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RESEARCH ARTICLE

Effects of Different Degrees of Cold Stress on FIAF Expression in Pigs

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Abstract

Cold stress is the main stressor restricting the development of animal husbandry in cold regions. Fasting-induced adipose factor (FIAF), also known as angiopoietin-like protein 4 (ANGPTL4), plays an important regulatory role in the metabolism of lipids. Its functions include inhibiting lipoprotein lipase (LPL) to eliminate triglycerides and free fatty acids in blood, reducing fat deposition and promoting adipose tissue degradation. This experiment was designed to investigate the effects of different degrees of cold stimulation on fat metabolism in finishing pigs. Growing and fattening pigs were randomly divided into different groups and exposed to temperatures of -10±2°C, -5±2°C, 0±2°C, 5±2°C and 21±2°C for 2 h. Serum, liver, neck, abdominal subcutaneous and mesenteric adipose tissues were collected and analyzed by Real-Time quantitative PCR (qRT-PCR), western blotting and enzyme-linked immunosorbent assay (ELISA) to examine FIAF expression. The results showed that a gradual increase in cold stress intensity resulted in a gradual increase in FIAF mRNA and protein expression levels in liver, neck, abdomen and mesenteric adipose tissues and FIAF concentration also gradually increased in the blood. It indicated that FIAF is involved in energy and fat metabolism in response to cold stress and may be regulated by the activation of peroxisome proliferator-activated receptor (PPAR) by free fatty acids in the blood induced by cold stress.

Keywords: *Cold stress, Fat tissue, Fasting-induced adipose factor, FIAF, Pig*

Farklı Derecelerde Soğuk Stresinin Domuzlarda FIAF Ekspresyonuna Etkileri

Öz

Soğuk stresi, soğuk bölgelerde hayvancılığın gelişmesini kısıtlayan ana stres faktörüdür. Anjiopoietin benzeri protein 4 (ANGPTL4) olarak da bilinen Fasting-induced adipose faktör (FIAF), yağların metabolizmasında önemli bir düzenleyici rol oynar. FIAF'ın fonksiyonları arasında, kandaki trigliseritleri ve serbest yağ asitlerini uzaklaştırmak için lipoprotein lipazı (LPL) inhibe etmek, yağ birikimini azaltmak ve yağ dokunun bozulmasını teşvik etmek bulunur. Bu çalışma, farklı derecelerde soğuk uyarımın, besili domuzlarda karaciğerin FIAF ve yağ metabolizması üzerine etkilerini araştırmak için tasarlanmıştır. Ergin ve besili domuzlar rastgele farklı gruplara ayrıldı ve 2 saat boyunca -10±2°C, -5±2°C, 0±2°C, 5±2°C ve 21±2°C sıcaklıklara maruz bırakıldı. Serum, karaciğer, boyun yağ dokusu, abdominal subkutanöz yağ doku ve mezenterik yağ doku toplandı ve FIAF ekspresyon analizi, kantitatif real-time PCR (qRT-PCR), Western Blot ve Enzim-işaretli immünosorbent test (ELISA) ile gerçekleştirildi. Sonuçlar, soğuk stres yoğunluğundaki kademeli artışın, karaciğer, boyun yağ dokusu, karın yağ dokusu ve mezenterik yağ dokusunda FIAF'ın mRNA ve protein ekspresyon seviyelerinde kademeli bir artışa neden olduğunu ve ayrıca kanda FIAF konsantrasyonunun da kademeli olarak arttığını gösterdi. FIAF'ın soğuk stresine yanıt olarak enerji ve yağ metabolizmasında rol oynadığını ve soğuk stresi ile indüklenen kandaki serbest yağ asitleri tarafından Peroksizom proliferatör-aktive reseptör (PPAR) aktivasyonu ile düzenlenebilir olduğunu gösterdi.

Anahtar sözcükler: *Domuz, FIAF, Soğuk stresi, Yağ doku*

INTRODUCTION

Cold stress is the main stress factor in the agricultural production process in cold regions. Low environmental temperatures in animal housing cause a series of physiological changes, including accelerated breathing, vasoconstriction, enhanced endocrine activities and accelerated nutrient and energy metabolism to maintain

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a normal body temperature [1]. Cold stress also causes accelerated fat mobilization and weight loss, decreased meat quality and impaired growth in animals, affecting animal welfare and resulting in significant impairments to the healthy development of the agriculture industry. Thermogenesis is required to maintain euthermia in changing external temperatures and is a physiological process performed predominantly by adipose tissue [2]. In addition, adaptive heat production mediated by adipose tissue is essential for chronic adaptation to cold. Studies have shown that long-term cold exposure can increase the rate of lipolysis in adipose tissue. Therefore, the study of fat metabolism in livestock and poultry subjected to cold stress would have theoretical and practical significance for animal production and fat metabolism in cold environmental conditions.

Adipose tissue acts as an endocrine organ and produces numerous bioactive factors, such as adipokines that communicate with other organs and modulate a range of metabolic pathways [3]. For example, adipocytokines are substances secreted by adipose tissue that have a variety of biological functions. In the process of fat metabolism, they are directly involved in the hydrolysis of triglycerides, the oxidation of free fatty acids and free fatty acid mobilization in non-adipose tissues. Adipocytokines in animals mainly include leptin, adiponectin, resistin and fasting-induced adipose factor (FIAF) [4]. FIAF, also known as angiopoietin-like protein 4 (ANGPTL4), plays an important regulatory role in the metabolism of lipids, and its functions include the inhibition of lipoprotein lipase (LPL) to eliminate triglycerides and free fatty acids in the blood, the reduction of fat deposition and the promotion of adipose tissue degradation [5]. The action of adipokines is mainly mediated by binding to their respective receptors on the membrane of target cells, which triggers intracellular signaling pathways [3]. Previous studies have found that some food nutrients, fasting, endurance training and gut microbes can affect FIAF concentrations in blood [6]. The aim of this study was to assess FIAF expression levels in various tissues and in blood to identify the role of FIAF in fat metabolism of pigs under cold stress.

Material and Methods

Sample Collection

Pig breed: Junmu No. 1 pig, provided by: Jida original pig farm (Changchun, China). The environment of the pig house is so clean that the pigs are well fed with feed and drinking water every day. A total of 30 healthy Junmu No. 1 pigs with an average body weight of 60±5 kg was randomly divided into five groups, with six pigs in each group. The control group of pigs were kept at 21±2°C prior to slaughter. The test groups of pigs were exposed to -10±2°C, -5±2°C, 0±2°C or 5±2°C for 2 h prior to slaughter. Both the control group and the test groups were slaughtered in the Jida original pig farm. The blood was collected from the femoral artery of the pig to separate serum before slaughter. And the liver tissue, abdominal fat tissue, neck fat tissue, mesenteric fat tissue was collected after slaughter. All samples were labeled and immediately stored in liquid nitrogen. The protocol was approved by the Animal Ethics Committee of Jilin University.

qRT-PCR

Approximately 100 mg tissue samples (liver tissue, abdominal fat tissue, neck fat tissue, and mesenteric fat tissue) were separately collected and placed in liquid nitrogen. Total RNA was then extracted using the one-step TRIZOL method (Invitrogen Corp., Carlsbad, CA, USA). A PrimeScript^{T}M RT-PCR Kit (Takara Biotechnology Co., Dalian, China) was used to reverse transcribe total RNA samples. The primer and probe sequences of FIAF and the reference gene, GAPDH, are shown in *Table 1* (synthesized by GeneCore Biotechnology Co., Shanghai, China). The PMD18-T vector was used to construct the recombinant gene plasmid (Takara), which was transformed into Escherichia coli DH5α host bacteria. After screening for ampicillin resistance, a BioTeke Plasmid DNA miniprep Kit (BioTeke Corp., DP1001 Beijing, China) was used to extract standard plasmid to create a standard curve. A Mastercycler® ep realplex Real-time PCR instrument (Eppendorf, Hamburg, Germany) was used to measure gene expression. The reaction system included 12.5 μL Premix Ex Taq™ (2×) (Takara), 10 μM upstream primer, 0.5 μL downstream primer, 1 μL probe, 2 μL cDNA and 8.5 μL ddH_2O. The template-free negative control (NTC) was set as the control. The obtained standard curve indicated that the amplification efficiency of the two genes was 100%. Therefore, the $2^{-\triangle\triangle Ct}$ method was used to calculate the relative expression levels of genes [7]. Each sample was measured in triplicate.

Western Blotting

Protein samples were ground into homogenates after being weighed, and a Bicinchoninic acid (BCA) protein assay kit (BioTeke., Beijing, China) was used to measure total protein concentration. Proteins were separated on 5% and 12% polyacrylamide gels (Bio-Rad Laboratories Inc., Hercules, CA, USA), and a Trans-Blot SD semi-dry transfer tank (Bio-Rad) was used to transfer separated proteins onto a polyvinylidene difluoride membrane (Millipore Corp., Burlington, MA, USA). The membrane was blocked, incubated with detection antibody (1: 2000 dilution) followed by the horseradish peroxidase-conjugated secondary antibody (1: 2000 dilution) for 2 h and developed in the dark-room. Antibody information was shown in *Table 2*.

ELISA

The concentration of FIAF in serum was measured with a Porcine of Angiopoietin-Like Protein 4 ELISA Kit (R&D Systems Inc., Shanghai, China) according to the manufacturer's instructions.

Table 1. *List of primers and probes used for quantitative real-time PCR*

Target	Accession Number	Primers and Probe	Amplicon Length (bp)
FIAF	AY751522	F: 5′-CTGGTGGTTGGTGGTTTGG-3′ R: 5′-GCTGCCGAGGGATGGAAT-3′ P: 5′-(FAM) TGACCTCCGCGCCCTGGC (Eclipse) -3′	75
GAPDH	NM_001206359.1	F: 5′-CTGACCTGCCGTCTGGAGAA-3′ R: 5′-TAGCCCAGGATGCCCTTGAG-3′ P: 5′-(FAM) CCTCGGACGCCTGCTTCACCACCT (Eclipse)-3′	95

Table 2. *List of antibodies used in this study*

Antibody Name		Species in which the Antibody was Raised	Dilution used in Western Blotting	Manufacturer of the Antibody
Primary antibody	FIAF	Rabbit	1: 500	Bioss
	β-actin	Goat	1: 2000	Santa cruz
Secondary antibody	FIAF	Goat Anti-Rabbit IgG	1: 1000	Santa cruz
	β-actin	Rabbit Anti-Goat IgG	1: 1000	Santa cruz

Statistical Analysis

Statistical analysis and comparisons between groups were performed using one-way ANOVA. All data were expressed as mean ± standard deviation (mean±SD). P≤0.05 was considered statistically significant. All analyses were performed by SPSS 17.0 software and Graphpad Prism (version 7.0).

Results

Expression of FIAF mRNA in Different Tissues

Fig. 1, 2, 3, 4 showed that a decrease in ambient temperature resulted in a gradual increase in levels of FIAF mRNA in different tissues. In pigs exposed to 5°C for 2 h, FIAF mRNA levels in neck fat tissue were significantly higher than those of the control group (21°C) (P<0.01). In pigs exposed to 0°C for 2 h, FIAF mRNA levels in liver tissue (P<0.05) and neck fat tissue (P<0.01) were significantly higher than those of the control group. In pigs exposed to -5°C for 2 h, FIAF mRNA levels in mesenteric fat tissue (P<0.01), abdominal fat tissue (P<0.05), liver tissue (P<0.01) and neck fat tissue (P<0.001) were significantly higher than those of the control group. In pigs exposed to -10°C for 2 h, FIAF mRNA levels were significantly higher in liver tissue (P<0.01), abdominal fat tissue (P<0.01), mesenteric fat tissue (P<0.01) and neck fat tissue (P<0.001) than those of the control group.

Moreover, there were significant differences between the -5°C group and other three groups (5°C, 0°C and -10°C) in liver tissue (P<0.05). There were significant differences between the -10°C group and other two groups (5°C and 0°C) in liver tissue (P<0.01). For the abdominal fat tissue, there were significant differences between the -10°C group and other groups (P<0.01), and there were significant differences between the -5°C group and other two groups (5°C and 0°C) (P<0.05). For the neck fat tissue, there were significant differences in FIAF mRNA levels among all groups (P<0.001). As for the mesenteric fat tissue, there were significant differences in FIAF mRNA levels between -5°C group and other two groups (5°C and 0°C) (P<0.05), and there were significant differences between other groups (P<0.01).

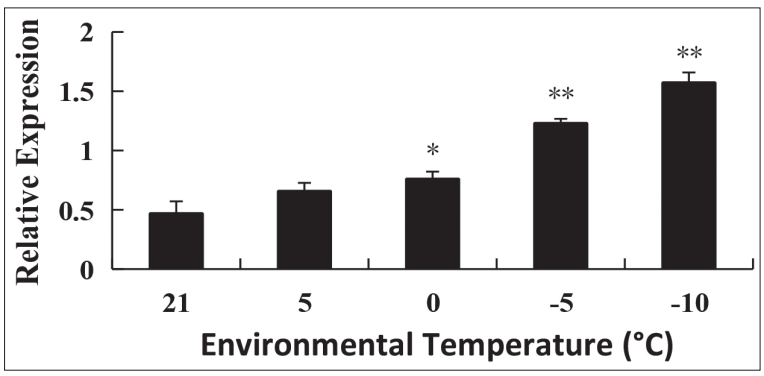

Fig 1. The effect of different environmental temperatures on FIAF mRNA levels in liver tissue. Compared to control * P<0.05; ** P<0.01

Expression of FIAF Protein in Different Tissues

As shown in *Fig. 5,* it was found that FIAF was expressed in liver tissue, abdominal fat tissue, neck fat adipose tissue and mesenteric fat tissue, and the protein molecular weight was consistent with the actual size. The molecular weight of FIAF protein was about 50 KD and β-actin was 42 KD.

FIAF Concentrations in Serum

As shown in *Table 3,* FIAF concentrations in the blood of pigs were low at 21°C (approximately 32.78 pg/L). As the ambient temperature decreased,

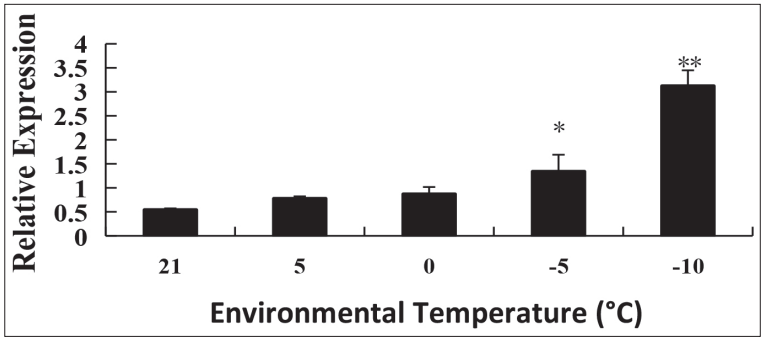

Fig 2. The effect of different environmental temperatures on FIAF mRNA levels in abdominal fat tissue

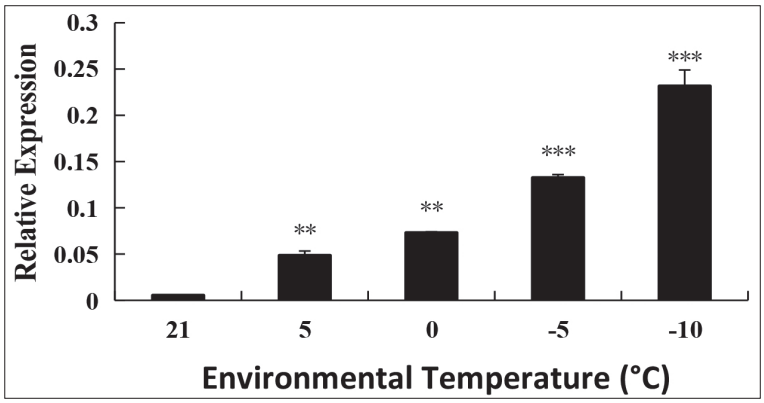

Fig 3. The effect of different environmental temperatures on FIAF mRNA levels in neck fat tissue. Compared to control *** P<0.001

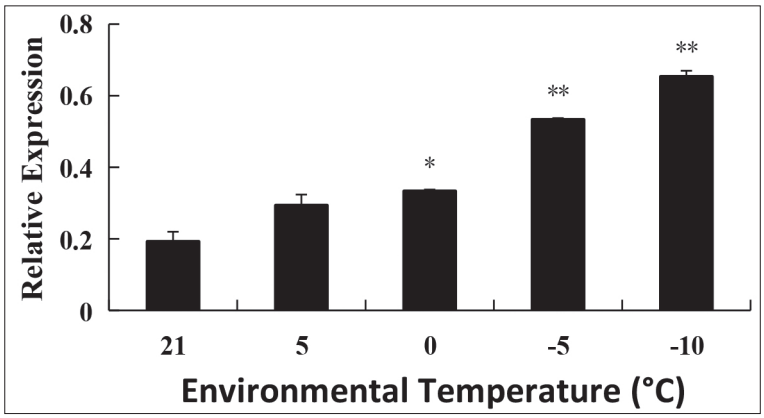

Fig 4. The effect of different environmental temperatures on FIAF mRNA levels in mesenteric fat tissue

FIAF concentrations in the blood increased. In pigs exposed to 5°C for 2 h, FIAF concentrations in blood were significantly higher than in the control group (P<0.05). In pigs exposed to 0°C, -5°C and -10°C for 2 h, FIAF concentrations in blood were significantly higher than in the control group (P<0.01). At an ambient temperature of -10°C, FIAF concentrations in blood reached 104.68 pg/L. Besides, in pigs exposed to -10°C for 2 h, FIAF concentrations in blood were significantly higher than in the -5°C group (P<0.05), the 0°C group (P<0.01) and the 5°C group (P<0.01). There were significant differences between other groups (P<0.01).

Discussion

Cold is a common stress factor for animals, especially newborn animals, at high altitudes and in cold regions. Cold stress can slow down animal growth, impair disease resistance and even cause death in severe cases, resulting in major losses to the farming industry. It is also one of the important limiting factors restricting agricultural development in regions at high latitudes. A previous study showed that acute cold stress increased the metabolic rate of animals, enhanced sugar, protein and fat catabolism and raised peripheral blood metabolites, including free fatty acids (FFA), glucose and arginine [8]. Under cold stress conditions, the metabolic activity of adipose tissue is regulated by neural and endocrine pathways. Adipocytokines were produced and secreted by adipose tissue also play important roles in maintaining energy metabolism stability and regulating lipid and carbohydrate metabolism *in vivo*. The previous research showed that the expression of leptin and adiponectin mRNA in the neck, back and mesenteric adipose tissue of pigs gradually decreased with the increase of cold stress intensity, but it had little effect on the expression of resistin mRNA [9]. It has also been confirmed that adipocytokines play a regulatory role in the fat metabolism process under cold stress.

Table 3. *The effect of different environmental temperatures on the concentration of FIAF in serum (pg/L)*

Group	Temperature	The Concentration of FIAF
Control	21±2°C	32.78±5.49
Test 1	5±2°C	52.44±4.34*
Test 2	0±2°C	76.30±2.57**
Test 3	-5±2°C	97.12±9.11**
Test 4	-10±2°C	104.68±10.92**

*Significant labeling based on the comparison between the control group and the four test groups. Compared to control * P<0.05; ** P<0.01*

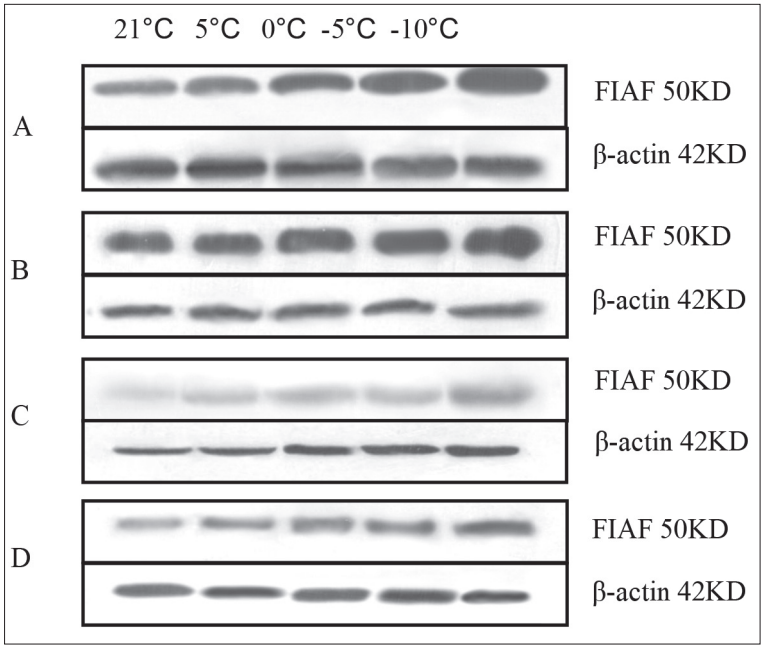

Fig 5. The effect of different environmental temperatures on FIAF protein levels in different tissues. **A:** liver tissue, **B:** abdominal fat tissue, **C:** neck fat tissue, **D:** mesenteric fat tissue

FIAF is a target gene of peroxisome proliferator-activated receptor (PPAR) and an endocrine signal that regulates energy metabolism. Fasting can cause an increase in FIAF concentrations in serum, whereas a high-fat diet may lead to decreased FIAF concentrations in serum. PPAR agonist can also cause elevated FIAF concentrations in serum [10]. The angiopoietin-like proteins (ANGPTLs) have emerged as key regulators of plasma lipid metabolism by serving as potent inhibitors of the enzyme, LPL [11]. Injection of FIAF has been shown to increase monomeric triglyceride fatty acids, glycerol, total cholesterol and high-density lipoprotein concentrations, inhibit the activity of apolipoprotein enzyme, enhance fatty acid oxidation and promote the expression of the fat uncoupling protein, UCP, in rat plasma, resulting in reduced adipose tissue weight [12].

Zhang et al.[13] found that the FIAF gene was expressed in the liver, fat, heart, small intestine and large intestine of pigs, and was most abundant in adipose tissue, followed by liver, large intestine, heart and small intestine. They also confirmed that *Bacteroides thetaiotaomicron* inhibited FIAF gene expression in ileal epithelial cells in the intestinal canal of pigs. In the present study, FIAF mRNA and protein expression levels were high in liver and abdominal fat tissues, whereas levels were low in neck fat. Exposure to low temperatures enhanced FIAF mRNA and protein expression levels in the liver and adipose tissue of various organs and elevated FIAF concentrations in serum. As the ambient temperature decreased, changes in FIAF expression levels increased. Elevated FIAF concentrations promote the degradation of adipose tissue, increasing free fatty acid and triglyceride levels in blood and enhance fatty acid oxidation, all of which are beneficial for adaptation to the high demands of energy metabolism in cold environments.

Under cold stress, changes in leptin and adipo-nectin expression are regulated by the sympathetic nervous system. Injection of β-adrenergic receptor agonist imitates the effects of acute cold exposure and inhibits leptin and adiponectin gene transcription levels in adipose tissue [14,15]. Intravenous injection of fat emulsion, other PPAR agonists (e.g., fenofibrate, pioglitazone, rosiglitazone, etc.) or β-adrenergic receptor agonist (albuterol) increases FIAF expression levels in blood, and significantly increases free fatty acids in blood. However, injection of salbutamol, a β-adrenergic receptor agonist, and acipimox, a fat degradation inhibitor, significantly reduces FIAF levels in blood [6]. Under cold stress, the sympathetic nerve is stimulated, resulting in increased secretion of adrenal medullary catecholamines, which mobilize adipose tissue to promote catabolism, and changes in free fatty acid concentrations in the blood. A recent study found that cold stress induces rat mesenteric fat cells to synthesize norepinephrine and epinephrine, and increase catecholamine synthetic enzyme content in stromal vascular fraction cells, indicating that an independent catecholamine synthesis system exists in fat cells and plays an important role in adipose tissue mobilization induced by cold stress [16]. Free fatty acids in the blood activate PPAR, and FIAF is a downstream target gene of PPAR. Previous studies have found that cold stress elevates PPARγ2 mRNA and protein levels in liver and adipose tissue [17]. Therefore, it was likely that the changes in FIAF concentrations in liver, adipose tissue and blood observed in pigs under cold stress were caused by the free fatty acid concentrations in the blood, which might be subject to joint control by the sympathetic nerve in the catecholamine sympathetic system of fat tissues and activation of PPAR.

This study confirmed that cold stress increased the levels of FIAF mRNA and protein in liver, neck, abdomen and mesenteric fat tissues of pigs, as well as FIAF concentrations in blood. This study also found that FIAF expression levels in liver and fat tissues were related to the intensity of the cold stress, and lower ambient temperatures resulted in higher FIAF expression. The results of this study would be beneficial for deeper understanding of the impact of cold stress on fat metabolism and for studying the neuroendocrine regulation of fat metabolism in pigs.

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Conflicts of Interest

The authors declare no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

Author Contributions

Hong JI designed the experiment. Ying LIU and Ziyi SHAO performed the experiment and analyzed the data. Hong JI and Ziyi SHAO made pictures or tables, and wrote the paper. Chunyang NIU revised the manuscript. All authors reviewed and approved the final manuscript.

References

1. Scharf I, Wertheimer K, Xin JL, Gilad T, Goldenberg I, Subach A: Context-dependent effects of cold stress on behavioral, physiological, and life-history traits of the red flour beetle. *Insect Sci*, 26 (1): 142-153, 2019. DOI: 10.1111/1744-7917.12497

2. Cline DL, Short LI, Forster MAM, Gray SL: Adipose tissue expression of PACAP, VIP, and their receptors in response to cold stress. *J Mol Neurosci,* 68 (3): 427-438, 2019. DOI: 10.1007/s12031-018-1099-x

3. Luo L, Liu M: Adipose tissue in control of metabolism. *J Endocrinol*, 231 (3): R77-R99, 2016. DOI: 10.1530/JOE-16-0211

4. Harwood Jr HJ: The adipocyte as an endocrine organ in the regulation of metabolic homeostasis. *Neuropharmacology*, 63 (1): 57-75, 2012. DOI: 10.1016/j.neuropharm.2011.12.010

5. Yang J, Li X, Xu D: Research progress on the involvement of ANGPTL4 and loss-of-function variants in lipid metabolism and coronary heart disease: Is the „prime time" of ANGPTL4-targeted therapy for coronary heart disease approaching? *Cardiovasc Drugs Ther*, 2020. DOI: 10.1007/s10557-020-07001-0

6. Kersten S, Lichtenstein L, Steenbergen E, Mudde K, Hendriks HFJ, Hesselink MK, Schrauwen P, Müller M: Caloric restriction and exercise increase plasma ANGPTL4 levels in humans via elevated free fatty acids. *Arterioscler Thromb Vasc Biol*, 29 (6): 969-974, 2009. DOI: 10.1161/ATVBAHA.108.182147

7. Livak KJ, Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta Ct}$ method. *Methods*, 25 (4): 402-408, 2001. DOI: 10.1006/meth.2001.1262

8. Li SZ, Yang YY, Yang HM, Ni J: Study on comparative-physiology of some endocrinal activities on cold exposure and cold acclimation in rats and chicks. *Chin J Appl Physiol*, 24 (1): 23, 2008. DOI: 10.13459/j.cnki.cjap.2008.01.002

9. Fu SP, Ren BY, Liu Y, Li SN, Li Y, Wang MP, Yang ZQ, Wei LB, Li ZQ, Liu JX, Wang W, Guo WB, Hu ZM: Adiponectin and its receptors mRNA levels in swine adipose tissue in the condition of cold stress. *Chin Anim Husb Vet Med,* 39 (3): 28-32, 2012.

10. Kersten S, Mandard S, Tan NS, Escher P, Metzger D, Chambon P, Gonzalez FJ, Desvergne B, Wahli W: Characterization of the fasting-induced adipose factor FIAF, a novel peroxisome proliferator-activated receptor target gene. *J Biol Chem*, 275 (37): 28488-28493, 2000. DOI: 10.1074/jbc.M004029200

11. Dijk W, Kersten S: Regulation of lipid metabolism by angiopoietin-like proteins. *Curr Opin Lipidol*, 27 (3): 249-256, 2016. DOI: 10.1097/MOL.0000000000000290

12. Desai U, Lee EC, Chung K, Gao CH, Gay J, Key B, Hansen G, Machajewski D, Platt KA, Sands AT, Schneider M, Sligtenhorst IV, Suwanichkul A, Vogel P, Wilganowski N, Wingert J, Zambrowicz BP, Landes G, Powell DR: Lipid-lowering effects of anti-angiopoietin-like 4 antibody recapitulate the lipid phenotype found in angiopoietin-like 4 knockout mice. *Proc Natl Acad Sci U S A,* 104 (28): 11766-11771, 2007. DOI: 10.1073/pnas.0705041104

13. Zhang LP: Study on the relationship of gut microbiota with body fat and ANGPTL4 gene in different breeds of pigs. *Sichuan Agricultural University*, 2007.

14. Gettys TW, Harkness PJ, Watson PM: The beta 3-adrenergic receptor inhibits insulin-stimulated leptin secretion from isolated rat adipocytes. *Endocrinology*, 137 (9): 4054-4057, 1996. DOI: 10.1210/endo.137.9.8756584

15. Delporte ML, Funahashi T, Takahashi M, Matsuzawa Y, Brichard SM: Pre- and post-translational negative effect of beta-adrenoceptor agonists on adiponectin secretion: *In vitro* and *in vivo* studies. *Biochem J,* 367 (3): 677-685, 2002. DOI: 10.1042/BJ20020610

16. Kvetnansky R, Ukropec J, Laukova M, Manz B, Pacak K, Vargovic P: Stress stimulates production of catecholamines in rat adipocytes. *Cell Mol Neurobiol,* 32 (5): 801-813, 2012. DOI: 10.1007/s10571-012-9822-6

17. Liu Y, Ren WZ, Li SN, Li WH, Fu SP, Wu YK, Wang K, Hu ZM: PPARγ2 gene expression of swine adipose tissue liver heart and kidney in cold stress. *Chin J Vet Sci,* 32 (4): 592-597, 2012. DOI: 10.16303/j.cnki.1005-4545.2012.04.022