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THE ROLE OF COLLAGENS FOR INTRAMUSCULAR ADIPOSE DEVELOPMENT

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BACKGROUND

The grading of carcasses is influenced by the amount of intramuscular adipose tissue or marbling; the more marbling the higher the grade. Improvement in meat quality for ages by breeding selection, Japanese Black cattle has acquired the genetic ability to deposit intramuscular adipose tissue in greater quantities than other cattle (7, 9). However, information is still obscure for understanding the reason of producing such highly marbled beef. Furthermore, even the process of adipose accretion in muscle that accompany fattening is unknown so far.

OBJECTIVES

Because of the spatial allowance for fat accumulation, we were concerned about intramuscular connective tissue components, so-called extracellular matrix (ECM), such as collagens. The purpose of this study was; (a) to compare the distribution of several collagens in intramuscular connective tissue and adipose tissue of Japanese Black cattle, (b) to investigate how adipose conversion affects collagen properties by in vitro cell culture system, and for further analysis, (c) a specific inhibitor of collagen synthesis was used to study the role of collagens during differentiation of intramuscular preadipocytes derived from Japanese Black cattle.

METHODS

Antibodies.
The following rabbit antisera were used as primary antibodies: anti-bovine type I, IV, V and VI collagen. The secondary antibodies used were fluorescein-isothiocyanate (FITC)-conjugated to goat anti-rabbit Ig and goat anti-rabbit IgG labeled with ^{125}I.
Immunohistochemical staining.
Small pieces of bovine skeletal muscle from *M. longissimus thoracis* between the sixth and seventh rib of a Japanese Black steer (47 months old, 688 kg), fed at the National Institute of Animal Industry, were excised immediately after slaughter. Sections were serially cut and incubated with each primary antiserum and with FITC-conjugated Ig as second antibody. After culture on slides, cells were reacted as similar and then observed with epifluorescent illumination using a Zeiss Axiophot microscope.
Cell culture.
A stromal-vascular preadipocyte cell line, derived from intramuscular adipose tissue of Japanese Black cattle (1), were maintained in the preadipose condition by cultivation in the Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum, 100 IU/ml penicillin and 100 μg/ml streptomycin. Cells were inoculated at a density of 2.1×10^4 cells/cm^2 and grown for 2 days to obtain confluency. To initiate the formation of adipocytes, confluent cultures were shifted to an adipogenic medium, containing 50 ng/ml insulin, 0.25 μM dexamethasone, 10 mM acetic acid and 5 mM octanate. The medium was changed every other day and the cells were allowed to differentiate for 10 more days. Control cultures were grown after confluency in non-adipogenic medium as preadipose state. Ethyl-3,4-dihydroxybenzoate (EDHB) in ethanolic solution was added to the culture medium to study the role of procollagens during adipocyte differentiation.
^{125}I Binding assay.
The amount of collagens deposited over the cell monolayers was determined using radiolabeled secondary antibody.
Triglyceride assay.
Triglyceride (TG) in the cell lysate was extracted with chloroform -methanol and quantified enzymatically using a Triglyceride G Test Wako Kit.

RESULTS AND DISCUSSION

As generally accepted, sections from skeletal muscle of Japanese Black cattle showed that type I collagen predominated in the perimysium and type IV collagen located exclusively in the endomysium (2, 3, 8). Type V (Fig. 1A) and type VI collagens were abundant in both perimysial and endomysial sheaths. Interestingly, this result was quite different from previous reports on other species (2, 3, 6). On the other hand, extracellular space in intramuscular adipose tissue were strongly stained with all four collagens which we detected (Fig. 1B).

Due to the interactions of numerous systems involved in growth of adipose tissue, it is difficult to perform in vivo studies. To investigate how adipose development affects collagen biogenesis, we compared the morphology and the level of each collagen by

stromal-vascular cells from Japanese Black cattle under adipogenic and non-adipogenic culture conditions. When cultured preadipocytes underwent adipose differentiation, they produced fibrillar network of collagen type I and IV that was not present in undifferentiated cells. While some few fibrous structure were observed in preadipocytes (Fig. 2A), which were different from the former two collagens, type V and type VI collagen also formed a great amount of fibers in adipose state (Fig. 2B). In addition, iodinated antibody assay showed that there was a significant increase in amount of pericellular collagens upon adipocyte differentiation (Fig.3). Taken together, these data indicate that under conditions permissive for adipose conversion, active synthesis and organization of collagens give rise to adipose tissue in skeletal muscle with its own extracellular products.

In order to study the role of collagen synthesis in adipose development, EDHB was used as a specific inhibitor. EDHB is known to be a structural analog of ascorbate; it inhibits the activity of prolyl hydroxylase which converts a few residues of proline to hydroxyproline during the post-translational maturation of collagens (5). This modification is essential for triple helix formation and subsequent secretion. Addition of various concentration of EDHB to the adipogenic medium reduced the TG accumulation of cells in a dose dependent manner; inhibitory effect near 50% was obtained at 100 μM EDHB as against normal adipose conditions. The result strongly imply that collagens play a critical role in differentiation of preadipocytes into adipocytes, that is, in fat storage, as in agreement with those reported previously on mouse cells (4).

CONCLUSION

Deposition of intramuscular fat, as seen most remarkably in Japanese Black cattle which have entered the fattening, caused alteration in composition and construction of collagens in skeletal muscle which was newly organized by adipocyte itself during differentiation. Further investigation demonstrated that active synthesis of collagens in fat cells was not only for organization and inert support of fat lobules, but also necessity for intracellular lipid accumulation. Consequently, these results prompted us to postulate that collagens might regulate the growth of adipose tissue and texture tenderization that accompany fattening.

PERTINENT LITERATURE

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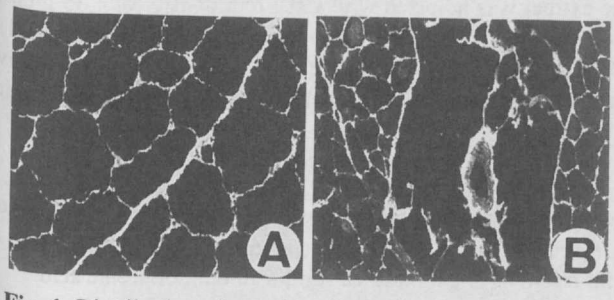

Fig. 1. Distribution of type V collagen in skeletal muscle (A) and intramuscular adipose tissue (B).

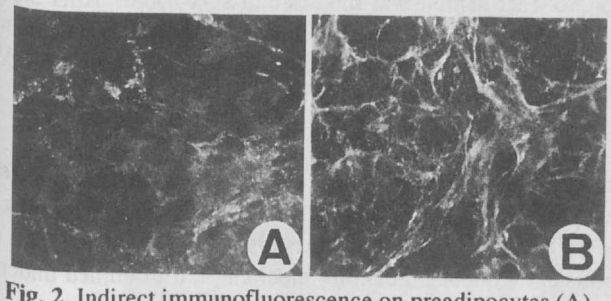

Fig. 2. Indirect immunofluorescence on preadipocytes (A) and adipocytes (B) using anti-type V collagen antibody.

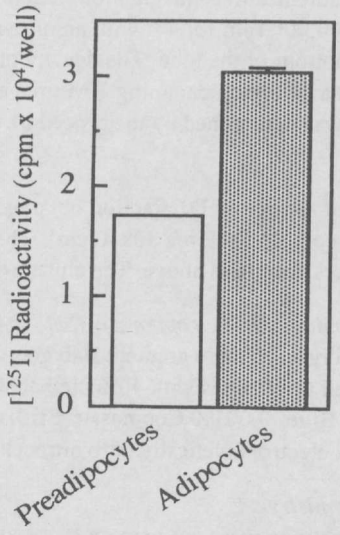

Fig. 3. Enhanced synthesis of type V collagen by adipocytes.