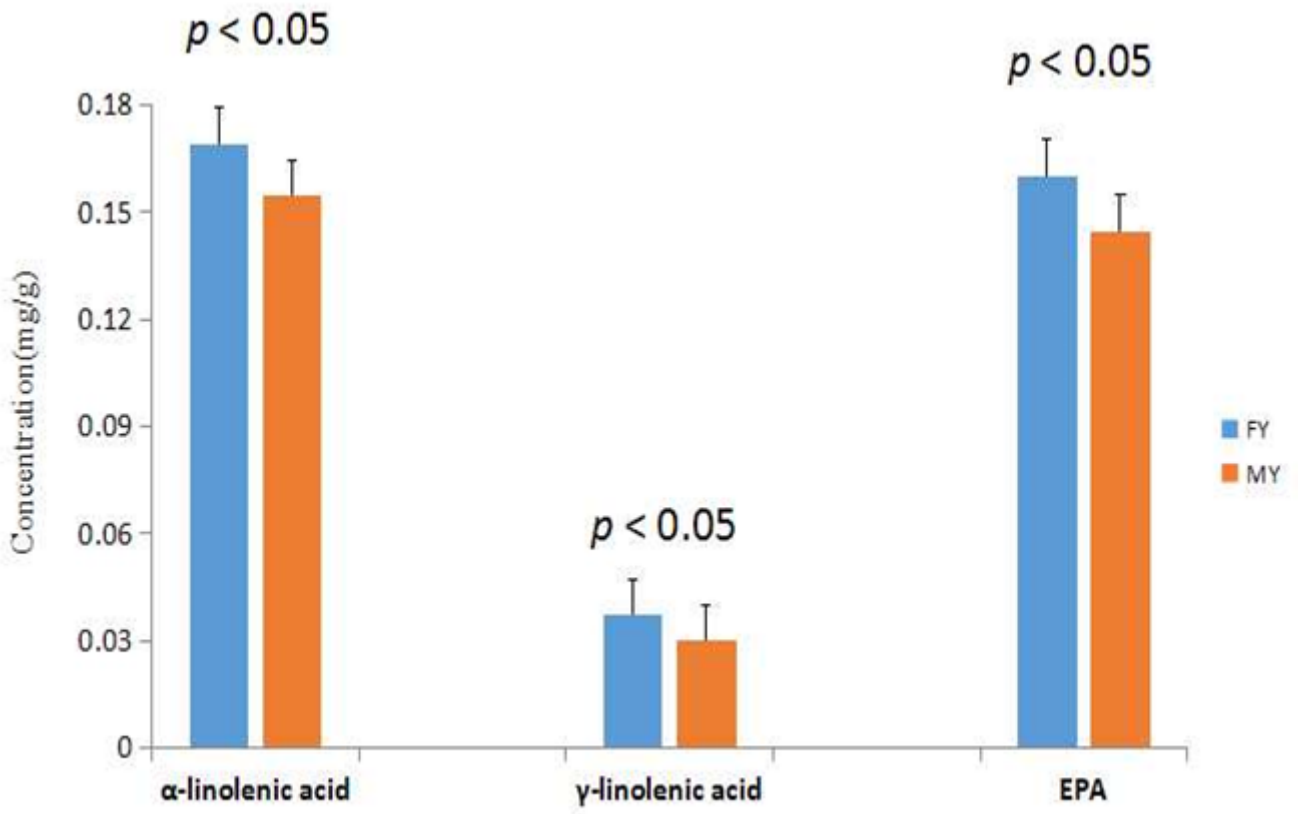

Figure 6

The absolute concentration of α-linolenic acid, γ-linolenic acid and eicosapentaenoic acid (EPA) in subcutaneous fat of female and male yaks by the gas chromatography (GC) analysis. FY, female yaks; MY, male yaks

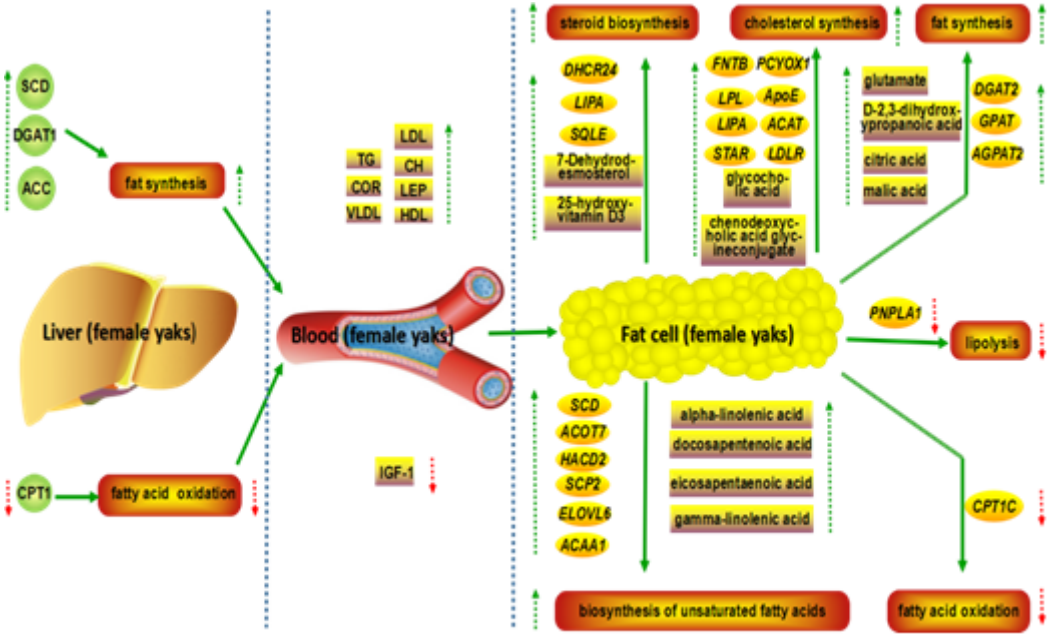

Figure 7

The fat metabolism in famle yaks by contrast with male yaks.↑: upregulation of gene expression or increased of metabolite or enhanced metabolic pathway;↓: downregulation of gene expression or decreased of metabolite or diminished metabolic pathway.

Figure 8

The regulatory mechanism of fat depossition in female yaks by contrast with male yaks.→: promote or result in;⊥: repress gene expression, diminish metabolic pathway or inhibit physiological process.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- TableS6.xlsx
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- TableS2.xls
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