——Research Note——

# The Relationship Between Fatty Acid Composition and the Size of Adipocytes from Subcutaneous Adipose Tissue of Holstein Steers During the Fattening Period

Mao Long HE, Sang-Gun ROH, Hideyuki OKA,
Satoshi HIDAKA, Nobuyoshi MATSUNAGA
and Hisashi HIDARI

Department of Animal Science, Obihiro University of Agriculture and Veterinary Medicine, Obihiro-shi 080

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**Abstract** The fatty acid composition and the diameter of subcutaneous adipocytes from 13 Holstein steers at different fattening stages were investigated. The mean diameter of adipocytes from individual steers was positively ($P<0.01$) correlated with the percentage of myristoleic acid (14 : 1) ($r=0.91$), palmitoleic acid (16 : 1) ($r=0.83$) or oleic acid (18 : 1) ($r=0.82$), and negatively ($P<0.01$) correlated with the percentage of stearic acid (18 : 0) ($r=-0.89$). The isolated adipocytes from the same finishing steer ($n=5$) were then separated by a 50$\mu$m mesh. The large adipocytes which remained on the mesh (118$\mu$m mean diameter) contained more ($P<0.01$) myristoleic acid (14 : 1), palmitoleic acid (16 : 1) and oleic acid (18 : 1), and less ($P<0.01$) palmitic acid (16 : 0), stearic acid (18 : 0) than the small adipocytes which were filtered through (29$\mu$m mean diameter). It is suggested that the growth of adipocyte is one of the important determinants of fatty acid composition in subcutaneous adipocytes of Holstein steers throughout the fattening period.

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**Key words** : Fatty acid, Adipocytes, Steer, Cell diameter

The ratio of monounsaturated to saturated fatty acids in the adipose tissue increased significantly with the progress of growth and fattening in the steers[2,4-5,10]. The accretion of a metabolically neutral lipid droplet increases in the adipocytes of steers during the fattening period, which causes the enlargement of the adipocytes. Adipose tissue is composed of adipocytes of different sizes. It has been assumed that the adipocytes are normally distributed about a mean diameter[1]. The mean diameter of the adipocytes increases with the age of the fattening steers[12].

Since there is little information about the differences in the fatty acid composition of the adipocyte as related to size, the present study aimed to investigate the relationship between fatty acid composition and the enlargement of subcutaneous adipocytes in Holstein steers at different fattening stages, and the difference of

ホルスタイン種去勢肥育牛の皮下脂肪細胞の大きさと脂肪酸組成との関連：何　茂龍・盧　尚建・岡　英之・日高　智・松長延吉・左　久（帯広畜産大学，帯広市 080）

the fatty acid compositions of the adipocytes distributed in the same subcutaneous adipose tissue of the same fattening steer.

## Materials and Methods

***Animals and tissue sampling***

Thirteen Holstein steers from 6 to 28 months of age fed mainly on a diet of concentrates plus hay were used in this experiment. All these fattening steers were kept in the same environment. To get the sample of adipose tissue, biopsies were performed two or three times, in the same animal, every 3 or 4 months. Subcutaneous adipose tissue samples were obtained from the region of the last thoracic vertebrae, about 20 to 30 cm to the right or left of the dorsal midline. Part of the tissue was transported to the laboratory in saline at 37℃ within 20 minutes while the rest were frozen in −80℃ for further analysis.

***Adipocyte isolation***

Bovine adipocytes were isolated from the adipose tissue using a modified version of Rodbell's method[9,11]. Each sample was dissected to free it of connective tissue and blood vessels, and placed in 25 m*l* polypropylene beakers containing M 199 (Medium 199, GIBCOBRL, Life Technologies, Inc. Grand Island, NY., USA) with 20 mM HEPES, 5 mM glucose, 5 mM acetate and 4% BSA (bovine serum albumin, fraction V, Sigma Chemical Co., St. Louis, USA) with 3 mg per m*l* collagenase (Worthing biochemical Co., New Jersey, USA) for isolation. After 90 minutes of digestion, cell suspensions were filtered through the polypropylene meshes with a pore size of 1,000 and 250 $\mu$m and washed with KRB buffer (Krebs-Ringer bicarbonate buffer, pH 7.4).

To estimate the possible changes in the fatty acid composition of the adipocytes during the isolation process, we compared the fatty acid composition of the adipose tissue and isolated adipocytes. There was no significant difference ($P>0.05$) in the fatty acid composition of the adipocytes and the adipose tissue taken from the experimental steers. The comparison suggested that the adipocytes which were isolated by this method still keep their original fatty acid composition that they had in the adipose tissue.

***Adipocyte separation***

The isolated adipocyte suspensions from five finishing steers with a mean body weight of 701 kg were then separated by a polypropylene mesh with a pore size of 50 $\mu$m into two parts : large adipocytes which remained on the mesh and small adipocytes which were filtered through the mesh. Both of these separated adipocytes were frozen in −80℃ for further analysis of fatty acid composition.

***Cell size of adipocyte***

Both the separated and isolated adipocytes, together with an objective micrometer (0.01 mm, Nikon, Japan), were immediately photographed using a micro photograph equipment (OPTIPHOT, Nikon, Japan). To estimate the mean diameter of the adipocytes, the diameters of 300 cells were measured from the photographs.

***Fatty acid analysis***

Adipose tissue and adipocytes (5 mg) were methylated in 5 m*l* methanol-choleric acid (95 : 5) for 3 hours at 100℃. The fatty acid methyl esters were extracted by hexane and were analyzed using a dual column gas chromatograph (HITACHI 163, Japan) equipped with a glass column (3 mm of diameter and 2 m in length) packed with 60/80 mesh chromosorb (DEGS, Uniport B, GL Sciences, Tokyo, Japan). The injection port and detector temperatures were 225℃ and 215℃ respectively. The oven temperature was 175℃. The flow rate of nitrogen was 25 m*l*/min. A standard of known composition was analyzed to verify the identity of the fatty acids in the samples. The fatty acids peak areas were quantified with an electric integrator (C-R 6 A, Chromatopac, Shimadzu Co., Kyoto, Japan).

***Statistical analysis***

Student's t-test was used to compare the change in composition of the fatty acids in adipocytes caused by isolation and the difference of the fatty acid composition between adipocytes with different sizes. Linear regression coefficient analysis was used to test the relationships between the mean diameter of the adipocytes and the age in terms of months, the composition of fatty acids and the mean diameter of the adipocytes.

## Results and Discussions

### *Fatty acid composition and mean diameter of adipocytes isolated from steers during different fattening periods*

The mean diameter of adipocytes from the subcutaneous adipose tissues of the steers increased advancing age. The relationship between the mean diameter of adipocytes (D, $\mu$m) and the age (A, months) was described by the following regression equation : $D=51.06\,LnA-41.02$ ($n=22$, $r=0.93$, $P<0.01$). The mean diameter of adipocytes, the age of months and the body weight of steers ranged from 41.8$\mu$m, 6 months and 205 kg to 137.9$\mu$m, 28 months and 747 kg, respectively. These values in Holstein steers are consistent with the mean diameter of 58.1$\mu$m in crossbred steers of Angus at 7 months of age[6], 115.3$\mu$m in Angus steers weighing 300 kg[8], and 151.9$\mu$m in Angus steers with 15 to 16 months of age[7].

The percentage of monounsaturated fatty acids in the adipocytes increased while the saturated fatty acid decreased with the enlargement of the isolated adipocytes (Fig. 1). The linear correlation coefficients ($n=22$) between the mean diameter and myristoleic acid (14 : 1), palmitoleic acid (16 : 1) or oleic acid (18 : 1) were 0.91 ($P<0.01$), 0.82 ($P<0.01$) or 0.83 ($P<0.01$), respectively. The linear correlation coefficients between the mean diameter and palmitic acid (16 : 0) or stearic acid (18 : 0) were $-0.37$ ($P>0.05$) or $-0.89$ ($P<0.01$), respectively.

These results were consistent with the reports[2,4,5,10] where fatty acid composition of

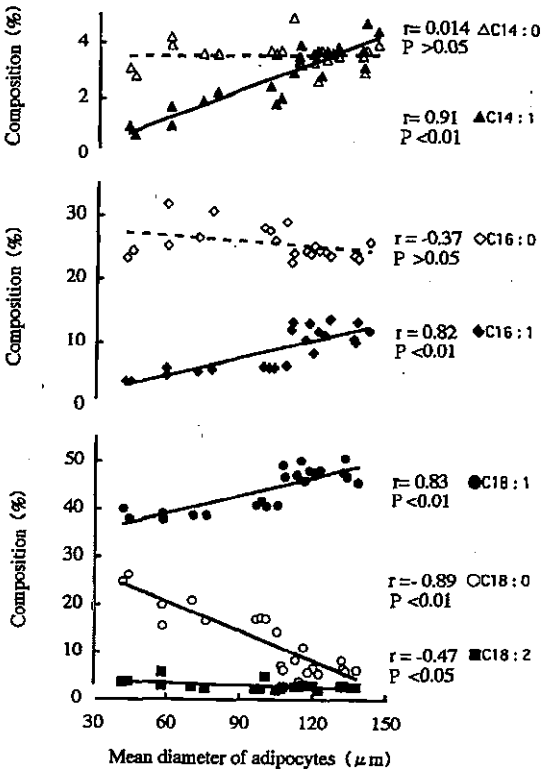


*Fig. 1.* Relationship between fatty acid composition and the mean diameter of subcutaneous adipocytes from Holstein steers during the fattening period ($n=22$).

subcutaneous fat was affected by aging and/or fattening. It suggests that while the mean adipocyte size of fattening cattle becomes larger, the fatty acid desaturase which converts stearic acid (18 : 0) to oleic acid (18 : 1) may become more active.

Because the adipocytes with different mean diameters were from different steers at different fattening stages, the relationship between the fatty acid composition and size of adipocyte that we showed here may be affected by age, body weight or diet. The best way to avoid the effect of differences in age, body weight or diet is to compare the fatty acid composition of adipocytes with different sizes distributed in same adipose tissue. In the present study, we found it practical to separate isolated adipocytes from individual steers

according size. Therefore, with a 50$\mu$m mesh, we separated adipocytes which were taken from same fattening steers into large adipocytes, those which remained on the mesh, and small adipocytes, those which were filtered through. These findings helped us clarify the relationship between fatty acid composition and the size of adipocytes.

***Fatty acid composition and the diameter of the separated adipocytes from the same adipose tissue***

In finishing Holstein steers (n=5), the adipocytes which remained on the 50$\mu$m mesh (118$\mu$m mean diameter) contained more ($P<0.01$) myristoleic acid (14 : 1), palmitoleic acid (16 : 1) and oleic acid (18 : 1), less ($P<0.01$) palmitic acid (16 : 0) and stearic acid (18 : 0) than the filtered adipocytes (29$\mu$m mean diameter) (Fig. 2).

Because the adipocytes with different sizes were obtained from the same adipose tissue of the animal, the differences in fatty acid compositions of adipocyte can only be explained by their difference in size. As the adipocytes became larger they became more unsaturated. The adipocyte size itself may dramatically influence the metabolism of the cell[1]. On the other hand, it may also be a reason that the change in size of adipocyte may cause the change in the ratio of the lipids in membranes to the lipids in lipid droplets which have different fatty acid compositions. Similar results were shown in Holstein steers during their early fattening stage (n=4) : The adipocytes which remained on the 50$\mu$m mesh with 100$\mu$m mean diameter contained more ($P<0.05$) oleic acid (18 : 1) and less ($P<0.05$) palmitic acid (16 : 0) than the filtered adipocytes with 31$\mu$m mean diameter (He *et al.*, unpublished). We can conclude that whereas the growth or fattening stage, body weight, age, sex, breed type and diet were important determinants in the fatty acid composition of adipocytes[10,12,13], the size of adipocyte itself was also an important determinant in the fatty acid composition.

From the present study, we found that

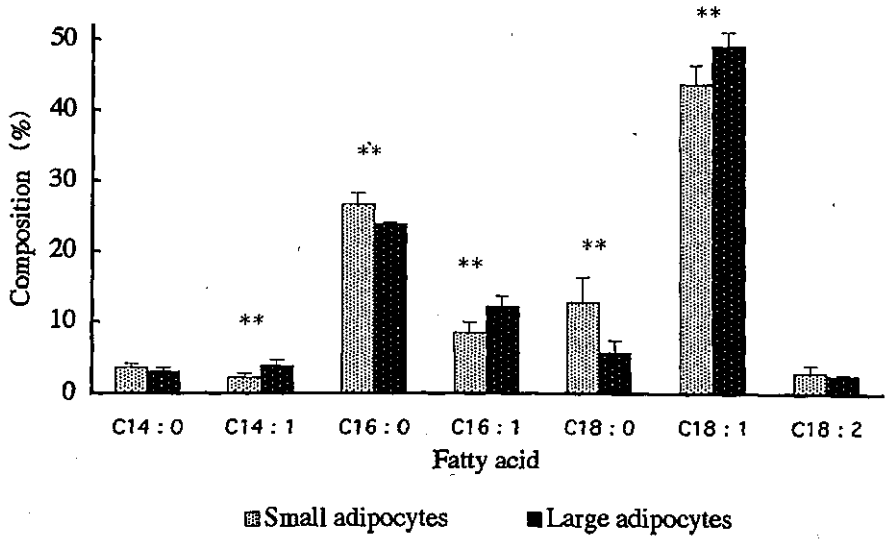


*Fig. 2.* Comparison of main fatty acid composition between the adipocytes with different sizes which were separated from the subcutaneous adipose tissue of Holstein steers (n=5) during the fattening period. Each column and vertical line represents the mean and standard deviation, respectively. This mark : ** means significantly different within each pair of columns ($P<0.01$).

adipocyte, even from the same finishing steers with different sizes have different fatty acid composition. This revealed a close relationship between the fatty acid composition and size of adipocyte. Further study is required to clarify the possible differences of lipogenesis or fatty acid desaturation enzyme in adipocytes with different sizes.

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