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Full Length Article

Impairment of bone growth of wistar rat fetuses of diabetic and hypercholesterolemic mothers

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ABSTRACT

Patients with either diabetes or hypercholesterolemia develop atherosclerosis and impair healing of bone. In the present work we illustrated their role in limb ischemia during bone cells differentiation of Wistar rat fetuses. Pregnant Wistar rats (n = 20 each) were arranged in three groups: control, diabetic or hypercholesterolemic. Diabetes was induced at the fifth day of gestation using streptozotocin, and hypercholesterolemia was carried by feeding virgin rats a diet containing 3% cholesterol for 6 wks prior to the onset of conception. At 13, 15, 17, and 19 d prenatal, pregnant rats were sacrificed, dissected, and fetuses were removed. Hind limbs were separated and subjected to histological and transmission electron microscopic examination, ossification, total calcium content of fetuses, isoenzymes alkaline and acid phosphatase and lactic dehydrogenase electrophoresis and DNA damage. Fetuses of diabetic or hypercholesterolemic mothers exhibited delayed histo-cytological differentiation of chondrocytes, and decreased periosteal ossification. Alkaline and acid phosphatase as well as lactic dehydrogenase isoenzymes showed altered diffusion rate and intensities of their bands reflecting their activities in both diseases comparing with the control. Assessments of bone calcium contents revealed marked reduction. Genomic expression of the degree of laddering (total DNA fragmented) or single-cell gel electrophoresis was found to be increased in cartilage and bone cells of fetuses of diabetic or hypercholesterolemic mothers. The authors concluded that both diseases had a selective, dramatic effect during fetus development in this model by retarding the histo- and cytological differentiation during limb bone growth. Both diseases increased the average cell death in skeletal elements and blood vessels as a consequence of altered alkaline and acid phosphatases and lactic dehydrogenase isoenzymes in accordance with DNA damage.

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1. Introduction

Hypercholsterolemia represents the common pathogenetic factor for bone disease [1,2]. Researchers are still figuring out exactly how diabetes changes cholesterol levels and contributes to vascular complications such as atherosclerosis [3]. Diabetes [4] or hypercholesterolemia [5] was interrelated with each other and increased the oxidative stress and cell damage.

Recently, we reported that maternal diabetic or hypercholesterolemic mothers exhibited massive damage of both blood vessels and myocardial fibers of their fetuses [6] as well as induce apparent hepatocyte damage [7] which may cooperate in bone defects.

A very few number of studies have reported that type 1 diabetes altered bone remodeling by reducing the formation of new bone, leading to osteopenia in humans and animals [8,9]. Type 1 diabetes was found to cause a significant delay in fracture healing [10](Lu et al., 2003) through a reduction of bone mineral density as assessed in the lumbar spine and proximal femur [11] and a decrease of bone formation compared with normal individuals [8].

Experimental studies on diabetic mice and rats revealed that the disease was concerned with reduction of bone formation, expression of osteocalcin, collagen types and decrease in bone healing [10], as well as increasing apoptosis of bone-lining cells [11]. Kayal et al. [12] observed a decrease of cartilage tissue and new bone area post 16 days of onset of diabetes in CD-1 mice. Won et al. [13] examined the inter-vertebral disk of OLETF (diabetic) rats at 6 and 12 months of age and detected increased incidence of apoptotic index of notochordal cells, which led to early intervertebral disc degeneration.

Atherosclerosis, like osteoporosis, is closely associated with each other [1]. The enhanced risk of bone fracture was the end result of accumulated damage [14]. Osteoclast formation and function were influenced by numerous inflammatory factors mediated in atherogenesis such as tumor necrosis factor -α which activated osteoclasts formation [15]. In experimental rats, Funaba et al. [16] reported a reduction of bone formation in 10-week-old hypercholesterolemic rats, suggesting inhibiting factor(s) for bone growth.

From the literature, there are no reports illustrating the direct effects on the differentiation of skeletal cells. The present study aims to outline the exact role of diabetes or hypercholesterolemia in histogenesis and cytological structures of bone elements, bone isoenzyme lactic dehydrogenase electrophoresis, and pattern of DNA damage of hind limb of Wistar rat during prenatal growth.

2. Materials and methods

2.1. Induction of diabetes

Experimental diabetes mellitus was induced by a single intraperitoneal injection of streptozotocin (60 mg/kg) in the citrate buffer (pH 4.5) on the fifth day of gestation [17]. Control animals received physiologic saline as a vehicle. Hyperglycemia was verified by measuring the blood glucose at approximately 350 mg/dL.

2.2. Induction of hypercholesterolemia

The experimental group was fed a hypercholesterolemic diet according to Enkhmaa et al. [18]. The component of the diet was illustrated in Table 1. The hypercholesterolemic diet was composed of 3% cholesterol and 15% cocoa butter in accordance with the standard diet formula. The rats were fed for 6 wks before the onset of gestation. The control group was supplied a standard diet free from atherogenic components.

2.3. Experimental animal work

This study and all procedures were approved by the Animal Care and Bioethics of the Egyptian Committee, and the animal work was done at Faculty of Science, Mansoura University. Eighty fertile male and virgin female rats of Wistar strain (*Rattus norvegicus*) (at a ratio of 1 male to 3 females) weighing approximately 125 g body weight, were obtained from Hellwan Breeding Farm, Ministry of Health, Egypt and used for experimentation. They were housed in cages with good ventilation on a 12-h light and dark cycle. Females were mated (1 male/3 females) overnight, and zero dates of gestation were determined the next morning by the presence of sperm in the vaginal smear. The pregnant rats were arranged into three groups (n = 20 per each) as follows: C, control; D, diabetic; H, hypercholesterolemic. Animals were maintained in free excess of diet as mentioned in Table 1 and water *ad Libitum*. At the end of treatment, they were sacrificed by light diethyl ether anesthesia and dissected at 13, 15, 17, and 19 d prenatal. The tibia regions from hind limbs were separated immediately from 13, 15, 17, and 19 d-old embryos and investigated as follows:

2.4. Light microscopic investigations

Hind limb of fetuses of both control and experimentally-treated fetuses were incised at 13-&15d-old and fixed in 10% phosphate buffered formalin (pH 7.4), dehydrated in ascending grades of ethyl alcohol, cleared in xylene and

Table 1 – Description of the percentages of diets constituents in control, diabetic and hypercholesterolemic groups.

Ingredient (%)	Control	Diabetic	Hypercholesterolemic
Crude protein	16	16	14
Butter fat, anhydrous	4	4	10
Choline bitartrate	1	1	1
Cholesterol	–	–	3
Cholic acid	–	–	2
Thiouracil	–	–	2
Cellulose	13	13	13
Casein	10	10	15
Soybean oil	5	5	15
Vitamins	2.5	2.5	2.5
Minerals	2.5	2.5	2.5
Moisture	7	7	7
Ash	4	4	3
Sucrose	10	10	5
Carbohydrates	10	10	5
Corn starch	15	15	5