A HISTOLOGICAL STRUCTURE AND SPECIFIC PROTEIN OF BROWN ADIPOSE TISSUE OF MONGOLIAN LAMBS

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ABSTRACT

Brown fat tissue of Mongolian lamb is divided histologically into a number of lobules with connective tissue and connective-muscular tissue septa,consists of fat cells, cytoplasm of which contains small vacuoles showing the presence of multiple lipid droplets (multilocular), and blood vessels are abundant there. However, lipid droplets in cytoplasm of brown adipose tissue /BAT/ cells of 7-day old lambs enlarge and cells are generally unilocular type. Immunohistochemical analysis of this fat tissue by using specific antiserum of sheep UCP1 protein (rabbit anti-serum againstUCP1) detected an antigen of UCP1protein and approximately 350 bp length DNA fragment encoding specific protein UCP1 was found in lamb genome by PCR.

KEY WORDS: Brown fat, thermogenin, UCP1, multilocular adipocyte, unilocular adipocyte

INTRODUCTION

Mongolian livestock has reproductive feature that they directly produce their offspring on the pasture during the spring season, when average air temperature is minimal and wind speed is maximal. Therefore, the body temperature of neonates, as well as keeping its homeostasis at the normal level requires thermal exchange and specific factor of thermal regulation, when the fetus shifts from constantly warm environment of maternal body to severely cool external environment.

Not only offspring of various mammals, but also human infants face the necessity of withstanding above mentioned natural challenges and during first moments of the neonatal period the body temperature drops (hypothermia)(S.John Martin, 2010) and then the body temperature becomes constant due to activation of specific mechanism of thermal regulation for a shorter period. Thus the essence of such stabilization of the body temperature for a shorter period is associated with that animal body heat production process is different from mature animals. A factor for essential and specific processes brown adipose tissue (BAT) is brown fat (Brown fat tissue). (B.Cannon, J.Nedergaard, 2003, J.C.Rauch, 1969, G.Purevdorj 2005, M.E.Symons 1991, S.Andrei 1991, G.Amgalanbaatar 2004)

Localization of brown fat differs with various animals as reported by many authors. For

example, normally grown calf brown fat is deposited in the perirenal region and it accounts for 27% of its total brown fat and 17.5% of subscapular brown fat, as well as significantly greater depositions are found in thoracic and abdominal cavities, along aorta, pericardium and axillary regions (PaivaiSoppela, 1986), and according to the study performed on mouse *Peromyscusmanicularis*, it is broadly scattered on interscapular, subscapular, axillary, perirenal, pericardial and intercostals regions (J.C.Rauch, 1969).

Once the parturition ends, a thermoregulatory center in the central nervous system is triggered due to effect of lower temperature of external environment, main physiological processes leading to heat production in animal body is stimulated, functions of BAT is intensified for a shorter period, and specific process of heat production takes place there. Maintenance of the body temperature at the normal level via stimulation of BAT functions results from such morphological characteristics as its innervations, blood circulations and localizations, as well as physiological factors including noradrenaline released at the sympathetic nerve ending, STH, TTH and ACTH of pituitary gland and hormones of thyroid and adrenal glands are also of greater significance (Toyoshi Endo and Tetsuro Kobayashi, 2008, Liu X, Li Q, Lin Q, Sun R., 2001, Mostovoi A.V. , IvanovS.L.,2004, Alison Sharpe Avram, 2005).

A specific protein called thermogenin (UCP1) was found in the inner membrane of cellular mitochondria plays a role of absorbing energy due to oxidation into blood in the form of heat energy (B.Cannon, J.Nedergaard, Abraham L, Alison Sharpe Avram).

In other words majority of chemical energy produced in BAT can be converted into thermal energy (B.Cannon, 2004, Cousin B., 1992, D.Enebish, 2003). Thus it is most important quality of BAT function and therefore this topic has been attracting greater interests in the field of both human health and veterinary sciences.

Within the framework of our research on structure and development of brown fat in the body of Mongolian lamb being adapted to such severe changes of temperature, the present study aimed to detect a specific histological structure of BAT, the gene of a specific protein (UCP1) in brown fat in the genome, and the antigen to UCP1 in brown fat during fetal development and early neonatal periods.

MATERIALSAND METHODS

The thoracic, pericardial, renal and flank BAT for our study were prepared by sacrificing and dissecting 12 fetuses obtained from 4-5 months pregnant ewes via Cesarean section, and 0, 3, 7 and 14-day old 6 healthy lambs.

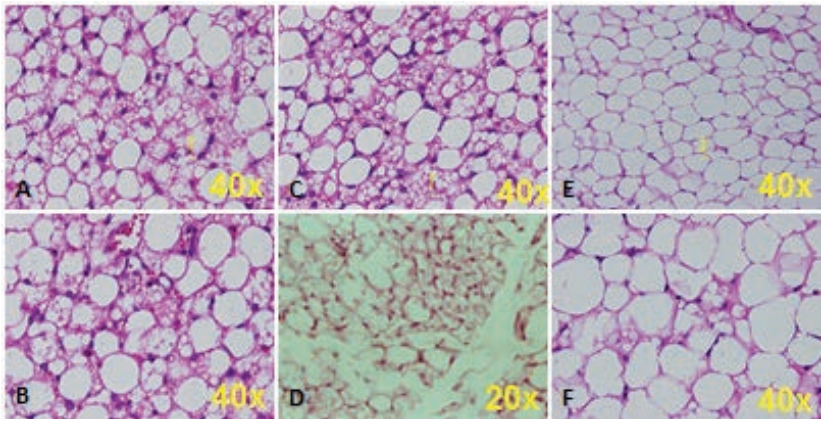
A histological structure of BATwas determined according to conventional methods, the antigen of UCP1 protein was detected by the immunohistochemical method using an antiserum to UCP1, and the gene of cell mitochondrial thermogenin (UCP1), was determined by PCR using specific primers of UCP1 (*forward-AGATCTCAGCGGGCCTAAC,reverse-GGCCTCCTTCATTAGGTCAT*).

RESULTS

Hematoxilin and eosin (HE) staining and microscopy of micro preparations of both thoracic superficial and deep, thoracic cavity, renal and flank brown fats obtained from fetuses and 0 to 3-day old neonatal lambs demonstrate that there are numerous lobules separated by connective tissue, in some cases connective-muscular tissue septa, cell sizes were not uniform (Picture 1), multilocular cells with smaller vacuoles evidencing cytoplasmic lipid droplets accounts for major part of adipocytes, whereas unilocular cells with larger vacuoles for a minor part. As well, presence of abundant blood vessels and

great number of intercellular capillaries makes brown fat very different from white fat.

Size of brown fat cells on above localizations in 7-day old lambs was slightly larger than those in 0 to 3-day old lambs, as well as fat cells as a whole are unilocular a type of white blood cells containing only large lipid droplet in its cytoplasm (Picture 1). In other words, there is a principle that brown fat is becoming typical to be white fat cells since the lamb is 7-day old. During the microstructural study, it is also observed that color of brown fat is changing into bright at this age.



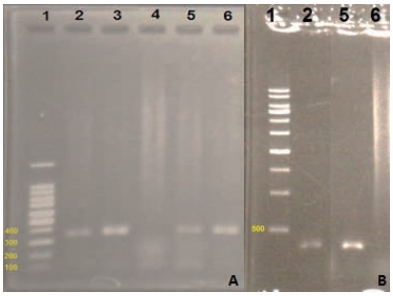
Picture 1.A histological structure of fetal BAT
A.Fetus thoracic, **B.**Fetus flank, **C.**0-day old lamb pericardium, **D.**0-day old lamb perirenal, **E.**7-day old thoracic, **F.**7-day old perirenalbrown fat

The BAT cell size is relatively smaller from fetal development period to 3-day old age and cellular diameter is approximately 18 to 25 μm in average. According to our measurements, BAT cells in thoracic and flank regions are slightly smaller than brown fat tissue cells in both thoracic and abdominal cavities or approximately 18 to 22 μm. From 7-day old age, the cell diameter becomes 25 to 30 μm, there was an observed principle that cells were slightly enlarged with typical changes (Table 1).

Table 1

Micro-measurements of brown fat tissue

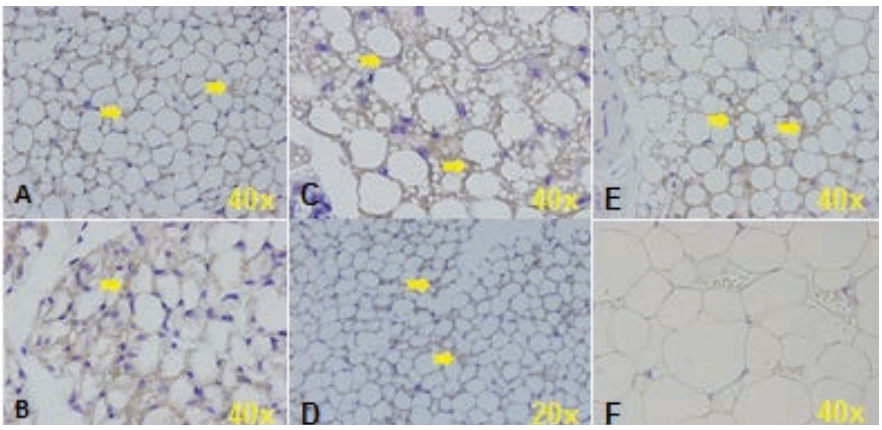
№	Localization of brown fat	Cell size with ages(μm)		Size of lipid droplet (μm)	
		Fetus and 0 to 3-day old lambs	7-day old lambs	Fetus and 0 to 3-day old lambs	7-day old
1	Thoracic brown fat	18-22	20-35	3-10	20-25
2	Flank brown fat	22			
3	Pericardium brown fat	20-25			
4	Perirenal brown fat	20-25			



Picture 2. Result of PCR
A - 1. Marker /100bp/, **2,3.** Fetal thoracic brown fat, **5,6.**Thoracic BAT of neonate /350bp/
B - 1. Marker /500bp/, **2.**Perirenal BAT of lamb, **5.**Periscrotum BAT of lamb /350bp/

DNA was extracted from brown fat of Mongolian lambs by using a phenol-chloroform method, then polymerase chain reaction was performed using specific primers of sheep UCP1 protein (*forward-AGATCTCAGCGGGCCTAAC, reverse GGCCTCCTTCATTAGGTCAT*), and a DNA fragment with approximately 350 bp length encoding specific protein UCP1 was detected in the genome.(Picture2)

Immunohistochemical analysis by using arabbit anti-sheep UCP1serum in brown fat of lambs thorax, scrotum, pericardium and kidneys revealed an antigen to UCP1 was detected. (Picture 3)



Picture 3.Results of immunohistochemical analysis
A.Thoracic brown fat, **B.**Scrotum brown fat, **C.**Perirenal brown fat,
D.Pericardium brown fat, **E.**Positive control, **F.**Negative control

DISCUSSIONS

Simple staining of micropreparation of brown fat of both fetuses and newborn lambs revealed the tissue was divided with connective tissue, in some cases with connective-muscular tissue septa into multiple lobules (Pictures 1, 2 and 3) and our results are in agreement with that multilocular cells with smaller vacuoles evidencing cytoplasmic lipid droplets accounts for the major part of adipocytes, whereas unilocular cells with large vacuoles for the minor part. As well, presence of abundant blood vessels and great number of intercellular capillaries makes brown fat very different from white fat(B.Cannon and J.Nederhaard, 2004, Abraham L, 2002, A.Ham, D.Кормак, 1983).Typical changes of fat cells since 7-day old age of lambs and enlargement of the cell size is also consistent with results of studies by other researchers.(Andrei S 1991, G.Purevdorj 2005)

In the present study, it was found that the genome of Mongolian lambs contains information on UCP1 protein and detection of the antigen to USP1 protein in brown fat in the localizations described by us results in creation of theoretical background that development of the brown fat, synthesis of its specific protein and their functions can be greater than the same species of animals under different ecological conditions and other non-hibernating animal species depending on our country's climate and specific ecological factors.

CONCLUSION

1. For histological structures, brown fat tissues are divided into lobules separated with connective tissue septa, it consists of 18 to 30 μm multilocular type cells with multiple small lipid droplets in their own cytoplasm, and capillary blood vessels are abundant between them.
2. DNA fragment with approximately 350 bplength encoding specific protein UCP1 was detected in the genome of Mongolian sheep lambs.
3. An antigen to UCP1 was detected by immunohistochemical analysis using rabbit anti-sheep UCP1 antiserum in brown fat of lambs thorax, scrotum, pericardium and kidneys.

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