Biochemistry and Cell Biology
Biochimie et biologie cellulaire

**Ameliorating effects of *Sporidiobolus pararoseus* extract on
dyslipidemia in mice with high-fat diet-induced obesity**

Journal:	<i>Biochemistry and Cell Biology</i>
Manuscript ID	bcb-2017-0332.R1
Manuscript Type:	Article
Date Submitted by the Author:	30-Mar-2018
Complete List of Authors:	Du, Chao; Ludong University, School of Food Engineering; Jiangnan University Ying, Danyu; Jiangnan University Guo, Yahui; Jiangnan University Cheng, Yuliang; Jiangnan University Han, Mei; Jiangnan University Zhang, Weiguo; Jiangnan University Qian, He; Jiangnan University
Is the invited manuscript for consideration in a Special Issue? :	N/A
Keyword:	Lipid metabolism, Dyslipidemia, High-fat diet-induced obesity, Unsaturated Fatty Acids, <i>Sporidiobolus pararoseus</i> extract

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Manuscripts

1 **Original Article**

2 **Ameliorating effects of *Sporidiobolus pararoseus* extract on dyslipidemia in mice with high-fat**
3 **diet-induced obesity**

4 **Running title: Hyperlipaemia-moderating effect of *S. p.* extract**

5 Chao Du^{1,2*}, Danyu Ying², Yahui Guo², Yuliang Cheng², Mei Han³, Weiguo Zhang³, He Qian^{12,4*}

6 ¹School of Food Engineering, Ludong University, 186 Middle Hongqi Road Yantai, Shandong Province, 264025, P. R. China

7 ²School of Food Science and Technology, Jiangnan University; ³School of Biotechnology, Jiangnan University

8 ⁴National Engineering Research Center for Functional Food, Jiangnan University

9 1800 Lihu Avenue Wuxi, Jiangsu Province, 214122, P. R. China

Authors' name	Email addresses	Affiliations	Postal addresses	Telephone numbers
Chao Du	516826782@qq.com	School of Food Engineering, Ludong University &School of Food Science and Technology, Jiangnan University	Ludong University, 186 Hongqi Road Yantai, Shandong Province, 264025, P. R. China	+86-535-669 5491
Danyu Ying	593924769@qq.com	School of Food Science and Technology, Jiangnan University	Jiangnan University 1800 Lihu Avenue Wuxi, Jiangsu Province, P. R. China	+86-510-8532 8713
Yahui Guo	601609769@qq.com			
Yuliang Cheng	wxfoodcyl@126.com			
Mei Han	hanmei.918521@163.com	School of Biotechnology, Jiangnan University		
Weiguo Zhang	zhangwg168@126.com			
He Qian	amtf168168@126.com	School of Food Science and Technology, Jiangnan University Synergetic Innovation Center Of Food Safety and Nutrition, Jiangnan University		

*Corresponding author; E-mail: 516826782@qq.com; ruindu@163.com

Tel: +86-510-8532 8713; Fax: +86-510-8532 9081;

The research was conducted in the School of Food Science and Technology of Jiangnan University.

1 **Abstract:**

2 The study aims to elucidate the intervention effect of *Sporidiobolus pararoseus* (*S. p.*) extract on the
3 lipid metabolism in Kunming mice with high-fat diet-induced obesity with Max EPA as a control. Ten
4 mice were randomly selected from 60 mice as control group and the remaining 50 mice were fed with a
5 high-fat diet to establish a dyslipidemia model. After 4 weeks, these 50 mice were randomly divided
6 into 5 groups: high-fat model group, Max EPA group, and the 3 groups of different doses of *S. p.*
7 extract (LD, MD and HD). After eight weeks, the mice were sacrificed and related parameters were
8 measured. Compared with the high-fat group, *S. p.* extract (HD) group showed the significantly
9 decreased body weight and serum TC, TG and LDL-c levels as well as the increased HDL-c level.
10 RT-PCR results showed that the mRNA expression of SREBP-1c, FAS and ACC in *S. p.* extract
11 supplementation group was lower than those in high-fat diet group whereas the expression of CPT-1 in
12 *S. p.* extract supplementation group was higher than that of the high-fat diet group. Our results implied
13 that the intake of *S. p.* extract might benefit the patients with dyslipidemia. Therefore, *S. p.* extract may
14 be developed as a dietary supplement for ameliorating lipid metabolism of obese people.

15
16 **Keywords:**²

17 *Sporidiobolus pararoseus* extract; Lipid metabolism; Dyslipidemia; High-fat diet-induced obesity;
18 Unsaturated Fatty Acids

Abbreviations:

TC: Total Cholesterol
TG: Triglyceride
HDL-c: High-Density Lipoprotein cholesterol
LDL-c: Low-Density Lipoprotein cholesterol
S. p.: *Sporidiobolus pararoseus*
S. p. extract-LD: low dose of *S. p.* extract
AI: Atherosclerosis Index
H&E: Hematoxylin And Eosin
SD: Standard Deviation
HFD: High-Fat Diet
FFAs: Free Fatty Acids
SREBP-1c: Sterol Regulatory Element-Binding Protein 1c
ACC: Acetyl-CoA Carboxylase
CPT-1: Carnitine Palmitoyltransferase 1

1. Introduction

Dyslipidemia is characterized by elevated total cholesterol (TC) and triglyceride (TG) and decreased high-density lipoprotein cholesterol (HDL-c) levels and usually co-exists with obesity.(Kim et al. 2016; Shih et al. 2013) Recently, dyslipidemia has become an increasingly serious social problem, and the treatment cost of obesity-related illness represents a significant expenditure in national healthcare budgets in both developed and developing countries.(Jall et al. 2017; Jing et al. 2013; Lee et al. 2013a) The over-consumption of industrialized foods containing rich fat is directly implicated in the above situation. The dyslipidemia individuals have the particular risk of Type 2 diabetes, hyperlipidemia, cardiovascular problems and certain cancers. (Bae et al. 2017; Choi et al. 2011; Vinuesa et al. 2016) Given the low efficacy and side effects of drugs, more attention had been paid to dietary prevention and intervention for dyslipidemia and related disorders. (Andrade et al. 2013; Jung et al. 2016; Park et al. 2015)

Sporidiobolus pararoseus (*S. p.*) belongs to the class *Urediniomycetes* and can biosynthesize intracellular oil in abundance (about 50% of dry weight). (Han et al. 2016a; Han et al. 2012; Han et al. 2016b) *S. p.* extract contains large amounts of unsaturated fatty acids and other nutrient substances, such as carotenoids, squalene and ergosterol.(Du et al. 2017a, b) Unsaturated fatty acids have a favorable effect on the regulation of blood lipids and can also effectively prevent cardiovascular diseases.(Siscovick et al. 2017) Ergosterol is a plant sterol and has been used as a novel functional ingredient for it can lower plasma cholesterol level by inhibiting the absorption of cholesterol. (Gil-Ramírez et al. 2014) Carotenoids are well known as natural antioxidants, which have inhibition effects on the proliferation of various tumor cells.(Du et al. 2015, 2016) Accordingly, *S. p.* extract may have the good regulation effect on blood lipids, but its regulation effect has not been reported.

Recently, emerging evidences have shown that sterol regulatory element-binding proteins, such as SREBP-1c, are the target of a variety of treatments for metabolic diseases. SREBP-1c plays an important role in the regulation of cholesterol and fatty acid biosynthesis. Moreover, it also targets the genes involved in fatty acid metabolism, such as FAS, ACC and CPT-1.(Li et al. 2016) To our knowledge, the role of *S. p.* extract in SREBP-1c signaling regulation has not been reported.

The study aims to investigate the effect of *S. p.* extract supplement on ameliorating dyslipidemia and regulating lipid metabolism in the high-fat diet-induced obese mice model. The effects of *S. p.* extract supplement on dyslipidemia-related parameters, such as serum levels of TC, TG, HDL-c, and LDL-c, were evaluated. The histological changes and relative molecular mechanisms were also investigated.

2. Materials and Methods

2.1 Regents

Sporidiobolus pararoseus yeast cells were cultivated and enriched according to a previously published method. (Han et al. 2012) Cell walls of *S. p.* were broken by high-pressure homogenization and then the lipid was extracted with ethyl acetate. The general composition of *S. p.* extract was determined by High Performance Liquid Chromatography (Supplementary material). Other chemicals and reagents with the commercially available highest degree of purity were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China).

2.2 Mice and experimental design

Eight-week-old Kunming male mice obtained from SLAC Laboratory Animal (Shanghai, China) were used in all the experiments. The mice were housed in standard cages under the temperature (23 ± 2 °C) and humidity (60 ± 5 %) with a 12-h light/dark cycle and fed with commercial standard chow (SLAC

1 Laboratory Animal, Shanghai, China) and water ad libitum. After 1 week of acclimatization, 10 mice
2 were randomly selected from 60 mice as the control group and the remaining 50 mice were fed with a
3 high-fat diet (78.8% basic forage, 1% cholesterol, 20% lard and 0.2% cholic acid sodium) to establish
4 an obesity model. After 4 weeks, the 50 mice were randomly divided into 5 experimental groups (n =
5 10) and fed with different diets (Table 1).

6 During the experimental process, *S. p.* extract, Max EPA or normal saline was administered
7 intragastrically by gavage every day. The control group mice were fed with basic forage and
8 intragastric administration was performed with normal saline once a day. Food consumption and
9 weight gain were respectively measured daily and weekly. At the end of the experimental period, the
10 mice were anesthetized with aether withholding food for 12 h, and blood was drawn from eyeball to
11 determine the plasma biomarkers. The liver and epididymal white adipose tissue were removed after
12 blood collection, rinsed with a physiological saline solution, weighed, and processed for histological
13 (H&E) staining and further analysis. (Du et al. 2016; Jing et al. 2013)

14 All experimental procedures involving animals in this research were carried out in accordance with the
15 Guide for the Care and Use of Laboratory Animals (1996, published by National Academy Press, 2101
16 Constitution Ave. NW, Washington, DC 20055, USA). The approval of this experiment was obtained
17 from the Institutional Animal Ethics Committee of Jiangnan University (Wuxi, China). All animal
18 protocols were reviewed and approved by the Animal Care and Use Committee of Jiangnan University
19 (JN No. 20160301-20160531[13]).

20 **2.3 Serum chemistry analysis**

21 Mice were fasted for 12 h before gathering blood samples for the serum chemistry analysis. The serum
22 was collected and serum total cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol

(HDL-c) and low-density lipoprotein cholesterol (LDL-c) were measured using a Roche Cobas C501 Automatic Analyzer (Roche, Ltd., Germany). Levels of atherosclerosis index (AI) were calculated according to the following formula: $AI = (TC - HDL-c) / HDL-c$. (Lim et al. 2015)

2.4 Histological analysis of liver and white adipose tissue

Liver and epididymal white adipose tissue were removed from the mice and fixed in 10% buffered formalin. Tissues were processed and paraffin blocks were made according to conventional tissue processing steps. For morphological analysis, paraffin blocks were cut into 5- μ m thick sections and mounted on the slides. The slides were subsequently stained with hematoxylin and eosin (H&E) for evaluation under a standard light microscope. (Li et al. 2016)

2.5 RNA extraction and quantitative real-time PCR

Total RNA was extracted using the TRIzol reagent (Shanghai Generay Biotech Co., Ltd., China). The concentration and purity of the RNA samples were determined spectroscopically. RNA was reversely transcribed into cDNA with Revert Aid TM M-Mu LV Reverse Transcriptase (Thermo Scientific, Rockford, USA) according to manufacturer's instructions. For each target mRNA, 1 μ L of cDNA was mixed with SYBR Green PCR Premix (TaKaRa, Dalian, China) and 0.8 μ L of each specific forward/reverse primer in a final volume of 10 μ L. The sequences of the primer sets are shown in Table 2 and synthesized by Generay Biotechnology (Shanghai, China). The PCR amplification was monitored in real time with the ABI 7900 Fast Real-Time PCR system (Applied Biosystems, Carlsbad, CA, USA). Gene expression was quantified by the method described before. (Du et al. 2017a; Vinuesa et al. 2016)

2.6 Statistical analysis

Data are expressed as the mean $\pm$ standard deviation (SD). Experimental data were analyzed by

1 one-way analysis of variance (ANOVA) using SPSS version 21.0. P -value < 0.05 was considered to be
2 statistically significant.

3

4 **3. Results:**

5 ***3.1 Effects of *S. p.* extract supplement on body weight and food intake***

6 Average weekly weight gain for 8 weeks in the *S. p.* extract (LD, MD and HD) supplementation groups
7 was significantly lower than that in the high-fat diet (HFD) group (1.28 ± 0.15 , 1.11 ± 0.18 , 1.00 ± 0.10 vs
8 1.56 ± 0.21 g/week). Among various *S. p.* extract groups of different doses, *S. p.* extract HD group
9 particularly exhibited the more significant response of suppressing weight gain (Fig. 1a). Moreover, *S.*
10 *p.* extract-LD supplement exhibited a similar effect on the weight gain as Max EPA in the end of the
11 experiment. In addition, *S. p.* extract supplement did not affect food intake (Fig. 1b) in all high-fat diet
12 groups. (Lee et al. 2013b)

13 ***3.2 Effects of *S. p.* extract supplement on serum lipid profiles***

14 To determine the dyslipidemia-ameliorating effects of *S. p.* extract on HFD-fed mice, circulating lipid
15 levels in serum, including TC, TG, HDL-c, and LDL-c, were measured (Fig. 2). HFD led to
16 significantly higher TC and LDL-C levels ($P < 0.05$) compared with the control group, whereas *S. p.*
17 extract supplement resulted in a significant reduction in serum levels of TC and LDL-c compared with
18 the HFD group. In contrast, serum TG or HDL-C levels showed no significant difference among the
19 groups. Atherosclerosis index (AI) had been considered as an important indicator of vascular risks and
20 significant predictors of atherosclerosis. Therefore, AI values of all groups were also determined. The
21 AI values of the HFD group were significantly higher than that of the control group. Besides, the *S. p.*
22 extract (LD, MD and HD) supplements effectively reduced AI values by 5.80%, 27.03% and 37.94%

compared with the HFD group.(Lee et al. 2014)

3.3 Effects of *S. p.* extract supplement on hepatic and adipose tissue morphology

S. p. extract supplement reduced the hepatic lipid droplets and average epididymal adipocyte size compared with the HFD group. In mouse livers of the control group, normal lobular architecture and cellular structures were accompanied by a few fat droplets (Fig. 3a). In contrast, the livers of HFD mice exhibited severe steatosis, whereas *S. p.* extract supplementation groups exhibited a lower grade of steatosis and a different distribution of fat droplets. Moreover, the *S. p.* extract-HD supplementation groups had the much smaller average epididymal adipocyte size compared with the HFD group (Fig. 3b).(Norris et al. 2017)

3.4 Effects of *S. p.* extract supplement on the mRNA expressions of *SREBP-1c*, *FAS*, *ACC*, and *CPT-1* in liver

To investigate the molecular mechanisms of *S. p.* extract supplement in the regulation of lipid metabolism in liver tissues, gene expressions of *SREBP-1c*, *FAS*, *ACC*, and *CPT-1* were analyzed (Fig. 4). These genes play key roles in fatty acid synthesis and β -oxidation.(Li et al. 2016) As shown in Fig. 4, mRNA expressions of *SREBP-1c*, *FAS*, *ACC* and *CPT-1* were up-regulated in the HFD group compared with the control group ($P<0.05$). In contrast, *S. p.* extract supplement exhibited significantly lower mRNA levels of *SREBP-1c*, *FAS*, and *ACC* than those in the HFD group ($P<0.05$) and the significantly higher expression levels of *CPT-1* than that in HFD group. Interestingly, the regulating effects on the mRNA expression of certain genes of groups treated with *S. p.* extract-HD were comparable to that of Max EPA.

4. Discussion:

1 Dyslipidemia is a metabolic disorder, which is characterized by abnormally high levels of serum
2 triglyceride (TG) and/or cholesterol (TC), increased low-density lipoprotein (LDL) cholesterol, and
3 decreased high-density lipoprotein (HDL) cholesterol. (Jing et al. 2013; Norris et al. 2017)
4 *Sporidiobolus pararoseus* (*S. p.*) extract contains large amounts of unsaturated fatty acids as well as
5 other nutrient substances, such as carotenoids, squalene and ergosterol.(Du et al. 2017a, b) Both
6 unsaturated fatty acids and ergosterol had a favorable effect on the regulation of lipid metabolism, thus
7 effectively preventing dyslipidemia and related diseases.(Gil-Ramírez et al. 2014; Siscovick et al. 2017)
8 In the present study, we investigated the effects of *S. p.* extract on obesity and dyslipidemia and
9 analyzed the underlying mechanisms with high-fat diet-induced (HFD) obese mice. We found that *S. p.*
10 extract significantly reduced body weight gain and plasma TC and LDL-c levels. Furthermore, *S. p.*
11 extract regulated the mRNA expressions of lipid metabolism-related genes in the *S. p.* extract
12 supplementation group compared with those in HFD-induced obese mice. These results suggest that *S.*
13 *p.* extract possesses ameliorating effect on dyslipidemia in obese mice.
14 In our study, eight-week-old Kunming mice were fed with either basic forage or high-fat diet (HFD) for
15 12 weeks. There was no statistically significant difference in initial mouse weight among the
16 experimental groups. The effects of the HFD on weight gain were observed after the model was
17 successfully established. *S. p.* extract supplement significantly reduced final body weight compared
18 with mice fed with HFD alone under the same food intake, indicating that *S. p.* extract had an attractive
19 effect on the body weight control of HFD-induced obesity.(Choi et al. 2011)
20 Previous studies suggested that HFD-induced obesity changed lipid metabolism including metabolic
21 parameters such as TC, TG, HDL-c and LDL-c. Increased levels of TC and LDL-c accompanied by
22 low levels of HDL-c were major risk factors for atherosclerosis and cardiovascular diseases.(Kim et al.

2016) Thus, the increased level of (TC-HDL-c)/HDL-c was considered as major predictors of atherosclerosis.(Lim et al. 2015) In the study, *S. p.* extract supplement significantly improved the lipid profiles by lowering the levels of TC and LDL-c in MD and HD groups, morphological improvements in livers and epididymal white adipose tissues were also observed through histological analysis. These results indicated that *S. p.* extract had beneficial effects on ameliorating dyslipidemia through body weight control and lipid metabolism regulation.

To further understand the beneficial effects of *S. p.* extract on lipid metabolism in obese-related disorders, it is necessary to investigate the molecular mechanisms. The liver is a major fat metabolizing organ where saturated free fatty acids (FFAs) are synthesized, esterified, and packaged into low-density lipoprotein for export, thereby preventing the retention of fat. Excessive dietary fat intake can increase serum FFAs levels and enhance the delivery of FFAs to the liver. As a result, these excessive FFAs are commonly associated with metabolic syndromes, including obesity, dyslipidemia and atherosclerosis.(Lim et al. 2015; Park et al. 2015)

Therefore, we investigated the gene expressions related to lipid metabolism in liver. Sterol regulatory element-binding protein 1c (SREBP-1c) plays a great role in fatty acid synthesis and is highly expressed in adipose, liver and brain tissues of mice and humans. SREBP-1c enhances the expression levels of other lipogenic genes, including FAS, an enzyme which can stimulate the de novo synthesis of fatty acids and catalyze the synthesis of saturated FFAs. Moreover, FAS also plays an important role in regulating body weight and dietary intake. During dyslipidemia, when FFA synthesis is stimulated by some enzymes such as FAS and acetyl-CoA carboxylase (ACC, a regulator of fatty acid oxidation), the processes of mitochondrial and peroxisomal β -oxidation are suppressed. Under normal circumstances, peroxisomal β -oxidation is a minor pathway of fatty acid oxidation compared with the mitochondrial

1 β -oxidation. However, when an animal is under a lipid-overloaded state and metabolic lipids are
2 dysregulated, peroxisomal β -oxidation is stimulated. Carnitine palmitoyltransferase 1 (CPT-1) is a
3 rate-limiting enzyme in mitochondrial fatty acid oxidation and the increased expression of CPT-1 is
4 associated with an up-regulation of fatty acid β -oxidation. (Jing et al. 2013; Lee et al. 2013b; Wakutsu
5 et al. 2012) In the study, the gene expressions of SREBP-1c, FAS and ACC were found to be reduced in
6 liver tissues from *S. p.* extract supplementation group compared with their mRNA expression in HFD
7 only mice, while the expression level of CPT-1 was increased. In *S. p.* extract-HD group, SREBP-1c,
8 FAS and ACC gene expressions were respectively significantly reduced by 20.31%, 18.73%, and
9 30.26%, whereas CPT-1 expression in *S. p.* extract-HD group was enhanced by 149.44% compared
10 with that in the HFD group. These results implied that *S. p.* extract supplement ameliorated
11 dyslipidemia and obesity through FFA synthesis inhibition and fatty acid oxidation acceleration.
12 In conclusion, the data in the study demonstrated that *S. p.* extract supplement reduced body weight
13 gain and prevented the development of dyslipidemia through improving blood lipid profiles and
14 lowering lipid accumulation in the liver of mice with an HFD. Furthermore, these effects appeared to
15 be mediated through the inhibition of the mRNA expressions of SREBP-1c, FAS and ACC as well as
16 the activation of CPT-1 expression. Thus, these results provide valuable insights into the possible
17 application of *S. p.* extract as a dietary supplement for the prevention of obesity-related metabolic
18 disorders, and further studies are ongoing.

19

20 **Acknowledgment:**

21 This article is supported by National Key Technology R&D Program in the 12th Five year Plan of
22 China (No.2015BAD16B01), Forestry science and technology innovation and extension project of

1 Jiangsu Province (LYKJ[2017]26) and Policies to guide class plan (cooperative) in Jiangsu Province
 2 (BY2016022-01).

3 **Conflict of interest:**

4 The authors declare that they have no competing interests.

5

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Figure Legends:

Figure 1 Supplementary effects of *S. p.* extract on (a) body weight and (b) food intake in mice fed with a high-fat diet. Data are expressed as mean $\pm$ SD (n = 10). After the dyslipidemia model was established, male Kunming mice were fed with or without various doses of *S. p.* extract (0.8 g/kg bw, 1.6 g/kg bw and 3.2 g/kg bw) by oral gavage once daily for eight weeks.

Figure 2 *S. p.* extract improved serum lipid profile and dyslipidemia in mice with high-fat diet-induced obesity. (a–d) Serum total cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol (HDL-c) and low-density lipoprotein cholesterol (LDL-c) concentrations. (e) Effects of *S. p.* extract on atherosclerosis index (AI) in obese mice. Data are expressed as mean $\pm$ SD (n = 10). The values with different letters are significantly different ($P < 0.05$). *S. p.* extract–LD (low dose of *S. p.* extract, 0.8 g/kg bw), *S. p.* extract –MD (middle dose of *S. p.* extract, 1.6 g/kg bw); *S. p.* extract–HD (high dose of *S. p.* extract, 3.2 g/kg bw).

Figure 3 Hematoxylin and eosin staining of (a) hepatic tissue and (b) adipose tissue in mice from different treatment groups. Original magnification, $\times 200$.

Figure 4 mRNA expression of SREBP-1c, FAS, ACC and CPT-1 in the liver of mice from different treatment groups. Data are expressed as mean $\pm$ SD (n = 6). The values with different letters are significantly different ($P < 0.05$). β -actin was used as the control. *S. p.* extract–LD (low dose of *S. p.* extract, 0.8 g/kg bw), *S. p.* extract –MD (middle dose of *S. p.* extract, 1.6 g/kg bw); *S. p.* extract–HD (high dose of *S. p.* extract, 3.2 g/kg bw).

20

Figure 1

254x93mm (300 x 300 DPI)

Figure 2

247x123mm (300 x 300 DPI)

Figure 3

236x229mm (300 x 300 DPI)

Figure

280x191mm (300 x 300 DPI)

262x163mm (300 x 300 DPI)

Table

Table1 Intragastric administration substances and doses in each group

Groups	Intragastric administration substances	Doses
High-fat model group	Normal saline	
Max EPA group	Max EPA	0.8 g/kg bw
<i>S. p.</i> extract-LD	Low dose of <i>S. p.</i> extract	0.8 g/kg bw
<i>S. p.</i> extract-MD	Middle dose of <i>S. p.</i> extract	1.6 g/kg bw
<i>S. p.</i> extract-HD	High dose of <i>S. p.</i> extract	3.2 g/kg bw

Table 2 The sequence of primers used for quantitative real-time PCR

Gene name	Forward Primer (5'-3')	Reverse Primer (5'-3')
SREBP-1c	CTGCTTGGCTCTTCTCTTT	CTTGTTTGCGATGTCTCC
FAS	TTCGGTGTATCCTGCTGT	GGCTTGTCCTGCTCTAACT
ACC	GGCAGCAGTTACACCACATAC	TCATTACCTCAATCTCAGCATAGC
CPT-1	GAGAGGGGAGGACAGAGA	ACACCACATAGAGGCAGAAG
β -actin	GGGAAATCGTGCGTGAC	AGGCTGGAAAAGAGCCT