

Ultrastructural Effects of Retinyl Palmitate on Brown Adipocytes of Fetal Mice

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Abstract: Brown adipose tissue (BAT) has a thermogenic function in animals. Mitochondrial uncoupling protein (UCP) is responsible for the thermogenic function in BAT cells. Vitamin A affects proliferation and differentiation in cells. Retinoic acid, a derivative of vitamin A, stimulates the expression of the UCP gene, which is found in BAT cells. Retinoic acid performs its function regardless of differentiation stage of BAT cells. It is known that vitamin A is teratogenic at therapeutic doses in pregnancy. In this study, effects of retinyl palmitate applied in a therapeutic dose were examined in mice fetal BAT cells. Tissue samples were subjected to a routine electron microscopic procedure for this purpose and then examined. In the control group cells, mitochondria were found to have short cristae, the glycogen granules occupying the cytoplasm were large and the same as those in developing BAT cells, and the lipid granules were few. Glycogen granules constituted smaller clusters in the cytoplasm of cells in the vitamin A group. As compared with the control group, cristae of mitochondria were elongated and matured. In this group, the amount of lipid granules was more striking than that in the control group. In conclusion, this study indicated that vitamin A derivative applied in therapeutic dose during pregnancy accelerates the developmental period of BAT adipocytes in fetal mice.

Key Words: Retinoic acid, brown adipocyte, ultrastructure, development

Retinil Palmitatin Fetal Fare Kahverengi Yağ Hücrelerinin İnce Yapısı Üzerine Olan Etkileri

Özet: Kahverengi yağ dokusu (KYD) hayvanlarda ısı üretici olarak işlev yapar. Mitokondriyal uncoupling protein (UCP) ise KYD hücrelerinde ısı üretiminden sorumludur. A vitamini hücrelerin çoğalmasında ve farklanmasında etkindir. A vitamini türevlerinden olan retinoik asit KYD hücrelerinde bulunan UCP geninin ekspresyonunu uyarır. KYD hücreleri hangi farklanma aşamasında olursa olsun, retinoik asit işlevini yerine getirmektedir. Gebelikte, tedavi dozunda uygulanan A vitamininin teratojenik olduğu bilinmektedir. Bu çalışmada, tedavi dozunda uygulanan retinil palmitatin fetal fare KYD hücrelerinin ince yapısı üzerine olan etkileri incelendi. Rutin elektron mikroskop takibi için kesitler alındı. Kontrol grubuna ait hücrelerdeki mitokondriyonların kristalleri kısaydı. Sitoplazmada geniş yer kaplayan glikojen granülleri gelişmekte olan KYD hücrelerindeki gibiydi. Lipid granülleri de azdı. A vitamini gurubundaki glikojen granülleri sitoplazma içinde küçük kümeler oluşturuyordu. Kontrol grubuyla karşılaştırıldığında mitokondriyonların kristalleri daha uzun ve olgun görünümdeydi. Bu grupta lipid granüllerinin miktarı kontrol grubuna göre daha fazlaydı. Sonuç olarak gebelere tedavi dozunda uygulanan A vitamini türevi, KYD hücrelerinin gelişim sürecini hızlandırmıştır.

Anahtar Sözcükler: Retinoik asit, kahverengi yağ dokusu hücreleri, ince yapı, gelişim

Introduction

Brown adipose tissue (BAT) plays an important role in thermogenesis and also is responsible for warming the blood that passes through it. In the developmental period, the amount of BAT in different parts of the animals is higher in newborns than in adults. BAT is found in both sides of neck, the axillary region, around the main vessels of mediastinum, thorax and pararenal region in newborns. It is essentially located in the interscapular region of newborn mice. Thermogenesis occurs via a

group of uncoupling agents in BAT. It was reported that oxidative phosphorylation did not occur in the mitochondria of BAT cells (1-4).

In the mitochondrion, electron transport and phosphorylation can be separated from each other by the compounds, which results in an increase in the permeability of mitochondrion inner membrane to protons, called uncoupling agents. These agents cause fast electron transport, and so the energy is transformed to heat without producing ATP (5).

BAT is composed of specialized cells, which produce heat by separating electron transport from phosphorylation under adrenergic stimuli. It was found that UCP (uncoupling protein) plays the key role in thermogenesis of BAT. UCP is also the molecular marker of BAT (6-9).

Vitamin A is a fat-soluble vitamin and is effective in proliferation and differentiation of cells (4,10). Bonet et al. (11) found that retinoids are physiological regulators of BAT. Scarpace et al. (12) reported that retinoic acid increases UCP-1 mRNA levels in BAT. Alvarez et al. (10) determined that retinoic acid also increased the amount of retinoic acid receptors in BAT.

Carmona et al. (5) reported that, at the differential stage in cell culture, the level of UCP-2 mRNA and transcription of UCP-2 gene is increased in brown adipocytes.

Retinoic acid is teratogenic, and when used during pregnancy, skeletal system deformities possibly result. There are few studies about the effects of vitamin A on the fine structure of BAT cells (13-15).

The aim of this study was to evaluate the effects of retinoic acid, a derivative of vitamin A, on the fine structure of BAT cells.

Materials and Methods

Swiss-Albino mice were used in order to examine the effects of retinoic acid on fetal mouse BAT. In each control and experimental group there were 6 female mice housed on a 12/12 hour light/dark cycle. Three female mice and a male mouse were housed overnight together. The following day, the mice that had sperm-positive vaginal plugs were considered to be in the first day of their pregnancy. On the 7th day of their pregnancy, 60 mg/kg retinyl-palmitate (Sigma) in 9% NaCl solution was applied intraperitoneally to mice in the experimental group. Same amount of 9% NaCl solution was also applied to mice in the control group. On the 18th day of pregnancy, BATs were taken from fetal interscapular region.

Tissues were fixed in phosphate buffered 2.5% glutaraldehyde solution, at pH 7.4 and +4 °C. After washing in phosphate buffer, tissues were fixed and stained with phosphate buffered 1% osmium tetroxide. Tissues were again washed with buffer solution, and they

were dehydrated with various degrees of ethanol. After staining with uranyl acetate, they were again dehydrated with various degrees of ethanol. Then in order to eliminate alcohol from the tissues, propylene oxide was applied. Tissues were put in araldide-DDSA-BDMA-propylene oxide pre-embedding material overnight and embedded in gelatine capsules containing araldide-DDSA-BDMA embedding material. Semithin sections of blocks were stained with toluidine blue. After marking the regions to be examined, thin sections were taken, stained with uranyl acetate and lead citrate and examined (Carl Zeiss EM 900).

Results

In the control group, BAT cells were large and round or roundish. Their nucleoli were euchromatic. Heterochromatin was clustered under the nuclear membrane, and their nucleoli were prominent. In the cytoplasm, triglyceride droplets were not present or were few. Mitochondria had in typical fetal mitochondrial structure, having a very large, abundant matrix and short and dull cristae. Cisterns of rough endoplasmic reticulum were prominent. Glycogen granules occupying large areas in cytoplasm had the same morphologic characteristics as the ones in developing BAT cells (Figures 1, 2) (Table).

In the cytoplasm of BAT cells in the test group, glycogen granules were scattered as small clusters throughout the cytoplasm. The number and diameter of triglyceride droplets were increased. In this group, rough endoplasmic reticulum cisterns were absent but smooth endoplasmic reticulum was present as small vesicles. The outstanding finding in this group was in mitochondria; when compared with the group of control cristae the mitochondria were elongated and showed tubular structures as the ones in matured BAT cells (Figures 3-5) (Table).

Discussion

In BAT adipocytes, there are capillaries, nerves and quantities of connective tissue altering with age. Adipose tissue consists of lobes and lobules. Adipocytes are wide radiated large cells. There are lots of triglyceride droplets in the cytoplasm of adipocytes. These cells have many large mitochondria that have well developed cristae. Besides, there are lots of smooth endoplasmic reticulum,

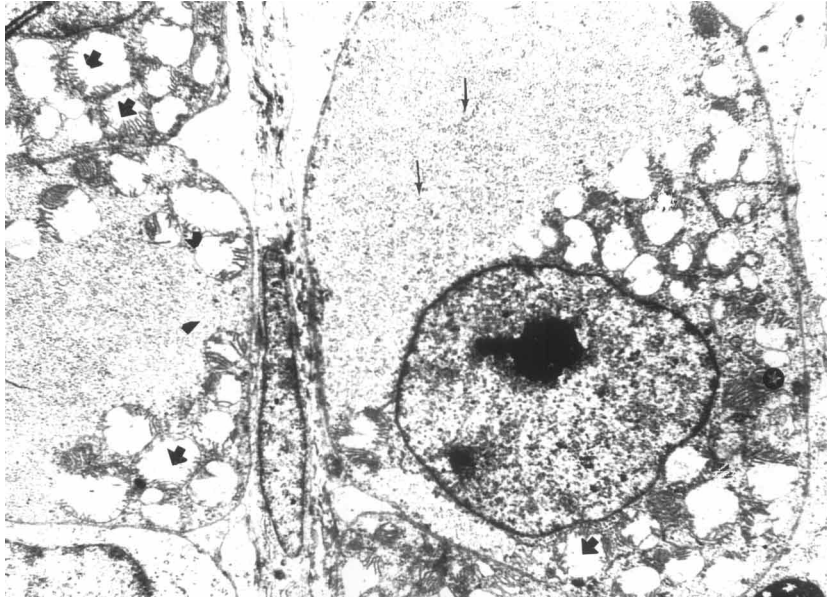


Figure 1. The control group. Mitochondria having short cristae (thick arrows). The areas containing granules of glycogen (thin arrows). The cisternae of rough endoplasmic reticulum (*). Uranyl acetate-lead citrate x 9000.

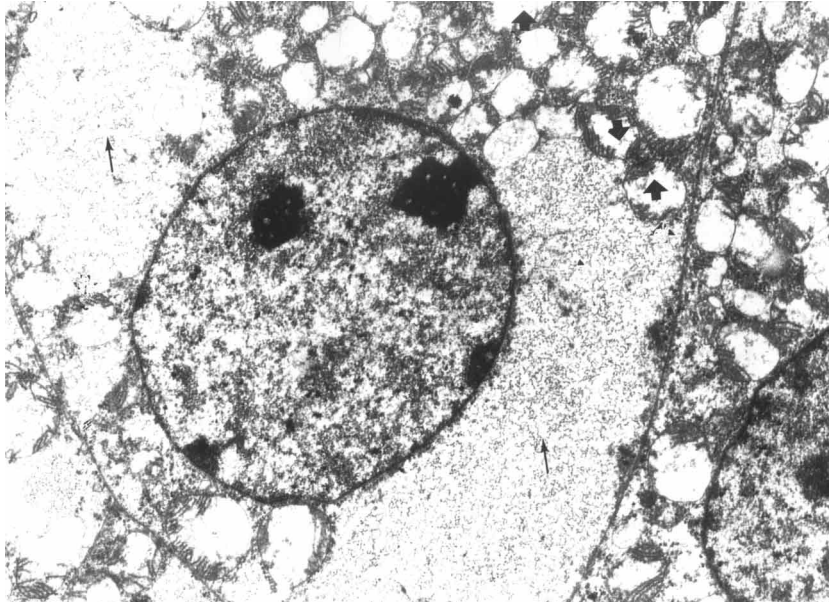


Figure 2. The control group. Mitochondria with short cristae (thick arrows) and large glycogen areas (thin arrows) but no triglyceride droplets. Uranyl acetate-lead citrate x 13,200.

and a few rough endoplasmic reticulum in their cytoplasm (16).

Recently, studies regarding rats have enabled us to learn more about BAT. During the 6th day of antepartum

period, the thin structure of rough endoplasmic reticulum cisterns were much in number, while the triglyceride droplets are found as low in BAT. The mitochondria are few and consist of short cristae (17).

Table. The structural results in control and treatment group.

Structures	The control group	The vitamin A group
Mitochondria	Many and have short, dull cristae	Less and have long cristae
Triglyceride droplets	None or few	Contain
Areas of glycogen	Large areas in cytoplasm	Small areas in cytoplasm
Endoplasmic reticulum	Rough endoplasmic reticulum	Smooth endoplasmic reticulum

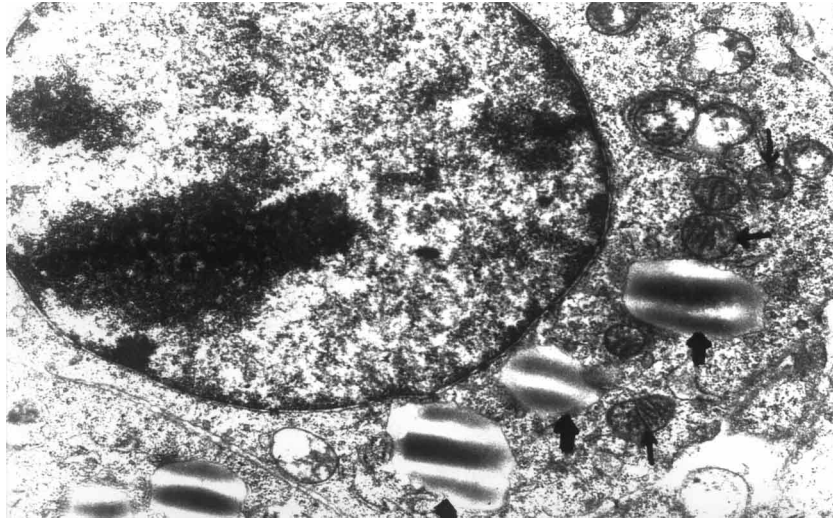


Figure 3. In the vitamin A group, there are a few triglyceride droplets not seen large in glycogen areas. Uranyl acetate-lead citrate x 21,000.

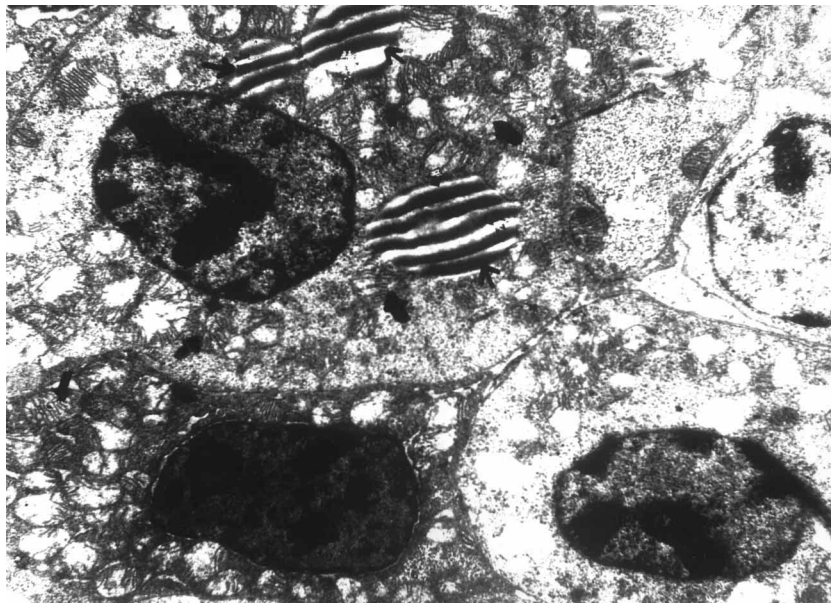


Figure 4. This figure of vitamin A group demonstrates (thick arrows) mitochondria with long cristae with respect to control group and more triglyceride droplets (thin arrows) Uranyl acetate-lead citrate x 13,200.

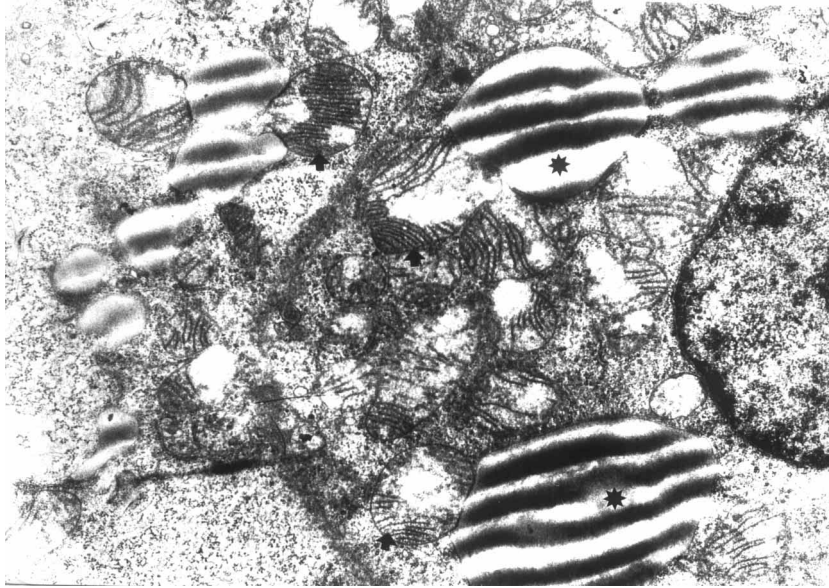


Figure 5. The higher magnification micrograph of the vitamin A treatment group. Matured mitochondria having long cristae (thick arrows). Triglyceride droplets (*). Uranyl acetate-lead citrate x 21000.

During the 3rd day of antepartum period, an increase in the number and size of triglyceride droplets in BAT is observed. Also, there are wide areas of glycogen granules with increased numbers of mitochondria in the adipocyte cytoplasm (18-20).

The gestation period for rats is about 21 to 23 days, while this period is about 19 to 21 days for mice. In this study, the period between antepartum 6th and 3rd days for rats, matches for the antepartum 3rd day of mice, for collection of the specimens. The histological properties of BAT for rats and mice are known to be similar (17).

In this study, our findings are compatible with the literature, as we evaluated the adipocytes of BAT of control fetal specimen during the 3rd day of antepartum period. In this group of rats, the cristae of the mitochondria are short, dull and few, as indicated in the 6th and 3rd days of antepartum period. The cisterns of rough endoplasmic reticulum can be distinguished in the cytoplasm, while wide glycogen containing areas are evident in our control group as evaluated in the 3rd day of antepartum period in other studies (17).

In the literature, few areas of glycogen granules have been noted during the 5th day of postpartum period and the number and size of triglyceride droplets were found to be increased. Mitochondria were found to be abundant, and contained long cristae (19,20).

After application of retinyl palmitate, an esteric form of retinoic acid, at a dose of 60 mg/kg intraperitoneally to the mother during the 7th day of gestation, some variations in the adipocytes were observed (1,21).

In the vitamin A group, an increase was measured in the number and the size of the triglyceride droplets in adipocytes. The glycogen granules were evaluated in the cytoplasm, although they did not form wide areas as in control subjects. In this group, the cristae of rough endoplasmic reticulum were not present, while smooth endoplasmic reticula were evaluated as small vesicles. Besides, the mitochondria of this group contain wide and more cristae in relation to the control group.

The ultrastructural variation in fetal BAT begins in the antepartum period and continues for a few weeks in the postpartum period. The adipocytes of control subjects are found to have similar ultrastructural features with the antepartum 6th and 3rd day of rats, and retinoic acid shows a maturation effect in BAT cells (22).

In conclusion, vitamin A, which is known as teratogenic for many other tissues, increased the normal development of BAT cells and it was allowed BAT cells to gain their postpartum structural properties before birth. Adipocytes, which play an important role in thermoregulation, were not affected by the teratogenicity of vitamin A.

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