ORIGINAL

Adiponectin plays a role in energy metabolism for musk secretion in scent glands of muskrats (*Ondatra zibethicus*)

Tianxiang Zhang^{1)*}, Weixuan Li^{1), 2)*}, Lei Qi^{1)*}, Mengyuan Fan¹⁾, Jiachen Shen³⁾, Yihua Wang¹⁾, Wenxia Wang¹⁾, Xiaolong Hu¹⁾, Ruibo Cai¹⁾, Ran Zhou¹⁾, Yuting Wei¹⁾, Juntong Zhou¹⁾, Shuang Yang¹⁾, Defu Hu¹⁾ and Shuqiang Liu¹⁾

¹⁾ College of Nature Conservation, Beijing Forestry University, Beijing 100083, People's Republic of China

²⁾ Zhongshan School of Medicine, Sun Yat-sen University, Guangzhou 510080, People's Republic of China

³⁾ College of Biological Science and Technology, Beijing Forestry University, Beijing 100083, People's Republic of China

Abstract. Adult male muskrats (*Ondatra zibethicus*) secrete musk from their scent glands to attract females for seasonal mating. The goal of the present study was to investigate whether the changes in energy metabolism related to musk secretion during the breeding and non-breeding seasons are mediated by adiponectin. We found that the secretion of musk during the breeding season was markedly greater than that during the non-breeding season. The serum adiponectin concentration measured using an ELISA kit was higher during the breeding season than during the non-breeding season. Glandular cells, interstitial cells, epithelial cells and glandular cavities were detected in scent glands using histological methods. Immunohistochemical methods were used to show that AMP-activated protein kinase- γ -1 (AMPK γ 1), and glucose transporter 1 (GLUT1) were more strongly expressed in glandular cells during the breeding season than the non-breeding season, whereas the immunoreactivity for acetyl-CoA carboxylase 1 (ACC1) was stronger during the non-breeding season. Consistent with these qualitative results, RNA-Seq analysis indicated that the expression of *AdipoR1* mRNA was not significantly different during the two seasons. However, *AMPK γ 1* and *GLUT1* mRNA levels were higher in scent glands during the breeding season than during the non-breeding season, whereas *ACC1* mRNA levels notably decreased during the breeding season. These results suggest that greater musk secretion requires additional energy, which may be provided by an adiponectin-mediated increase in β -oxidation and glucose absorption.

Key words: Male muskrat, Scent gland, Musk secretion, Energy metabolism, Adiponectin

FOUR stages of musk secretion have been characterized: a non-breeding period, a scent-gland-growth period, a vigorous musk-secretion period, and a prolonged musk-secretion period. During the non-breeding season, from October to February, no musk is secreted from the scent gland. Because the scent gland is not swollen and contraction is unnecessary at this time, it is filled with connective tissue. However, during the vigorous musk-secretion period from April to May, the scent gland expands, and the connective tissue is progressively replaced with glandular tissue. The inner surface is smooth, the mucosa unfolded and

full of blood vessels. The scent gland mainly consists of glandular cells, epithelial cells and excretory ducts [1, 2].

Adiponectin is a cytokine released by adipose cells to circulate in the bloodstream as a hormone. Adiponectin plays an important role in resistance to insulin, regulation of glucose and lipid metabolism, and resistance to atherosclerosis as well as other physiological functions through its binding to adiponectin receptors (AdipoRs) [3]. The adiponectin protein comprises four functional areas: N-terminal signal peptides, non-homologous regions, collagen repeats and globular carboxyl terminals [4]. Adiponectin monomers exist only in fat cells, being secreted out of these cells to function after convergence to polymers inside the cells.

The AdipoR is in a family of cell-surface receptors that contains seven transmembrane domains but is significantly different than the G-protein-coupled

Submitted Dec. 25, 2015; Accepted Apr. 22, 2016 as EJ15-0720

Released online in J-STAGE as advance publication May 13, 2016

Correspondence to: Shuqiang Liu, No.35, Qinghua East Road, Haidian District, Beijing, China, Zip code: 100080 010.

E-mail: liushuqiang@bjfu.edu.cn

* These authors contributed equally to this work.

receptor family [5]. Its amino terminal lies inside the cell, whereas its carboxyl terminal is outside the cell. AdipoRs can be characterized into AdipoR1 and AdipoR2 subtypes, with 66.7% homology between them. The AdipoR1 has a high affinity to the spherical structure of adiponectin but a relatively low affinity to full-length adiponectin [6, 7].

Previous research was conducted to investigate the regulation of adiponectin and AdipoR expression in liver and skeletal muscle tissue [8-10]. Li *et al.* (2005) found that AdipoR1 is widely distributed in various tissues of normal Wistar rats, with the highest expression detected in the testis and skeletal muscle [11]. AdipoR1 may function to regulate insulin sensitivity and energy metabolism by controlling other cytokines. AdipoR2 exists primarily in liver and may function to regulate insulin sensitivity and the output of glycogen. It has also been suggested that adiponectin regulation may occur in a paracrine or autocrine manner [11]. Adiponectin binds the AdipoR1 present in skeletal muscle to activate a series of cascade reactions, which first activate and phosphorylate AMP-activated protein kinase (AMPK). The gene expression of the key enzyme in fatty acid synthesis, acetyl-CoA carboxylase (ACC), is then inhibited and peroxisome proliferator activated receptor alpha (PPAR α) is activated, which increases fatty acid oxidation in mitochondria. In addition, the phosphorylation of AMPK triggers the expression of the glucose transporter 4 (GLUT-4) gene to promote glucose utilization [12-14].

Few studies have examined the mechanism of musk secretion in male muskrats. Estrogen has been shown to have important functions in musk secretion. Estrogen receptor alpha and beta are expressed in the scent glands of the muskrat, with the expression of estrogen receptor alpha especially strong in glandular cells, interstitial cells and epithelial cells [15]. Lu *et al.* (2014) found stronger expression of the androgen receptor in scent glands during the breeding season, which suggests that the scent gland may be an androgen target organ [16]. However, the regulation of the mechanism for musk secretion in muskrats by other factors is still unclear. Musk secretion is a process requiring high energy metabolism, as abundant energy must be transferred by tissues or organs to synthesize musk. Adiponectin plays an important role in energy metabolism. However, whether adiponectin functions in the process of musk secretion, is unknown. Therefore, in the present study, we investigated the

function of adiponectin in the metabolic energy pathways related to musk secretion from the scent gland, providing insight into the mechanism of adiponectin-mediated musk secretion.

Materials and Methods

Musk collection

This animal study was approved by the Chinese Association for Laboratory Animal Sciences. Adult male muskrats were purchased from Jinmu Technologies Limited Company (Xinji, Heibei, China). Three animals were purchased during the breeding season (April) and three during the non-breeding season (October). Muskrats were conventionally housed. Food and water were provided *ad libitum*. Musk was collected three times during a single month for each group. For musk collection, the thumb and forefinger were used to locate and gently massage and squeeze the scent gland to collect the musk into a 30 mL glass tube held snug around the penis (Liang 2000). The collected musk was weighed on an electronic analytical balance.

Sample management

Blood from both groups of muskrats was obtained from the heart, collected into tubes containing EDTA (1 mg/mL blood) and aprotinin (500 Units/mL blood) (Sigma) (CR *et al.*, 2010), and centrifuged for 15 min at 2,000 rpm. The supernatant was then collected. The scent glands were removed and preserved in liquid nitrogen.

ELISA analysis of adiponectin

Serum levels of adiponectin were assayed with an ELISA reagent kit (Rat Adiponectin ELISA kit, Beijing Dong Songs Biological Technology, Beijing, China), according to the manufacturer's instructions. Briefly, samples were diluted and added to a standard. The diluted samples (50 μ L) were then added to each well for final concentrations of 90 μ g/L, 60 μ g/L, 30 μ g/L, 15 μ g/L, and 7.5 μ g/L and incubated for 30 min at 37°C. The liquid was discarded and the wells were dried. The horseradish peroxidase-conjugated reagent (50 μ L) was added to each sample well, incubated for 30 min at 37°C, and washed. Equal volumes (50 μ L) of Chromogen Solution A and B were added to each well and incubated in the dark for 15 min at 37°C. The Stop Solution was added, and the absorbance of each sample was read at 450 nm with the blank considered zero. Adiponectin

concentrations were determined in duplicate, and the intra-assay coefficient of variation was 6.5%.

Immunohistochemistry

The scent glands preserved in liquid nitrogen were analyzed as frozen sections because the tissue collected during the non-breeding season was fragile. Immunohistochemical localization of AdipoR, AMPK, ACC and GLUT was conducted using rabbit polyclonal antibodies against AdipoR1, AMPKG1, ACC1 and GLUT1 (Hua Xing Bo Chuang Biological Technology Center, Beijing, China), respectively. The tissue was sectioned into 7 μ m thick slices at -25°C and mounted onto adhesive microscope slides. The samples were fixed in acetone for 10 min, washed in 0.02 M phosphate-buffered saline (PBS) three times for 5 min each. Standard paraffin section methods were used for the following procedures. Endogenous peroxidase was blocked by incubating the sections in 3% hydrogen peroxide in methanol for 10 min. The sections were washed in 0.02 M PBS three times for 5 min each. After nonspecific binding was blocked with 10% normal sheep serum in PBS, the sections were incubated with the antibodies (diluted 1:50) overnight at 4°C. After further washing in PBS, the sections were processed using a PV-9001 kit (Zhong Shan Jin Qiao Co., Beijing, China), and the peroxidase activity was detected with diaminobenzidine (Sigma; St Louis, MO). Finally, the sections were counterstained with hematoxylin and mounted using conventional methods.

RNA-Seq analysis of the scent glands

High-throughput RNA-sequencing (RNA-Seq) technology was used to analyze the expression of mRNA. The samples were sent to Biomarker Technologies Company (Beijing, China) for sequencing. The tissue RNA was extracted, the cDNA amplified from the RNA, and a library was constructed. The sequencing was performed using an Illumina HiSeq H2000 (Illumina, San Diego, CA, USA) at 50 base pairs, paired end, and 25 million paired reads per sample, following the manufacturer's protocol. Multiplexed single-read runs were conducted with a total of 10^7 cycles [17].

The unigenes were annotated by comparison with deposited sequences in the NR, Swiss-Prot, Go, COG, Kegg database using BLAST software. The reads of the samples were compared with the transcript using Bowtie software. The comparison information was used in RSEM software to evaluate the expression level

of the unigenes. The result was presented as RPKM value. In this study, we selected the expression level of GAPDH as a reference. The expression level of the mRNA we concerned with was presented as the quotient of the RPKM value of target genes to GAPDH.

Western blotting analysis of the scent glands

The musk gland tissues were kept at -80°C. The samples were from three individuals in April and another three in November. Take approximately 0.1g tissue from each individuals. Homogenize the tissue in a homogenizer containing 300 μ L of 10 mg/mL PMSF stock and incubated on ice for 30 min while maintaining the temperature at 4 °C throughout all the procedures. Primary incubation of the membranes was carried out using a 1:2,000 dilution of rabbit anti-rat AdipoR, AMPK, ACC and GLUT for 60 min. Secondary incubation of the membrane was then carried out using a 1:40,000 dilution of goat anti-rabbit IgG tagged with alkaline phosphatase for 60 min.

Statistical analysis

In this study, the statistical comparisons were made with the Student *t*-test and One-way analysis of variance.

Results

Decrease in musk weight of muskrats in the non-breeding season

Previous studies reported that musk secretion in muskrats decreases and even stops during the non-breeding season [18]. To explore the relationship between musk secretion and adiponectin, we measured the amount of musk secretion in muskrats during the breeding and non-breeding seasons by weighing the collected musk. We found a marked decrease in the musk secretion of muskrats during the non-breeding season compared with that during the breeding season (Fig. 1, $p < 0.05$).

Decrease in adiponectin levels of muskrats in the non-breeding season

Adiponectin is relevant to sugar and lipid metabolisms. We measured the adiponectin concentration of serum in muskrats during both the breeding and non-breeding seasons with an ELISA reagent kit. Compared with that in the breeding season, the adiponectin concentration in muskrats during the non-breeding season was markedly reduced (Fig. 2, $p < 0.05$).

Seasonal morphology profile of musk gland

Morphological observations during the breeding and non-breeding musk glands sections are compared in Fig. 3. During the breeding season (Fig. 3A), the glandular cavities are obvious and abundant, and glandular cells are diffusely distributed. Glandular cells are located around the glandular cavity, and a single

layer of epithelial cells surrounds the glandular cells and the glandular cavity. During the non-breeding season (Fig. 3B), the glandular cavity is no longer apparent, and glandular cells are no longer separately distributed. Nuclei of glandular cells during the non-breeding season are bigger than those during the breeding season.

Fig. 1 Musk secretion in muskrats during breeding and non-breeding seasons
Musk secretion is markedly lower during the non-breeding season than during the breeding season. Data are presented as the mean $\pm$ SEM from at least three independent experiments, and error bars indicate SEM. * Denotes a statistically significant difference between breeding and non-breeding seasons ($p < 0.05$).

Fig. 2 Serum concentration of adiponectin
Serum adiponectin concentrations, analyzed using an ELISA kit, are markedly lower during the non-breeding season compared with those during the breeding season. Error bars indicate SEM. * Denotes a statistically significant difference between the breeding and non-breeding seasons ($p < 0.05$).

A Breeding season negative control

B Non-breeding season negative control

Fig. 3 Immunohistochemical staining (negative control) in muskrat scent glands during breeding and non-breeding seasons
(A) During the breeding season, the glandular cavities are obvious and abundant, and glandular cells are diffusely distributed. Glandular cells are located around the glandular cavity, and a single layer of epithelial cells surrounds the glandular cells and the glandular cavity. (B) During the non-breeding season, the glandular cavity is no longer apparent, and glandular cells are no longer separately distributed. Nuclei of glandular cells during the non-breeding season are bigger than those during the breeding season. IC, interstitial cells; GC, glandular cells; EC, epithelial cells; GCa, glandular cavity; ED, excretory ducts. Scale bars represent 50 μ m.

Immunohistochemical expression of AdipoR1, AMPKG1, GLUT1 and ACC1 protein

Adiponectin is an endogenous insulin sensitizing-agent and functions to regulate glucose metabolism and inhibit oxidative stress or to produce other physiological functions upon binding to its receptor. Our immunohistochemical results showed that AdipoR1 was observed in the membranes of glandular and epithelial cells during the breeding season (Fig. 4A). Similarly, the expression of AMPKG1 and GLUT1 was stronger in the cytoplasm of glandular and epithelial cells dur-

ing the breeding season (Fig. 5A, Fig. 6A) than during the non-breeding season (Fig. 5B, Fig. 6B). The levels of ACC1, a key enzyme in lipid anabolism, were measured in the cytoplasm of epithelial and glandular cells. In contrast to the results for AMPKG1 and GLUT1, the expression of ACC1 was stronger in glandular and epithelial cells during the non-breeding season (Fig. 7B) than during the breeding season (Fig. 7A), with no expression of ACC1 observed in the glandular cells during the breeding season.

Fig. 4 Adiponectin receptor 1 (AdipoR1) immunolocalization in muskrat scent glands during breeding and non-breeding seasons. AdipoR1 was examined in the membrane of glandular cells and epithelial cells during the breeding season (A). IC, interstitial cells; GC, glandular cells; EC, epithelial cells; GCa, glandular cavity; CM, cell membrane. Scale bars represent 50 μ m.

Fig. 5 AMP-activated protein kinase-gamma-1 (AMPKG1) immunolocalization in muskrat scent glands during breeding and non-breeding seasons. Strong positive immunoreactivity for AMPKG1 is observed in the cytoplasm of glandular cells, epithelial cells and interstitial cells during the breeding season (A), but little positive staining is detected in the glandular cells during the non-breeding season (B). IC, interstitial cells; GC, glandular cells; EC, epithelial cells; GCa, glandular cavity; Cyt, cytoplasm. Scale bars represent 50 μ m.

Fig. 6 Glucose transporter 1 (GLUT1) immunolocalization in muskrat scent glands during breeding and non-breeding seasons. GLUT1 staining is clearly observed in glandular cells during the breeding season (A), whereas little positive staining is detected in the glandular cells during the non-breeding season (B). IC, interstitial cells; GC, glandular cells; Cyt, cytoplasm; GCa, glandular cavity. Scale bars represent 50 μ m.

Fig. 7 Acetyl-CoA carboxylase 1 (ACC1) immunolocalization in muskrat scent glands during breeding and non-breeding seasons. Immunoreactivity for ACC1 is apparent in the cytoplasm of epithelial cells during both breeding and non-breeding seasons (A, B), but stronger staining is observed in the glandular and epithelial cells during the non-breeding season (B). No ACC1 immunostaining is detected in the glandular cells during the breeding season. IC, interstitial cells; GC, glandular cells; EC, epithelial cells; GCa, glandular cavity; Cyt, cytoplasm. Scale bars represent 50 μ m.

Expression of *AdipoR1*, *AMPKG1*, *GLUT1* and *ACC1* mRNA

RNA-Seq analysis was used to determine the gene expression for the proteins analyzed above. We found that *AdipoR1* mRNA levels were not significantly different during the breeding and non-breeding season. The mRNA levels of *AMPKG1* and *GLUT1* were higher in muskrats during the breeding season than during the non-breeding season, whereas *ACC1* mRNA levels were higher during the non-breeding season (Fig. 8, $p < 0.05$).

Expression of *AdipoR1*, *AMPKG1*, *GLUT1* and *ACC1* protein

We conducted Western blotting (Fig. 9) to explore the protein expression levels of *AdipoR1*, *AMPKG1*, *GLUT1* and *ACC1*. The results shows a similar conclusion to the transcriptome analysis. The *AdipoR1* and *AMPKG1* protein levels are not significantly different during the breeding and non-breeding seasons. However, the protein level of *GLUT1* is markedly higher ($p < 0.01$) in the breeding season than during the non-breeding season, whereas the mRNA level of *ACC1* is lower ($0.01 < p < 0.05$) in the breeding season (Fig. 10).

Fig. 8 Transcriptome analysis

The *AdipoR1* mRNA levels are not markedly different during the breeding and non-breeding seasons. However, the mRNA levels of *AMPKG1* and *GLUT1* are higher in the breeding season than during the non-breeding season, whereas the mRNA level of *ACC1* is lower in the breeding season. Data are presented as the mean $\pm$ SEM from at least three independent experiments, and error bars indicate SEM. * Denotes a statistically significant difference between breeding and non-breeding seasons ($p < 0.05$).

Discussion

The scent gland of the muskrat secretes musk and is mainly composed of glandular tissues. During the breeding season, the cells in the scent gland are distributed loosely. The glandular cells, interstitial cells, epithelial cells and glandular cavity can be recognized, with cellular junctions common among the glandular cells and epithelial cells distributed as a single layer outside the glandular cells (Fig. 3A). By contrast, during the breeding season, the cells in the scent gland are closer together and the glandular cavity disappears (Fig. 3B). We found that musk secretion is also seasonal, with a greater amount secreted during the breeding season than the non-breeding season (Fig. 1, $p < 0.05$), consistent with results of previous studies [19, 20].

Adipose tissue is a site of energy storage and an essential endocrine organ. It releases cytokines and hormones that modulate whole-body metabolism, inflammation and the immune response [21, 22]. Adiponectin is an adipokine that participates in glucose and lipid metabolism and provides resistance to atherosclerosis [4, 23]. The results of our ELISA analysis showed that serum levels of adiponectin in muskrats during the breeding season are higher than those during the non-breeding season (Fig. 2, $p < 0.05$), suggesting that the scent gland requires adiponectin to secrete musk.

Fig. 9 The grey scale results of *GAPDH*, *AdipoR1*, *AMPKG1*, *GLUT1* and *ACC1* in the scent glands during the two seasons.

Fig. 10 Western blotting analysis

The *AdipoR1* and *AMPKG1* protein levels are not significantly different during the breeding and non-breeding seasons. However, the protein level of *GLUT1* is markedly higher in the breeding season than during the non-breeding season, whereas the mRNA level of *ACC1* is lower in the breeding season. Data are presented as the mean $\pm$ SEM from at least three independent experiments, and error bars indicate SEM. * and ** Denotes a statistically significant differences between breeding and non-breeding seasons (*, $0.01 < p < 0.05$; **, $p < 0.01$).

When bound to AdipoR, its cell-surface receptor, adiponectin regulates the expression of downstream factors to activate physiological effects. In the present study, AdipoR1 was expressed on the membrane of glandular cells and epithelial cells during the breeding season (Fig. 4A). The signaling molecules downstream of AdipoR mainly include AMPK, p38 mitogen-activated protein kinase and PPAR α . AMPK is a key signaling molecule in the adiponectin-related signal transduction pathway as a protein kinase involved in energy and oxidative metabolism [24]. We found that the AMPKG1

protein was strongly immunolocalized in the cytoplasm of glandular and epithelial cells during the breeding season (Fig. 5A), with little staining detected during the non-breeding season (Fig. 5B). The phosphorylation of AMPK induces gene expression of GLUT and the transfer of GLUT protein to the cytomembrane, which increases glucose absorption and energy provision in myocardial tissue [25]. We found that the expression of GLUT1 in the cytoplasm of glandular cells was stronger during the breeding season (Fig. 6A) than the non-breeding season (Fig. 6B). Our immunohistochemical result is similar to the results of a study that found that adiponectin upregulates the phosphorylation of ACC, which increased the fatty acid oxidation of myocardial cells [26]. The expression of ACC1 was detected in the cytoplasm of epithelial cells but not in glandular cells during the breeding season in the present study (Fig. 7A); however, the expression of ACC1 observed in epithelial cells was stronger during the non-breeding season (Fig. 7B). We speculate that musk secretion in the scent gland requires an abundant amount of energy and that this physiological process is mediated by adiponectin as it changes the expression of downstream signaling molecules. The RNA-Seq results in our study were consistent with our immunohistochemical staining results. There was no apparent difference in adiponectin mRNA levels during the breeding and non-breeding seasons. However, higher mRNA expression of *AMPKG1* and *GLUT1* was detected during the breeding season than the non-breeding season. Conversely, mRNA expression of *ACC1* was lower during the breeding season than the non-breeding season (Fig. 8, $p < 0.05$). And the Western blotting results continually confirm the results of transcriptome analysis (Fig. 10). However, the *AdipoR1* and *AMPKG1* protein levels are not significantly different during the breeding and non-breeding seasons. The protein expression level of *GLUT1* is significantly higher in the breeding season ($p < 0.01$), while *ACC1* is higher in the non-breeding season ($0.01 < p < 0.05$).

In conclusion, musk secretion, serum levels of adiponectin, and protein and mRNA expression of *AMPKG1* and *GLUT1* were all higher in muskrats during the breeding season compared with those during the non-breeding season, whereas the opposite was found for protein and mRNA expression of *ACC1*. In addition, the *AdipoR1* mRNA level was not notably different during the breeding and non-breeding seasons, consistent with a previous study conducted by Bauche *et al.*, suggesting that adiponectin does not influence the expression of *AdipoR1* in

adipose cells [27]. These results suggest that the process of musk secretion requires abundant energy and that this energy metabolism is mediated by adiponectin. Adipose cells release the adipokine adiponectin into the peripheral blood around the scent gland to bind to *AdipoR1*, a membrane receptor located in the glandular and epithelial cells of the scent gland. *AdipoR1* may participate in the process transitionally and not be upregulated or downregulated by adiponectin. The receptor is activated after binding to adiponectin, allowing adiponectin to pass into cells and cause downstream changes. The epithelial cells are closely linked to the glandular cells and may play an auxiliary function for the glandular cells to participate in musk secretion since both cells mediate energy homeostasis. The effect of adiponectin binding to *AdipoR1* on the glandular and epithelial cells is to activate the phosphorylation of *AMPKG1*, upregulating the gene expression of *GLUT1* and downregulating the expression of *ACC1*. Higher mRNA expression of *GLUT1* is transcribed into GLUT1 protein, which is transported from the cytoplasm to the cytomembrane of cells to utilize peripheral glucose for glycolysis. A reduction in the *ACC1* protein level in the cells stimulates β -oxidation in mitochondria. Two changes occur, the increase in glycolysis and β -oxidation, to strengthen energy production in the scent gland. The process of providing energy is mediated by adiponectin, which functions through an AMPK signal transduction pathway. Thus, factors related to this signaling pathway change with the altered energy requirements for musk secretion during different seasons in muskrats. Further studies are underway to elucidate the mechanism by which other factors in the adiponectin signaling pathway affect the energy metabolism of musk secretion in muskrats.

Acknowledgements

The present study was supported by the Species Salvation Program of the Department of Fauna and Flora and Nature Reserve Management, State Forestry Administration of China (No. Forest musk deer 2013). The experimental facilities were provided by the College of Nature Conservation Innovation Laboratory, Beijing Forestry University.

Disclosure

None of the authors have any potential conflicts of interest associated with this research.

References

- Chen Y (2007) Histological Observation on Musk-secreting Scented Gland in Muskrat. *Chinese J Zool* 42: 91-95 (In Chinese).
- Chen Y, Jin S, Tong Y, Qao X, Zhou A, et al. (1996) Studies on morphology of cystgland and histological structure in the periods of secreting fragrant of muskrat. *Acta Theriologica sinica* 16: 151-154 (In Chinese).
- Cui XB, Han Y, Li L, Wu LL (2011) Research progress in regulation of adiponectin receptors expression. *Sheng Li Ke Xue Jin Zhan* 42: 169-174 (In Chinese).
- Berg AH, Combs TP, Scherer PE (2002) ACRP30/adiponectin: an adipokine regulating glucose and lipid metabolism. *Trends Endocrinol Metab* 13: 84-89.
- Dong B, Meng H, Gu Z, Gong D (2007) Research Advance of Adiponectin and Adiponectin Receptor. *China Ani Husb Vet Med* 34: 44-48 (In Chinese).
- Luo J (2011) Regulation Mechanism of Adiponectin Receptors Expression. *Chinese J Ani Nutr* 23: 1651-1657 (In Chinese).
- Yamauchi T, Kamon J, Ito Y, Tsuchida A, Yokomizo T, et al. (2003) Cloning of adiponectin receptors that mediate antidiabetic metabolic effects. *Nature* 423: 762-769.
- Wang PH, Ko YH, Liu BH, Peng HM, Lee MY, et al. (2004) The Expression of Porcine Adiponectin and Stearoyl Coenzyme a Desaturase Genes in Differentiating Adipocytes. *Asian-Aust J Anim Sci* 17: 588-593.
- Yu HK, Wu LL (2009) Recent advances on adiponectin receptor. *Pro in Mod Biomed* 9: 375-378 (In Chinese).
- Inukai K, Nakashima Y, Watanabe M, Takata N, Sawa T, et al. (2005) Regulation of adiponectin receptor gene expression in diabetic mice. *Am J Physiol Endocrinol Metab* 288: E876-882.
- Li C, Gong Y, Tian H, Lu J, Li M, et al. (2005) Distribution and expression of adiponectin receptors in different tissues of normal Wistar rats. *Jie Fang jun yi xue za zhi* 30: 718-719 (In Chinese).
- Heiker JT, Kosel D, Beck-Sickinger AG (2010) Molecular mechanisms of signal transduction via adiponectin and adiponectin receptors. *Bio chem* 391: 1005-1018.
- Kadowaki T, Yamauchi T (2005) Adiponectin and adiponectin receptors. *Endocr Rev* 26: 439-451.
- Tomas E, Tsao TS, Saha AK, Murrey HE, Zhang Cc, et al. (2002) Enhanced muscle fat oxidation and glucose transport by ACRP30 globular domain: Acetyl-CoA carboxylase inhibition and AMP-activated protein kinase activation. *Proc Natl Acad Sci U S A* 99: 16309-16313.
- Lu L, Zhang HL, Lv N, Ma XT, Tian L (2011) Immunolocalization of Androgen Receptor, Aromatase Cytochrome P450, Estrogen Receptor Alpha and Estrogen Receptor Beta Proteins during the Breeding Season in Scent Glands of Muskrats (*Ondatra zibethicus*). *Zool Sci* 28: 727-732.
- Lu L, Liu S, Li Q, Huang S, Bao L, et al. (2014) Seasonal expression of androgen receptor in scented gland of muskrat (*Ondatra zibethicus*). *Gen Comp Endocrinol* 204: 1-7.
- Royse KE, Zhi D, Conner MG, Clodfelder-Miller B, Srinivasasainagendra V, et al. (2014) Differential Gene Expression Landscape of Co-Existing Cervical Pre-Cancer Lesions Using RNA-seq. *Front Oncol* 4: 339.
- van Dorp DA, Klok R, Nugteren DH (1973) New macrocyclic compounds from the secretions of the civet cat and the musk rat. *Recl Trav Chim Pays-Bas* 92: 915-928 (In Dutch).
- Jiang F, Zhang X, Han X, Han C, Yue Z, et al. (2006) Morphology of Muskrat Scent Glands and Initial Culture of the Glands Epithelium. *Chinese Agri Sci Bull* 22: 40-43 (In Chinese).
- Chen Y, Tong Y, Gao X, Wei H, Li Z, et al. (1996) A preliminary study on the scent gland development and the collection of scent secret in living musk rats. *Acta Theriologica Sinica* 16: 43-47 (In Chinese).
- Saltiel AR (2001) You are what you secrete. *Nat Med* 7: 887-888.
- Spiegelman BM, Flier JS (2001) Obesity and the Regulation of Energy Balance. *Cell* 104: 531-543.
- Matsuda M, Shimomura I, Sata M, Arita Y, Nishida M, et al. (2002) Role of adiponectin in preventing vascular stenosis. The missing link of adipo-vascular axis. *J Biol Chem* 277: 37487-37491.
- Hosch SE, Olefsky JM, Kim JJ (2006) APPLIED mechanics: uncovering how adiponectin modulates insulin action. *Cell Metab* 4: 5-6.
- Xie Y, Wang J (2007) AMPK and mycodial ischaemia-reperfusioninjury. *Int J Pathol Clin Med* 27: 516-520.
- Li L, Wu L, Wang C, Liu L, Zhao Y (2007) Adiponectin modulates carnitine palmitoyltransferase-1 through AMPK signaling cascade in rat cardiomyocytes. *Regul Pept* 139: 72-79.
- Bauche IB, Ait E, Mkaem S, Rezsahazy R, Funahashi T, et al. (2006) Adiponectin downregulates its own production and the expression of its AdipoR2 receptor in transgenic mice. *Biochem Biophys Res Commun* 345: 1414-1424.