Journal of Animal and Feed Sciences, 13, Suppl. 1, 2004, 389–392

In vitro differentiation of preadipocytes from bovine adipose tissue*

O. Grageola[1], A. González[1], A. Shimada[1], F Castro ML[3], A. Varela[2] and O. Mora[1,4]

[1]Facultad de Estudios Superiores – Cuautitlán, UNAM, México
[2]Instituto de Neurobiología–UNAM, México
[3]Centro de Investigación y Estudios Avanzados - Zacatenco, México

ABSTRACT

To study differentiation of bovine adipose tissue preadipocytes into adipocytes, two tissue sources, two animal ages, and two differentiation media were tested. While no differentiation was observed with cells obtained from adult adipose tissue, foetal perirenal fibroblasts differentiated into adipocytes in the following medium: DMEM/F-12 1:1; 10 µg/mL insulin; 0.25 µM dexamethasone; 10 µg/mL very low-density lipoproteins; 100 µg/mL streptomycin; and 100 U/mL penicillin.

KEY WORDS: bovine, differentiation, adipocytes, *in vitro*

INTRODUCTION

White adipose tissue (AT) is the main site of energy depots in animals. It is used as a triglyceride reservoir in periods where an excess of energy is present and it is mobilized when energy is scarce (Dani, 1999; Prokesch, 2002). AT appears late in embryonic development. In humans, it starts developing during the last two thirds of gestation and major proliferation of preadipocyte populations occurs after birth and during puberty (Dani, 1999). AT is macroscopically undetectable during embryogenesis. It can only be identified when it contains adipocytes (completely differentiated or terminal cells). No specific markers for identifying adipoblasts or preadipocytes *in vivo* have been found. Furthermore, information concerning the ontogeny of this type of tissue is scanty (Dani, 1999).

AT growth is the combined result of both hyperplasia and hypertrophy. Hyperplasia occurs by preadipocyte proliferation and its subsequent

* Supported by CONACYT, 41337Z
[4] Corresponding author: e-mail: mora@mail.inb.unam.mx

differentiation, mainly in young animals. Hyperplasia is controlled by hormones and growth factors such as insulin-like growth factors I and II (IGF-I and IGF-II), acid and basic forms of the fibroblast growth factor (aFGF and bFGF) and tumor necrosis factor (TNF-α), among others (Prokesch, 2002). Adipocytic cells develop from a branch of pluripotent cells of mesodermal origin, which also give rise to muscle, bone and cartilage. However, the mechanisms regulating differentiation of this mesenchymal branch into an adipocytic lineage remain largely unknown (Rosen and Spielgeman, 2000). In order to differentiate fibroblast into adipocytes of bovine adipose tissue a serial subcultures were carried out to obtain a suitable model to address the molecular mechanisms and environmental cues that drive somatic stem cells to become preadipocytes and subsequently mature adipocytes.

MATERIAL AND METHODS

AT was obtained from two sources: 1. Subcutaneous and omental adipose tissue of 3-4 year-old Holstein adult animals, and 2. Perirenal and omental adipose tissue from Holstein foetuses (last third of gestation). All samples were collected immediately after sacrifice in the Querétaro city municipal abattoir.

Samples were maintained and transported in DMEM medium containing 100 U/mL penicillin, 100 µg/mL streptomycin and 10% bovine foetal serum at 4°C. AT was dissociated with 2 ml digestion solution (DMEM containing 20 mM HEPES, pH 7.4, 2 mg/mL type II collagenase, and 4 mg/mL bovine serum albumin) per 0.5 g of sample (Wu et al., 2000). The resulting cell pellet was resuspended in basal medium (DMEM containing 4 µM biotin, 200 µM ascorbic acid, 100 U/mL penicillin, 100 µg/mL streptomycin, and 10% bovine foetal serum). Subsequently, the resuspended cells were plated in 60 mm culture dishes and incubated at 37°C under a 5% CO_2 atmosphere. Cells were maintained in these conditions until preconfluence was reached in approximately four days. At this point cells were subcultivated by detaching them with trypsin and replating them at 10^5 cells per 100 mm diameter culture. To induce adipocytic differentiation the subcultured cells were plated into 24 well plates at a density of 2×10^4 cells/well. When cultures reached preconfluence (48 h), cells were treated independently with each of the two following culture media to promote differentiation: Medium 1 was DMEM containing 20% bovine foetal serum, 10^{-7} M dexamethasone, 50 µM indomethacin, 2 mM glutamine, 100 U/mL penicillin and 100 µg/mL streptomycin sulphate (Prokesch, 2002). Medium 2 was DMEM/F-12 1:1, containing 10 µg /mL insulin, 0.25 µM dexamethasone, 10 µg very low-density lipoproteins, 100 µg/mL streptomycin and 100 U/mL penicillin (Wu et al., 2000).

Cultures were maintained in the differentiation media for at least 8 days, changing medium every third day. The presence of fat droplets in cellular cytoplasm was detected under phase-contrast microscopy.

Cultures carried out in parallel were fixed at the end of the differentiation and stained with oil red O (Ramírez-Zacarías et al., 1992).

As an additional indicator of differentiation, G3PDH activity was determined spectrophotometrically (Wise and Green, 1979; Castro-Muñozledo et al., 1987).

A regression analysis was carried out to establish the best fitting model to describe cellular proliferation for the different tissue. The results of oil red O staining were analysed by SAS general linear models (SAS, 1992). Means were compared using the least square difference method.

RESULTS AND DISCUSSION

Serial preadipocyte subcultures were established for adult omental, adult subcutaneous, foetal omental, and foetal perirenal adipose tissues. Results show that there were no differences in the proliferation rates of the different cultures. The equations for each curve were, respectively: $y = -26.03 + 24.69x - 1.40\ x^2$ ($r^2 = 0.98$, $P<0.01$); $y = -0.12 + 18.35x - 0.73x^2$ ($r^2 = 0.99$, $P<0.01$); $y = 59.84 + 39.27x - 2.63x^2$ ($r^2 = 0.97$, $P<0.01$), and $y = -17{,}66 + 22.49x - 0.95\ x^2$ ($r^2 = 0.93$, $P<0.01$), where y is the cellular proliferation (acumulative yield). No obvious morphological differences were observed between any of the cultures and no lipid droplets were detected either. Upon reaching subconfluence, the cultures were detached with trypsin and plated in each of the two differentiation media described in methods. Adipocytic differentiation was observed within approximatley 13 days but only in the culture of foetal perirenal adipose cells and only when medium 2 was used. Abundant lipid droplets were detected in the differentiated cells. For these cultures of foetal perirenal AT cells, the average incorporation of oil red per well in absorbance units was 0.169a, 0.115a and 0.206b (SEM 0.031) for cultures in basal medium, and differentiating media 1 and 2, respectively. The largest absorbance value corresponded to differentiating medium 2 ($P<0.01$).

These results indicate that different tissues from different ages have distinct adipogenic properties, at least in the *in vitro* conditions tested in our study. Preadipocyte differentiation has been successful for different adipose tissue depots; nevertheless, results have not been conclusive.

CONCLUSIONS

Bovine foetal perirenal adipose tissue cells are capable of differentiating into mature adipocytes *in vitro* by the addition of insulin, dexamethasone and very low-density lipoproteins. This reveals a differential ability of preadipocytes of different sources and ages to undergo adipocytic differentiation *in vitro*. This culture system may be used as a tool to study molecular mechanisms of adipocytic differentiation in this species.

REFERENCES

Castro-Muñozledo F., Marsh-Moreno M., Beltrán-Langaríca A., Kuri-Harcuh W., 1987. Commitment of adipocyte differentiation in 3T3 cells is inhibited by retinoic acid and expression of lipogenic enzymes is modulated through cytoskeleton stabilization. Differentiation 36, 211-219

Dani C., 1999. Embryonic stem cell-derived adipogenesis. Cell Tissues Organs 165, 173-180

Prokesch A., 2002. Large scale gene expression profiling during adipogenesis of murine bone marrow stromal cells. Master Thesis. Institute for Biomedical Engineering, University of Technology, Graz (Austria) and the National Institute of Health Bethesda (USA)

Ramírez-Zacarías J.L., Castro-Munozledo F., Kuri-Harcuch W., 1992. Quantitation of adipose conversion and triglycerides by staining intracytoplasmic lipids with oil red O. Histochemistry 97, 493-497

Rosen E.D., Spielgelman B., 2000. Molecular regulation of adipogenesis. Annu. Rev. Cell. Dev. Biol. 16, 145-171

SAS Institute, Inc., 1992. SAS/STAT® User´s Guide. Version 6.02. SAS Institute, Inc., Cary, NC

Wise L., Green H., 1979. Participation of one isozyme of cytosolic glycerophosphate dehydrogenase of 3T3 cells. J. Biol. Chem. 254, 273-275

Wu P., Sato K., Suzuta F., Hikasa Y., Kagota K., 2000. Effects of lipid-related factors on adipocyte differentiation of bovine stromal-vascular cells in primary culture. J. Vet. Med. Sci. 62, 933-939