# The Relationship Between Lipogenic Activity and the Size of Adipocytes from Subcutaneous Adipose Tissue of Holstein Steers During the Fattening Period

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**Abstract** Isolated adipocytes were used to evaluate the lipogenic activity in subcutaneous adipose tissue of Holstein steers during various fattening periods. During the fattening period, both the mean diameter of adipocytes and the lipogenesis increased continually. There was a positive correlation between the mean diameter of adipocytes and the lipogenesis from glucose ($r=0.74$, $P<0.05$) or from acetate ($r=0.81$, $P<0.05$). Further, the relationship between the size of adipocyte and the lipogenic activity was investigated. It can be done through measuring the incorporation rate of substrates into lipids in each fraction of adipocytes which were separated by meshes. It was shown that both the incorporation rate of glucose or acetate into lipids and the relative contribution ratio of incorporation rate of acetate to glucose in the adipocytes taken from finishing steers, were significantly larger ($P<0.05$) than those from the early fattening steers. In the adipocytes which were taken from early fattening steers, the incorporation rate of glucose or acetate into lipids became larger with increment of the mean diameter from fraction 1 (33$\mu$m) to fraction 6 (139$\mu$m) (both : $r=0.72$, $P<0.05$). In the adipocytes which were taken from finishing fattening steers, the incorporation rate of glucose into lipids increased with the increment of the mean diameter from fraction 1 (36$\mu$m) to fraction 6 (164$\mu$m) ($r=0.79$, $P<0.05$), while the incorporation rate of acetate into lipids increased only with increment of the mean diameter from fraction 1 (36$\mu$m) to fraction 4 (116$\mu$m), then decreased with the increment of the mean diameter from fraction 4 (116$\mu$m) to those very large adipocytes in fraction 6 (164$\mu$m).

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**Key words** : Isolated adipocytes, Cell size, Lipogenesis, Fattening, Holstein steer

The development of adipose tissue of steers during the fattening period is very important in beef production. There were some studies about the contributions of acetate and glucose to the lipogenesis in adipose tissue taken from cattle[2,9,10,12,14,18-20]. Through the incubation of adipose tissue fragments *in vitro*, it was found that lipogenesis increased in several depots of adipose tissues from cattle during fattening period[20]. The proportion of fatty acids re-esterified also increased with age in adipose tissues from growing cattle[14]. However, the adipose tissue fragment comprises a variety of cell types, including not only adipocytes but also preadipocytes, endothelial cells, fibroblasts and macrophages[1], it would be

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more reasonable to measure the lipogenesis in isolated adipocytes than in adipose tissue fragment.

Size of adipocyte has been used as a marker of age, adiposity, or lipid accumulation to indicate possible metabolic alterations that accompany or follow the altered lipid accumulation in many studies. The size of adipocyte is changeable. It can continue to increase from 30 $\mu$m to 120~150 $\mu$m or more. Expressing metabolic results in relation to the size of adipocyte has been used to define the experimental conditions in many studies on adipose tissue. The size of adipocyte has been interpreted as having its own causative effect on metabolic activities, therefore, it can dramatically influence the metabolism of the cell[1]. Recently, we found a close relationship between the fatty acid composition and the size of adipocyte from steers during fattening period[4].

Little information about the relationship between the lipogenesis and the size of adipocyte in growing and fattening cattle was available[19]. In the present study, we measured the incorporation rates of glucose or acetate into lipids in isolated adipocytes from adipose tissue of Holstein steers during fattening period. Further, the relationships between the lipogenic activity and the size of isolated adipocytes within the individual adipose tissue from Holstein steers with different fattening stages were also investigated.

Experiment 1 was designed mainly for understanding the relationship between the lipogenic activity and the increment of mean diameter in isolated adipocytes from Holstein steers during fattening period. Experiment 2 was designed mainly for understanding the differences in the lipogenic activity of adipocytes with different diameters within the individual adipose tissue from Holstein steers in different fattening stages.

## Materials and Methods

### *Animals and tissue sampling*

In experiment 1, twelve Holstein steers from 4 to 28 months of age and in experiment 2, eight Holstein steers, in which four with 28 months of age and having mean body weight 761 kg, while the other four with 16 months of age and having mean body weight 483 kg, were given a daily diet of concentrates plus hay and water *ad libitum*, respectively. The concentrate consisted of : ground corn, 56% ; wheat bran, 24% ; soybean oil meal, 12% ; and required vitamins and salts. Subcutaneous adipose tissue samples were obtained from the region of the last thoracic vertebrae 20 to 30 cm to the right or left of the dorsal midline[4,5]. The tissue was transported to the laboratory in saline at 37℃ within 20 min and then rinsed with fresh saline. To get the sample of adipose tissue, in experiment 1, biopsies were performed in the steers with 4, 9, 13, 15, 23, 25 and 27 months of age.

### *Isolation of adipocyte*

The adipocyte isolation was based on the modified method of Rodbell[5,13,17]. Samples weighing approximately 2 g were dissected free from the connective tissue and blood vessels. The tissue was then placed in 25 m*l* polypropylene beakers containing 6 m*l* M 199 (Medium 199, pH 7.4 ; GIBCOBRL, Life Technologies, Inc. Grand Island, NY, USA) with Earle's salts, 4.2 mM $NaHCO_3$, 5.5 mM glucose and 0.61 mM sodium acetate, supplemented 20 mM HEPES, 4.5 mM glucose, 4.39 mM acetate, 4% BSA (bovine serum albumin, fraction V, Sigma Chemical Co., St. Louis, USA) and 3 mg per m*l* collagenase (Worthing Biochemical Co., New Jersey, USA). After minced and gassed for 30 s with a 95% $O_2$-5% $CO_2$ mix, the tissue was digested in a water bath at 37℃ with shaking 60 cycles per min for 90 min. After digestion, the cell suspensions were filtered through a polypropylene mesh with 1 mm pore size and washed with warm KRB (Krebs-Ringer

bicarbonate buffer, pH 7.4) supplemented with 20 mM HEPES, 10 mM glucose, 5 mM acetate and 4% BSA. Then, the cells were filtered through a polypropylene mesh with 250 $\mu$m pore size and washed 3 times.

***Lipogenesis in vitro***[5]

Aliquots of isolated adipocytes suspension (100 $\mu l$) were added to warm KRB supplemented with 20 mM HEPES, 10 mM glucose, 5 mM acetate, 4% BSA. Tubes containing 1 m$l$ medium with the same volume of cell aliquots were used to determine the density of cells in the medium for incubation. After 1 $\mu$Ci D-[U-$^{14}$C] glucose (specific activity : 283 $\mu$Ci per mmol, Amersham, England) or 1 $\mu$Ci [U-$^{14}$C] acetic acid, sodium salt (specific activity : 59 $\mu$Ci per mmol, Amersham, England) was added into the media, tubes were gassed for 1 min with a 95% $O_2$ : 5% $CO_2$ mix, capped and incubated for 2 h in a water bath at 37℃ with shaking 60 cycles per min. At the end of the incubation, 0.4 m$l$ of 2% osmium tetroxide was added into the tubes to terminate the incubation and to fix adipocytes. The total amount of lipids were extracted by the method of Dole[3]. Samples were evaporated to dryness, resuspended in 10 m$l$ of a scintillation cocktail (UL TIMA-FLO™M, PACKARD, USA), then counted in a liquid scintillation counter (LSC-5102, Aloka, Japan). The incorporation rate of $^{14}$C-labeled glucose or acetate into total amount of lipid material was calculated as nmol glucose or acetate incorporated per 2 h-incubation period per $10^5$ cells. Relative contributions of acetate and glucose to the lipogenesis in isolated adipocytes were expressed by the ratio of incorporation rate of acetate to the rate of glucose.

***Adipocyte separation***

In experiment 2, the isolated adipocytes which were incubated with $^{14}$C-labeled glucose or acetate were fixed through addition of 0.4 m$l$ 2% osmium tetroxide into tube containing 1 m$l$ of the medium and adipocytes, and were then kept at least 3 days at 4℃. All these cells were separated by polypropylene meshes with pore size of 25, 50, 70, 90, 110, 125 and 150 $\mu$m into six fractions. The total amount of lipids which was from the cells of each fraction were extracted by the method of Dole[3]. Samples were evaporated to dryness, resuspended in 10 m$l$ of the scintillation cocktail, and radioactivity was counted. The total number of the cells in each separated fraction was determined. The incorporation rate of $^{14}$C-labeled glucose or acetate into total amount of lipid was calculated as nmol glucose or acetate incorporated per 2 h-incubation period per $10^5$ cells. The method that we described here is a modified method of Rule *et al.*[12]. To estimate the possible changes in the incorporation rate of $^{14}$C-labeled glucose or acetate into lipids during the osmium fixation and separation process, we compared the rates in fresh adipocytes and in osmium-fixed adipocytes. There was no significant difference ($P>0.05$) between the incorporation rates in these adipocytes.

***Adipocyte cellularity***

The diameters of both fresh adipocytes before the metabolic incorporation experiments in experiment 1 and osmium-fixed adipocytes after separated by polypropylene meshes in experiment 2 were measured by a micro photograph method. The adipocytes were immediately photographed together with an objective micrometer (0.01 mm, Nikon, Japan) using micro photograph equipment (OPTIPHOT, Nikon, Japan). For every treatment more than 300 cells from the photographs were used for measuring the mean diameter and the diameter distribution.

***Statistical analyses***

Linear regression equation was used to describe the relationship between the lipogenic activity and the month of age, the body weight or the mean diameter of the adipocytes. Student's *t*-test was used to compare the differences in mean diameters of adipocytes between each pair of fractions from steers in early fattening and finishing stages. Analysis of

variance (ANOVA) was used as the model to test the effects of the size of adipocytes (Si, df=5), the fattening stage (St, df=1) and their interaction (Si×St, df=5). The Duncan mutiple-range test was used for mutiple comparisons of the means when a significant difference ($P<0.05$) was found[8].

## Results

***Experiment 1***

***The change in cellularity and lipogenesis of adipocytes during the fattening period***

The cell size which is expressed by mean diameter of adipocytes from the subcutaneous adipose tissues of the steers increased with the progress of fattening. The relationship between the mean diameter of adipocytes (D, $\mu$m) and the age (A, month) or body weight (W, kg) was described by the following regression equation : $D=46.54\,LnA-17.86$ ($n=27$, $r=0.91$, $P<0.01$) ; $D=68.85\,LnW-316$ ($n=27$, $r=0.92$, $P<0.01$).

The change in the cell size which is expressed by number frequency distribution of the diameters of adipocytes from steers in different fattening periods is shown in Fig. 1. The curves describe the distribution of the adipocytes diameter around 60, 70, 100, 110, 130 and 140 $\mu$m for the steers with 210, 326, 385, 483, 628 and 723 kg of body weight, and 4, 9, 13, 17, 23 and 27 months of age, respectively. The distribution rate of large cells became higher while that of small cells became lower with the increments of body weight and months of age.

In isolated adipocytes from the steers during the fattening period, the incorporation rate of

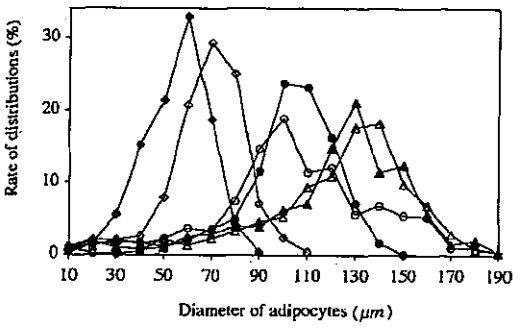


*Fig. 1.* The distributions of diameters of isolated adipocytes from Holstein steers during fattening periods (experiment 1). Each datum represented the average of data from adipocytes isolated from two steers with the same age. The months of age and the average body weight is described here : ◆ : 4 months old and 210 kg ; ◇ : 9 months old and 326 kg ; ● : 13 months old and 358 kg ; ○ : 17 months old and 483 kg ; ▲ : 23 months old and 628 kg ; △ : 27 months old and 723 kg.

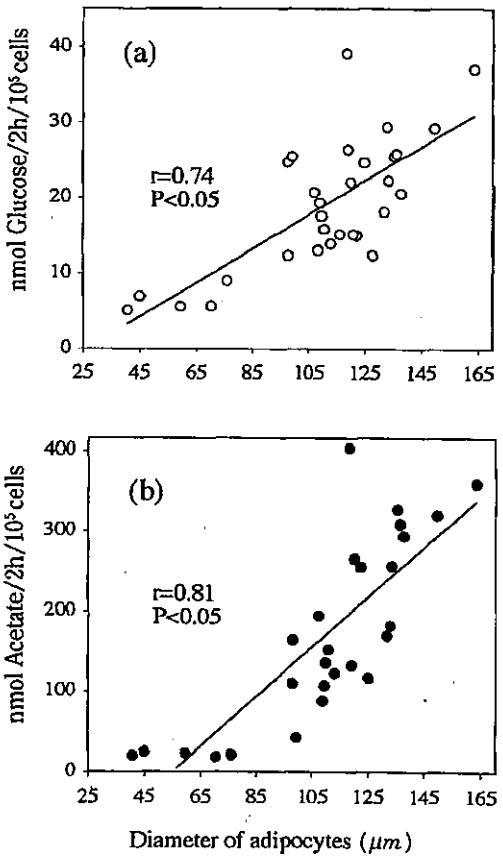


*Fig. 2.* The change in lipogenesis together with mean diameter of adipocytes from Holstein steers in different fattening periods (experiment 1). (a) : The change in the incorporation rate of glucose into lipids. (b) : The change in the incorporation rate of acetate into lipids.

glucose into lipids became higher with the increment of mean diameter (Fig. 2 a) ; the incorporation rate of acetate into lipids also had become higher since the mean diameter reached about 85$\mu$m, before this, they had kept a unchangeable low lipogenesis rate (Fig. 2 b). In relation to the size of the isolated adipocytes, the linear correlation coefficient between the incorporation rate of glucose or acetate into lipids and the mean diameter was 0.74 ($P<0.05$) or 0.81 ($P<0.05$), respectively.

***Experiment 2***

***Effect of cell size on the lipogenesis in adipocytes distributed in adipose tissue from steers with different fattening stages***

Separated by polypropylene meshes, the mean diameter of adipocytes in fraction 1, 2, 3 and 4 taken from the early fattening steers are similar to that of their corresponding fractions of adipocytes taken from finishing steers, but the mean diameters of adipocytes in fraction 5 of 124$\mu$m and 6 of 139$\mu$m separated by meshes in early fattening steers were smaller ($P<0.05$) than that of their corresponding fractions 5 of 146$\mu$m and 6 of 164$\mu$m in finishing steers (Table 1). It would be more reasonable to compare the incorporation rates between fraction 6 in the early fattening stage and fraction 5 in finishing stage, but it had no big difference in the results because of the close values of fraction 5 and 6 in finishing stage.

Both the cell size which was expressed as mean diameter and fattening stage had significant effects ($P<0.05$) on the incorporation rate of glucose or acetate into lipids in isolated adipocytes (Table 1). There was a interaction effect of mean diameter and fattening stage ($P<0.05$) on the incorporation rate of acetate into

*Table 1.* The incorporation rates of glucose or acetate into lipid in adipocytes distributed in same adipose tissue but with different sizes which were separated into 6 fractions by meshes in experiment 2 (mean±standard deviation)

| Fraction | Diamete | Distribution | Glucose** | Acetate** | Ratio** |
|---|---|---|---|---|---|
| | ($\mu$m) | (%) | (nmol/2 h/$10^5$ cells) | | Acetate/Glucose |
| Early fattening stage | | | | | |
| 1 | 33±2.4 | 3.0±1.5 | $10\pm4.2^{c}$ | $19\pm7.1^{e}$ | $1.8\pm0.5^{c}$ |
| 2 | 69±3.6 | 4.0±2.3 | $17\pm3.9^{bc}$ | $51\pm27^{de}$ | $3.1\pm1.5^{bc}$ |
| 3 | 89±1.2 | 19±10 | $20\pm6.8^{abc}$ | $77\pm28^{de}$ | $3.9\pm1.4^{bc}$ |
| 4 | 111±4.3 | 24±11 | $30\pm13^{abc}$ | $109\pm48^{cde}$ | $3.6\pm1.2^{bc}$ |
| 5 | 124±4.3 | 36±8.9 | $36\pm21^{abc}$ | $123\pm64^{cde}$ | $3.4\pm1.4^{bc}$ |
| 6 | 139±5.4 | 15±8.0 | $39\pm12^{ab}$ | $143\pm64^{cd}$ | $3.7\pm1.9^{bc}$ |
| Finishing fattening stage | | | | | |
| 1 | 36±0.9 | 2.8±0.8 | $10\pm2.5^{c}$ | $41\pm19^{de}$ | $4.3\pm1.4^{abc}$ |
| 2 | 69±1.4 | 5.6±3.0 | $28\pm10^{abc}$ | $211\pm25^{bc}$ | $7.6\pm2.8^{ab}$ |
| 3 | 92±1.8 | 5.9±2.3 | $38\pm11^{ab}$ | $371\pm64^{a}$ | $9.7\pm3.0^{a}$ |
| 4 | 116±2.8 | 7.7±14 | $41\pm14^{ab}$ | $381\pm60^{a}$ | $9.3\pm3.7^{a}$ |
| 5 | 146±3.6* | 30±14 | $43\pm9.3^{ab}$ | $297\pm24^{ab}$ | $6.9\pm0.8^{ab}$ |
| 6 | 164±3.7* | 48±19 | $45\pm11^{a}$ | $261\pm9.2^{b}$ | $5.8\pm1.1^{abc}$ |
| | | | | *** | |

* : Each pair of values with the same number of fraction differ significantly ($P<0.05$)

** : The lipogenic activities or the ratio of incorporation rate of acetate to that of glucose in adipocytes with different sizes and from steers in different fattening stages differ significantly ($P<0.05$).

*** : The interaction between size of adipocytes and fattening stage is significant ($P<0.05$).

$^{a-e}$ : Values within a column without the same superscripts differ significantly ($P<0.05$).

lipids in isolated adipocytes (Table 1).

In the adipocytes taken from early fattening steers, the incorporation rate of glucose into lipids became larger ($r=0.72$, $P<0.05$) with a mean diameter increment from fraction 1 (33 $\mu$m) to fraction 6 (139 $\mu$m) (Fig. 3 a), there was a significant difference ($P<0.05$) between fraction 1 of 10 nmol/2 h/$10^5$ cells to fraction 6 of 39 nmol/2 h/$10^5$ cells. The incorporation rate of acetate into lipids also became larger ($r=0.72$, $P<0.05$) with a mean diameter increment from fraction 1 to fraction 6 (Fig. 3 b), there was a significant difference ($P<0.05$) between fraction 1 of 19 nmol/2 h/$10^5$ cells to fraction 6 of 143 nmol/2 h/$10^5$ cells.

Likewise, in the adipocytes taken from finishing fattening steers, the incorporation rate of glucose into lipids became larger ($r=0.79$, $P<0.05$) with a mean diameter increment from fraction 1 (36 $\mu$m) to fraction 6 (164 $\mu$m) (Fig. 3 a), there were significant differences between fraction 1 and fraction 3, 4, 5 or 6 (Table 1). However the incorporation rate of acetate into lipids became larger only when the mean diameter increment from fraction 1 (36 $\mu$m) to fraction 4 (116 $\mu$m) (Fig. 3 b), there were significant differences ($P<0.05$) between fraction 1, 2 and 3 or 4 (Table 1). Afterwards, the incorporation rate became lower, there was a significant differences ($P<0.05$) between fraction 4 and fraction 6.

The ratios of incorporation rate of acetate to that of glucose in isolated adipocytes taken from finishing steers were significantly highter ($P<0.05$) than that from early fattening steers (Table 1). The ratios of incorporation rate of acetate to that of glucose in isolated adipocytes taken from early fattening steers were ranged from 1.8 to 3.9, while in isolated adipocytes taken from finishing steers were ranged from 4.3 to 9.7.

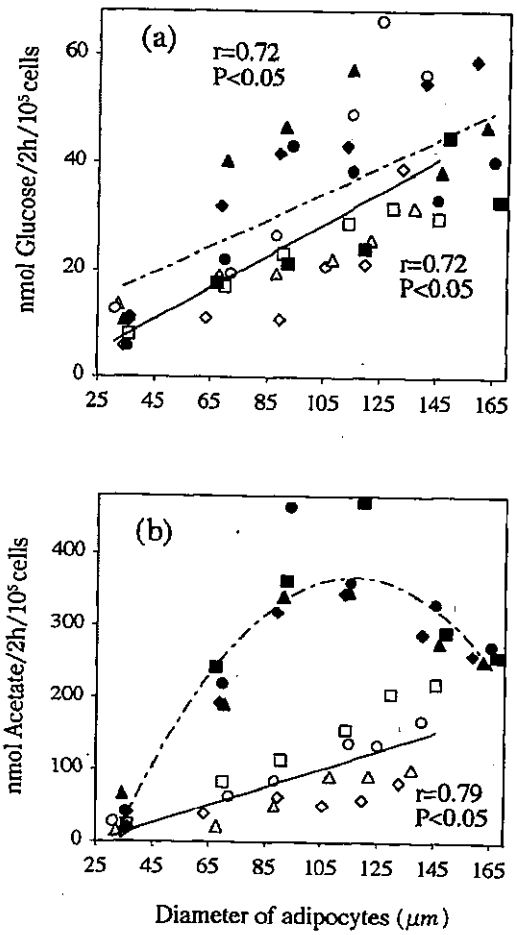


*Fig. 3.* The effect of adipocyte size on the lipogenesis (experiment 2). (a) : The effect of adipocyte size on the incorporation rate of glucose into lipids. (b) : The effect of adipocyte size on the incorporation rate of acetate into lipids. ◇□○△ : four early fattening steers. ◆■●▲ : four finishing steers.

## Discussion

The isolation procedure removes most of other cell types and leaves the isolated adipocytes, and is therefore one of preparation methods for studying adipocyte metabolism *in vitro*[1,17,21]. Based on the modifications of the Rodbell method and our previous study[5], we used M 199 supplemented HEPES and 4% BSA as basal medium for the collagenase digestion and measured the lipogenesis rate of glucose and acetate in the isolated adipocytes from adipose tissue of steers. Through using

modified method for separating the adipocytes from adipose tissue by Rule *et al.*[12], we fixed and separated the adipocytes from mixture of isolated adipocytes after incubation, and made it possible to understand the relationship between lipogenesis rate and the size of adipocytes distributed in adipose tissue from steers during different fattening periods.

In experiment 1, we found that there were positive correlation between the lipogenesis and the mean diameter of adipocytes taken from steers during fattening period. It is consistent with the report using adipose tissue fragments which showed that the rate of fatty acid synthesis from lactate and acetate increases between 11 and 19 months of age in several depots of growing cattle[19]. In sheep, parallel to the increase of the adipocyte mean volume, the rate of lipogenesis in subcutaneous adipocytes increased steadily after about 200 days of age[19].

The adipocyte size may influence the metabolism of the cell dramatically[1]. Rule *et al.* separated the adipocytes from an osmium-fixed adipose tissue which had been incubated with $^{14}$C-acetate and $^{14}$C-lactate, and measured the incorporation rate of the substances into lipid molecules according to cell size[12]. They studied the effect of adipocyte size on *in vitro* lipogenesis in subcutaneous adipose tissue of cattle at 6 months of age and showed that the lipogenesis per cell increased with increased cell size. In experiment 2, we found the effects of cell size and the fattening stage on the lipogenesis in adipocytes. There was a positive correlation between the lipogenesis from glucose and the diameter of adipocytes distributed in the adipose tissues. In adipocytes taken from the early fattening steers, there was also a positive correlation between the incorporation rate of acetate and the diameter of adipocytes. However, in adipocytes taken from the finishing steers, the incorporation rate of acetate became lower after the adipocytes reached a very large diameter. Up to now, we have no suitable explanation for this. Recently, some new functions of adipocytes were found. Adipocyte produces and secretes a variety of hormones and other factors related to the lipogenesis, such as IGF-I, adipsin, leptin[19]. The abilities to secrete these hormones or factors may be changeable with the growth of adipocyte. It may be one of the reasons that caused the change of lipogenesis in adipocytes with different sizes or from steers during different fattening periods. Further studies are needed to clarify the mechanism about the effects of adipocyte size.

In adipose tissue of adult ruminant, as a substrate for lipogenesis, acetate normally had a larger incorporation rate into lipids than glucose. In experiment 2, we found that the relative contribution ratio of acetate to glucose was higher in each fraction of isolated adipocytes from finishing steers than that from early fattening steers. As a substrate for lipogenesis in adipose tissue of adult ruminant, glucose is comparatively and constantly lower than acetate. With the fattening stage changed from early fattening stages to finishing stages, there were relatively small increment in the incorporation rate of glucose and large increment in the incorporation rate of acetate. It caused that the relative contribution ratio of acetate to glucose was higher in adipocytes from finishing steers than that from early fattening steers. There were some studies on the relative contributions of acetate, lactate and glucose to lipogenesis in bovine adipose tissue[10,15-16]. These studies showed that glucose carbon could be incorporated into glycerol, fatty acids and lactate, and the relative percentage may be variable with breed, age or incorporation medium. Although we did not trace the incorporation carbon from acetate and glucose which used as the substrates in present study, it is acceptable that acetate and glucose could incorporate into both glycerol and fatty acids moiety of the adipocytes. It is possible that the change on

the ratio of acetate to glucose is caused by the change on utilization of glucose and/or acetate. Glucose may contribute more to lactate, glyceride-glycerol and/or less to glyceride-fatty acids directly in adipocytes from finishing steers. Further studies are needed to compare the utilization of glucose in adipocytes with different sizes which are from steers during different fattening stages.

In conclusion, the present study showed that the lipogenesis has a close relationship to the size of isolated adipocytes from Holstein steers during fattening periods. In isolated adipocytes from subcutaneous adipose tissue, the lipogenic acitivities of the adipocytes were varied with their diameter. There is a decrease in incorporation rate of acetate into lipids in those very large adipocytes taken from finishing fattening steers, it can be suggested that the lipogenesis might be limited in these adipocytes.

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# 肥育ステージの異なるホルスタイン種去勢牛における皮下脂肪細胞の大きさと脂肪合成能との関連

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肥育過程における肉用牛の皮下脂肪組織の脂肪細胞の発達と，脂肪合成能との関連を明らかにするため，ホルスタイン種去勢牛における脂肪合成能を単離脂肪細胞におけるグルコースや酢酸の総脂質への取り込み量によって評価した．皮下脂肪細胞の平均直径は肥育過程の進行とともに増加し，単離脂肪細胞におけるグルコースや酢酸の総脂質への取り込み量はこの増加に伴い有意に増加した（$P<0.05$）．脂肪細胞の平均直径とグルコースや酢酸の脂質への取り込み量との間にはそれぞれ $r=0.74$，0.87 の相関が見られた（$P<0.05$）．また，肥育前期および後期牛の脂肪組織から得た単離脂肪細胞を，7 種類のナイロンメッシュで篩別し，6 分画に分け，直径の異なる単離脂肪細胞における脂肪合成能を調べた．酢酸，グルコースの総脂質への取り込み量は，肥育前期去勢牛から得た細胞より，肥育後期の方が多く，酢酸/グルコースの取り込み比も大きかった（$P<0.05$）．肥育前期牛の脂肪細胞におけるグルコースおよび酢酸の総脂質への取り込み量は，細胞の大きさによる分画間に有意な差があり（$P<0.05$），脂肪細胞直径の大きさの順に従って多くなり，細胞の大きさと脂質への基質取り込み量との間には酢酸，グルコースとも有意な相関が見られた（$r=0.72$ ; $P<0.05$）．肥育後期牛の脂肪細胞におけるグルコースの総脂質への取り込み量も，細胞の大きさによる分画間に，有意な差があり（$P<0.05$），直径の小さい細胞から大きい細胞の順で多くなり，取り込み量と細胞の大きさとの間の相関係数は 0.79 であった（$P<0.55$）．一方，肥育後期牛の脂肪細胞における酢酸の総脂質への取り込み量は，細胞径による分画間に，有意な差があり（$P<0.05$），直径 116$\mu$m 以下の脂肪細胞分画では，細胞直径の大きさの順に従って取り込み量は多くなったが，直径 116$\mu$m 以上の細胞では，直径の大きさの順に従って，その取り込み量が少なくなった（$P<0.05$）．

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