Cellularity of Adipose Tissue Obtained from Different Sex and Growth Stages of Hanwoo Cattle and Sheep[a]

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ABSTRACT : In order to understand the effects of sex or age on cellular characteristics of adipocytes from Hanwoo and sheep, samples were obtained from omental, subcutaneous, intermuscular and intramuscular adipose tissue depots of bulls, steers, heifers and cows in Hanwoo, and perirenal, omental and subcutaneous adipose tissues of fetal lambs, suckling lambs and wethers in sheep. In case of Hanwoo, mean diameter, surface area and volume of adipocytes from each depot were obtained by multisizer II (Coulter Co., UK). Osmium-fixed adipocytes were sized and counted using 560 μm aperture. For samples obtained from sheep, cellularity was measured by using microscope and MCV program of Texas Instrument. Bulls had less subcutaneous and kidney fat than steers even though their slaughter and carcass weight were heavier. The amounts of fat from cows were greater in subcutaneous, kidney and internal organs than heifers. Steers had larger adipocytes in subcutaneous, intermuscular and intramuscular adipose tissues than bulls, although the differences were significant only for the subcutaneous adipose tissue depots. Adipocytes appeared to be largest in omental and smallest in intramuscular adipose tissue, although there were no significant differences among tissues. In a comparison of heifers and cows, significant site effects ($p<0.05$) were shown in adipocyte diameter, surface area and volume, and adipocyte appeared to be largest in omental tissue. Statistical difference ($p<0.05$) was only shown in cell volume of intramuscular tissue which was higher in cow than heifer. Intramuscular adipose tissue tended to have relatively greater numbers of cells per gram tissue and reflect lesser maturity of intramuscular adipose tissue relative to other adipose tissues. In sheep, regardless of adipose tissue depots, wethers had the greater adipocyte diameters than those at any other growth stage of sheep. Within adipose depots, the ranking of cell size was the greatest in the omental tissue of wether and the lowest in the renal and subcutaneous adipose tissue depots of fetal lamb. The cell size of adipocyte became larger with age, especially from fetal to suckling lamb due to a rapid hypertrophy of both perirenal and subcutaneous adipocytes during the suckling period. (*Asian-Aus. J. Anim. Sci. 2000. Vol. 13, No. 2 : 155-160*)

Key Words : Adipose Tissue, Hanwoo, Steer, Bull, Cow, Age, Wether, Fetal Lamb, Suckling Lamb, Cellularity, Omental, Subcutaneous, Intermuscular, Intramuscular

INTRODUCTION

The primary function of adipose tissue is to store energy in the form of triglycerides. However, the deposition of fat in meat animals may be beneficial or undesirable depending on the quantity and the location of the deposit. The deposition of excess fat, particularly in the internal and subcutaneous adipose tissues of meat-producing animals, can detract from quality and often requires manual trimming from certain cuts of meat. Thus the quality and quantity of fat deposited are important in the energetics and economic efficiency of meat production.

Amount of adipose tissue in animal is dependent primarily upon the number and size of adipocytes (Waters, 1909). It implies that adipose tissue mass expands ultimately by either hyperplasia (cell proliferation), hypertrophy (cell enlargement) or a combination of the two (Bjorntorp and Sjostrom, 1971; Greenwood, 1974; Garbutt et al., 1979). Consequently, a description of adipocyte size and number of adipose tissue depots during growth is important to understand the etiology of fat accumulation in animals.

Cellularity studies on selected adipose tissues of pig (Anderson and Kauffman, 1973; Steele et al., 1974; Mersman et al., 1975; Enser et al., 1974; Hood and Allen, 1977), cattle (Hood and Allen, 1973; Thornton et al., 1974) and sheep (Burton et al., 1974) have been reported. Hood and Allen (1976) had reported the changes in adipose cell size and number during the growth of cattle.

In Korea, Hanwoo is a main beef cattle breed and has taken an very important part in animal industry economically and socially. Male Hanwoos are usually used for beef production and castrated at early ages for the production of high quality meat and Hanwoo cows are used for calf production and finally to produce beef after several calvings. However, there are few studies about the effect of castration and age on adipose development, especially cellularity of adipocytes

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[a] A part of Ph.D. Dissertation of Dr. H. J. Lee under the direction of Professor In K. Han, Seoul National University, Korea. This study was funded by MAF-SGRP (Ministry of Agriculture and Forestry-Special Grant Research Program), Korea.
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Received January 7, 1999; Accepted May 12, 1999

in Hanwoo.

Also, sheep can be a good model to understand the lipid metabolism in ruminants and adipose tissue metabolism, but there have been few reports about the cellular development of sheep adipose tissue from the early growth, fetal stages.

This study was designed to examine the effects of castration with males and age with females on cellularity at various depots from Hanwoo, and the changes of cellularity in omental, subcutaneous and perirenal adipose tissues for fetal, suckling and growing periods of sheep.

MATERIALS AND METHODS

Animals

Eight Hanwoo bulls and eight Hanwoo steers were fed and managed under a high quality beef production program. Steers were castrated at 3 months old and slaughtered at 24 months old. Eight heifers and eight cows were raised by the feeding standard of the National Livestock Research Institute and slaughtered at 20 months and 44 months old, respectively.

Finn×Dorset-Horn crossbred sheep were used. Suckling lambs were left with their mothers until slaughter at about 1 month of age. Growing sheep were 6 to 8 months old castrated males; pregnant sheep were 4~6 years old and about 115 days pregnant; growing sheep and pregnant sheep were fed on hay ad libitum plus concentrate (300 g/day and 800 g/day, respectively)

Tissue preparation

1) Hanwoo

Immediately after stunning and exsanguination, the muscle and fat portions between the 10th to 12th ribs were removed and the subcutaneous, intermuscular and intramuscular fat depots were sampled from this rib section for analysis of cellularity. Adipose tissue slices (<500 μm thick) of 100~150 mg were rinsed twice in 37℃, 0.154 M NaCl. Rinsing was done to minimize the amount of free lipid released from adipocytes ruptured during slicing. If the free lipid was not rinsed away, subsequent fixation with osmium tetroxide resulted in aggregation of fixed adipocytes entrapped by fixed, free lipid and, in subsequent treatment, urea was not able to penetrate the slice or aggregate. Thus, adipocyte number would be underestimated due to the adipocytes entrapped in the fixed lipid. The rinsed adipose slices were then fixed in osmium tetroxide by a modification of the method of Hirsch and Gallian (1968). A tissue slice was placed in a 2.5×5.0 cm vial (scintillation counting vial) containing 3ml of 50 mM collidine-HCl buffer, pH 7.4 and 5 ml of 3% osmium tetroxide in collidine buffer was added. Adipose tissue slices were left in the fixative for 72~96 h at room temperature in a ventilated fume hood. After fixation, the osmium-collidine buffer was removed and 10 ml of 0.154 M NaCl was added. After 24 hrs, the 0.154 M NaCl was removed and 10 ml of 8 M urea in 0.154 M NaCl was added, Following the addition of 8 M urea, samples were occasionally swirled by hand and left at room temperature. Within 24~48 h, fixed adipocytes had been liberated from the adipose tissue slice.

Fixed and urea-isolated adipocytes were separated by filtering through nylon mesh screens with pore diameter of 20 μm, and collecting the cells retained on the respective screens. Non-urea treated adipose tissue slices were rinsed with copious amounts of distilled water on a 250 μm screen to liberate fixed adipocytes. Isolated adipocytes were rinsed through the successive screens with 0.01% Triton X-100 in distilled water (v/v), pH 10. When the different sized cells on the screen were rinsed into beakers for eventual sizing and number determination, 0.01% Triton X-100 in 0.154 M NaCl, pH 10 was used. The rinsed volume was 80 ml which was sufficient to prevent clumping when the electrolyte, 0.154 M NaCl, was added to a final volume of 800 ml.

2) Sheep

Collagen digestion mixtures were made up and left in an incubator until dissolved; adipose tissue was added and covered with a stopper and then left for 4-5 h in an incubator at 37℃. During this period, it was swirled gently once or twice. Before counting, cells were placed in a 50 ml plastic beaker and a slide and coverslip were warmed for 10 min in advance. Coverslip was placed on slide and a drop of digestion mixture was transferred on it and the other side too.

Cellularity measurement

1) Hanwoo

Mean diameter, surface area and volume of adipocytes from each depot were obtained by multisizer II (Coulter Co., UK). Osmium-fixed adipocytes were sized and counted using 560 μm aperture.

2) Sheep

Cell diameter was measured using a ruler. 50 cells were randomly measured on one side and it was repeated for the other side (40×10 objective). MCV program on Texas Instrument was used to calculate the mean cell volume.

Statistical analysis

All data were analysed by analysis of variance;

depending on castration treatment, ages and developmental stages. The differences of means between treatments were compared by Duncan's multiple range test, using General Linear Model (GLM) procedures of SAS package (1989).

RESULTS AND DISCUSSION

Carcass characteristics in Hanwoo bulls and steers are shown in table 1. Amounts of fat from steers were greater in subcutaneous ($p<0.05$) and kidney fat than those from bulls even though their slaughter and carcass weight were lighter. Bull carcass yielded a higher percent of retail cuts than that of steer.

Table 1. Comparison of carcass characteristics of Hanwoo bulls and steers

Items	Bulls	Steers
Slaughter wt., kg	697.33[a]	647.00[b]
Cold carcass wt., kg	433.80[a]	405.95[b]
Retailed cuts, %	44.40[a]	40.00[b]
Subcutaneous fat, %	12.58[b]	14.34[a]
Kidney fat, %	3.22	3.38
Internal organs fat, %	5.04	4.87
Backfat thickness, 12th rib, cm	2.12	1.54

[a,b] Means in the same row that have different superscripts differ ($p<0.05$).

Arthaud et al. (1977) reported that backfat thickness in 12th rib were 0.6 and 1.1 cm in Angus bulls and 0.7 and 1.6 cm in steers at the age of 12 and 24 months, respectively.

Previous papers have shown that castration in sheep affected both the total amount, and the partitioning of dissected fat in the body. Butterfield et al. (1984) reported that castrated males, wethers, had a greater weight of carcass fat than males (rams) without significant difference in body weight. Furthermore, Butterfield et al. (1985) showed that mature wethers had a greater proportion of fat in the subcutaneous fat partition than mature rams with little change in the weight of dissected fat in the intermuscular fat partitions. Thomson et al. (1988) concluded that the increased weight of dissected fat in the subcutaneous fat wethers compared with rams could result from changes in the following factors: adipocyte volume, adipocyte number, or the proportion of chemical fat in the dissected fat.

Relative to live weight, the weight of total fat is higher in females than in males (Benevent, 1971). The greater adiposity observed in the female confirms the work of Thompson et al. (1988) who reported a sex effect on adipocyte volume, with mature ewes having larger adipocytes than mature rams in the carcass fat partitions (subcutaneous and intermuscular fat) with no effect in the non-carcass partitions (kidney fat, omental and mesenteric fat partitions). Similarly, Allen et al. (1976) observed that ewes had larger adipocytes in the subcutaneous fat than rams at the same age. Thomson et al. (1988) noted that the sex effect in sheep appeared confined to the subcutaneous partition, whereas in cattle, Jones et al. (1981) reported that heifers had a greater concentration of chemical fat in the subcutaneous, intermuscular and body cavity than bulls. These findings are quite consistent with the results from this study.

The difference in adipose mass between bulls and steers might result from the sexual endocrine status (Swartz and Wade, 1981). Wade and Gray (1979) reported that the quantity of rat adipose tissue was influenced by the sex steroid hormones.

Table 2 compares carcass characteristics in Hanwoo heifers and cows. It shows that the amounts of fat from cows were significantly ($p<0.05$) greater in subcutaneous and internal organs. Kidney fat and backfat thickness appeared to be higher in cows even though significant differences were not shown.

Table 2. Comparison of carcass characteristics of Hanwoo heifers and cows

Items	Heifers	Cows
Slaughter wt., kg	413.50[b]	503.00[a]
Cold carcass wt., kg	254.75[b]	314.95[a]
Retail cuts, %	41.64	39.29
Subcutaneous fat, %	8.62[b]	12.16[a]
Kidney fat, %	3.24	3.70
Internal organs fat, %	3.29[b]	4.38[a]
Backfat thickness, 12th rib, cm	1.46	2.26

[a,b] Means in the same row that have different superscripts differ ($p<0.05$).

Garcia-de-Siles et al. (1977) reported slightly higher backfat thickness and kidney fat in heifers and in steers, but Dolezal et al. (1993) reported that age and frame were significantly related to the absolute weight of all fat depots.

Table 3 shows adipocyte diameter, surface area and volume in different adipose tissues from Hanwoo bulls and steers. In calculation of cell diameters, the population of cells less than 31 μm in diameter were not included. It was considered that smaller cells than 31 μm could have been fragments resulting from freezing the samples prior to osmium fixation.

The size of adipocytes seemed to depend on the location of adipose tissue, having significant ($p<0.01$) site effect in diameter, surface area and volume. Adipocytes appeared to be largest in omental and smallest in intramuscular adipose tissue, although there were no significant differences among tissue sites.

Table 3. Cell diameter, surface area and volume in different adiposes tissues from Hanwoo bulls and steers

Items	Omental		Subcutaneous		Intermuscular		Intramuscular		Effect	
	Bull	Steer	Bull	Steer	Bull	Steer	Bull	Steer	Castration	Site
Diameter (μm)	199.58[a]	185.80[a]	135.35[a]	154.60[a]	140.08[a]	157.55[a]	120.17[a]	128.85[a]	NS	**
Surface area (μm^2)	130.84[a]	119.90[a]	52.56[b]	81.31[a]	68.15[a]	80.91[a]	49.24[a]	55.22[a]	NS	**
Volume (μm^3)	4,656[a]	4,080[a]	1,269[b]	2,312[a]	1,869[a]	2,287[a]	1,179[a]	1,324[a]	NS	**

[a,b] Means within a site in the same row that have different superscripts significantly differ (p<0.01).
** : Significant (p<0.01), NS : Not significant.

Table 4. Adipocyte size and volume in different adipose tissues from Hanwoo heifers and cows

Items	Omental		Subcutaneous		Intermuscular		Intramuscular		Effect	
	heifer	cow	heifer	cow	heifer	cow	heifer	cow	Age	Site
Diameter (μm)	173.88[a]	176.46[a]	154.53[a]	158.30[a]	158.70[a]	157.06[a]	129.62[b]	145.87[a]	NS	**
Surface area (μm^2)	93.68[a]	84.35[a]	63.69[a]	62.69[a]	51.56[a]	51.51[a]	47.06[a]	55.74[a]	NS	*
Volume (μm^3)	2,680[a]	2,790[a]	1,690[a]	1,688[a]	1,278[a]	1,302[a]	1,047[b]	1,363[a]	NS	**
Cell number (10^5/g tissue)	2.88[a]	4.99[a]	3.89[a]	5.16[a]	6.46[a]	4.84	6.58[a]	5.96[a]	NS	NS

[a,b] Means within a site in the same row that have different superscripts significantly differ (p<0.05).
* : Significant (p<0.01), ** : Significant (p<0.05), NS : Not significant.

For adipocyte cellularity, in 1909, Waters first reported that the size of adipocytes within a muscle was smaller compared with that of the kidney, subcutaneous or intermuscular adipocytes. He suggested that the relatively smaller size of intramuscular adipocytes might be due to mechanical pressure and drew an analogy to the small adipocytes distributed in the tough white connective tissue of the muscle. Hood and Allen (1973) reported that intramuscular adipose tissue was late maturing; it was still actively growing by both hypertrophy and hyperplasia in steers at 14 months of age and that the majority of the cells were 70-130 μm, average 90.9±5.9 μm, in diameter. Cianzio et al. (1985) demonstrated that average adipocyte diameter from intramuscular fat in the M. longissimus increased linearly (73 to 107 μm) from 11 to 17 months of age and then remained the same at 19 months of age; the amount of intramuscular fat in cattle, however, continued to increase during growth from 11 to 19 months of age. The results from the present study, even with older cattle, had a similar tendency to those findings, although showing higher mean cell volume.

Leat (1980) suggested that the size of adipocytes varied one site to another, the largest usually being found in the perirenal adipose tissue and smallest in intramuscular which reflects their order of development. In cattle, it is known that fat is first deposited in perirenal and internal tissue such as omental and mesenteric and next deposited between muscles, intermuscular and then subcutaneous adipose tissue. In later stages of fattening, fat is deposited between muscle fiber giving the meat marbling. He reported that usual range of adipose cell size is 50~100 μm and adipose cells from obese animals can be 250 μm. The results above were quite in agreement with earlier reports and those previous findings have been confirmed.

There were no statistical differences in mean adipocyte sizes between steer and bulls. Steers had larger adipocytes in subcutaneous, intermuscular and intramuscular adipose tissue than bulls, although the differences were significant (p<0.05) only for the subcutaneous adipose tissue depots. Bulls showed adipocytes larger in diameter and volume in omental adipose tissue depots. Castration resulted in an increase in adipocyte volume in some adipose tissue depots, although the magnitude of the increase varied between adipose tissue depots. There were a 1.82, 1.22 and 1.21 folds increase in adipocyte volume in steer relative to bull in the subcutaneous, intermuscular and intramuscular adipose tissues, respectively.

The above results for steers are similar to those obtained by Broad et al. (1980) and Thompson et al. (1988) in bulls and castration seems to have a large effect on the cellular characteristics of adipose tissues in Hanwoo, too. They reported that the differences in lipid metabolism of adipocytes from bulls and steers are most likely regulated by sex steroid hormones.

As shown in table 4, there were no age effects on adipocyte diameter, surface area and volume, and cell number per gram tissue in different adipose depots from heifer and cow. Significant site effects were shown in adipocyte diameter (p<0.01), surface area (p<0.05) and volume (p<0.05), and adipocytes appeared to be largest in omental tissue. Statistical difference (p<0.05) was only shown in cell volume of intra- muscular tissues which was higher in cows than heifers.

Intramuscular adipose tissue tended to have a

Table 5. Adipocyte size and volume in adipose tissue of sheep in different growth stage

Items	Fetal lamb		Suckling lamb		Wether		Age
	Perirenal	Subcutaneous	Omental	Subcutaneous	Omental	Subcutaneous	effect
Diameter (μm)	2.48	2.48	10.94	10.78	13.08	12.59	**
Volume (μm^3)	4.63	4.22	238.55	232.87	583.92[a]	381.30[b]	**

[a,b] Means within a stage of growth in the same row that have different superscripts significantly differ ($p<0.05$).
** : Significant ($p<0.01$).

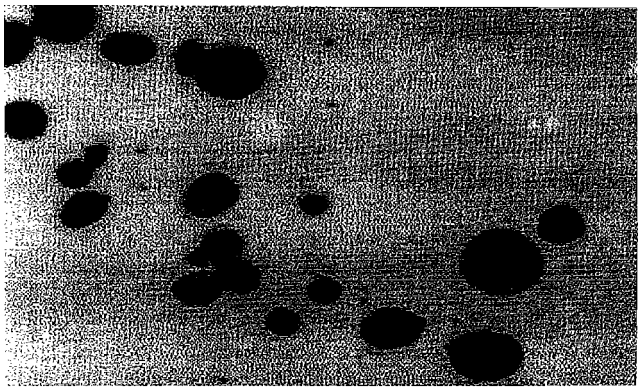

Figure 1. Hanwoo adipocyte stained with osmium tetroxide

relatively greater number of cells per gram tissue. These findings are in agreement with those of earlier studies (Hood and Allen, 1973; Smith and Crouse, 1984; Cianzio et al., 1985; Miller et al., 1991) and reflect the lesser maturity of intramuscular adipose tissue relative to other adipose tissues.

The diameters and volumes of adipocyte in the omental, subcutaneous and perirenal adipose tissue in fetal and suckling lamb and mature wether are presented in table 5. There were significant age effects ($p<0.01$) on adipocyte diameter and volume, and mature wethers had the greater adipocyte diameters than any other growing stage of sheep. Within adipose tissues, the ranking of cell size was greatest in the omental of wethers and the lowest in the renal and subcutaneous adipose tissues.

The cell size of adipocytes became larger with age, especially from fetal to suckling lamb due to a rapid hypertrophy of both perirenal and subcutaneous adipocytes during the suckling period (Vernon, 1986).

Vernon (1986) demonstrated that until about 400 days of age, the early stages of the fattening phase, the sizes of perirenal and subcutaneous adipocytes were the same, but as the fattening phase progressed perirenal and omental cells became progressively bigger than those of carcass depots.

That the fetal lamb had the smaller adipocyte volumes seemed to be reflected in a greater number of adipocytes per gram of adipose tissue compared with suckling lamb and mature wether. However, once preadipocytes were differentiated to adipose cells, these seems to synthesize and accumulate triglyceride inside, continuously enlarging the cell volume.

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