

<b>Original Article</b>	<b>Implication of Protein Restriction on the Developing Albino Rat Myocardium</b> <i>Adel Kamel Abdelmalek; Fathy Zaky Hassan; Manal M.S. El-Meligy and Hala Mohammed Hassenien</i> <i>Department of Human Anatomy and Embryology, Faculty of Medicine, Assiut University</i>
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### ABSTRACT

**Background:** Malnutrition, particularly protein deficiency, has become a serious problem in the densely populated and underdeveloped areas. It is well known that protein malnutrition can affect the structure and function of the heart.

**Aim of the work:** Was to investigate the effect of maternal low protein diet on the myocardial structure of albino rat offspring.

**Material and Methods:** A total number of 20 adult female albino rats were used. Female rats were divided into two groups: a control group and an experimental group; of 10 rats each. The first group was fed a normal-protein diet (NPD) that contained 20% casein. The second group was fed a low-protein diet (LPD) that contained 9% casein. All breeder rats were habituated to their respective diets for 2 weeks before mating, during pregnancy and lactation. The hearts of the pups were examined using a light and an electron microscopy at birth and at the age of one month.

**Results:** Low protein diet given to the mothers affected the structure of the pups' hearts in the form of wide spaces among cardiomyocytes, cytoplasmic vacuolization, interruption of myofibrils, loss of the cross striations and mitochondrial disruption. **Conclusion:** The protein energy under nutrition affects the structural organization of the cardiac muscle which would be reflected on the contractile function of the heart.

**Recommendation:** It is advisable to combat the protein energy malnutrition especially in the early stages of the heart development.

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**Key Words:** Myocardium, offspring, protein, under-nutrition.

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### INTRODUCTION

The earlier concepts that the heart was spared in malnutrition were shown to be incorrect. There have been disagreements about the structural changes induced in myocardial cells by a low protein diet. Some investigators reported mild or minor histological changes whereas others described more severe alterations (*Bayo et al., 1979; Isner et al., 1979; Reed and Fariss, 1984; Pierobon et al., 1989; Masayo and Torao, 1999 and Corstius et al., 2005*).

A little is known about the precise ultra structural response of myocardial cells to a low protein diet.

The aim of the present study is to investigate the effect of maternal low protein diet on the myocardial organization of the rat offspring.

### MATERIAL AND METHODS

#### Material

Twenty adult female albino rats were used. The animals were obtained from Animal House of Assiut University.

Female rats were divided into two groups; control and treated of 10 rats each. The control group was fed (NPD) that contained 20% casein. The treated group was fed (LPD) that contained 9% casein. Diet intake was monitored daily for 2 weeks before mating, during pregnancy and lactation. Breeder rats were housed individually and maintained at an ambient temperature of 21°C with a 12-h day/night cycle. Food and water were administered ad libitum (Office of Laboratory Animal Welfare, 2002). Female breeders were mated with male rats and the presence of

sperms in vaginal plug on light microscopic examination was considered as 0 day pregnancy (*Mahmoud and El-Badry, 2001*). The offspring were randomly selected. The pups' hearts were examined using a light and an electron microscopy at birth and at the age of one month (10 rats from each age group). Dealing of the animals was approved by Ethical Committee of Assiut Faculty of Medicine.

## **Methods**

### **1- Anaesthesia and sacrifice**

The pups were anaesthetized by ether. The chest was opened to expose the heart for intracardiac perfusion with 10 ml of an isotonic solution then Bouin's solution for the specimens that would be examined by light microscope, or gluteraldehyde in sodium coccodylate for the specimens that would be examined by electron microscope. The hearts were excised and trimmed of any excess tissue and stored in 10 % buffered formalin.

For light microscopic examination, some specimens were cut serially in a sagittal plane (7 microns thickness), stained with haematoxylin and Eosin (Hx and E) stain (*Bancroft and Gamble, 2002*) and photographed. Also, semi-thin sectioning at 1.0 micron, staining with 2% aqueous toluidine blue and photographing were done.

For electron microscopic examination, ultra-thin sectioning at 0.1 micron was done (*Bozzola and Russel, 1992; Bancroft and Gamble, 2002 and Kue, 2007*). The sections were examined by transmission electron microscope (Jeol-JEM-100 CXII) and photographed at Electron Microscope Unit of Assiut University.

## **RESULTS**

### **1) Newly born rats**

#### **A) Light microscopic examination**

The heart of the control offspring shows that myocardial cells have central ovoid nuclei. Blood capillaries are observed among cardiomyocytes (Figure 1).

The heart of the treated offspring shows thinning of myocardial cells, wide spacing and

denser and flattened nuclei (Figure 2). Some blood vessels show abnormal configuration, dilatation or rupture (Figures 2, 3).

#### **B) Electron microscopic examination**

The myocardial cell of the control pup shows regular arrangement of myofibrils and regular pattern of striations with well demarcated intercalated discs. Mitochondria are abundant (Figures 4, 5).

The myocardial cell of the treated pup shows interruption of myofibrils and loss of the regular pattern of striations. Nuclear membrane indentation and mitochondrial disruption are also observed (Figure 6).

### **II) One month aged rats**

#### **A) Light microscopic examination**

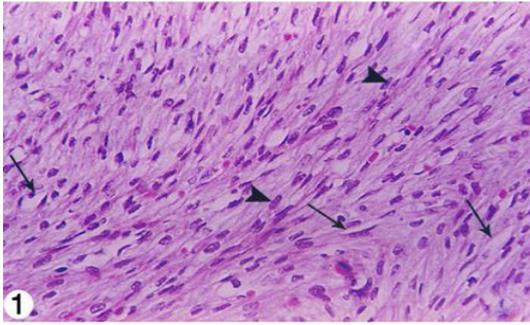
The heart of the control pup shows that the cardiac muscle fibres are elongated and branching with pale acidophilic sarcoplasm and central ovoid nuclei. At this age, the branching character of cardiomyocytes is well apparent and the cells become more differentiated (Figure 7).

The heart of undernourished offspring shows that the orientation of the cardiac muscle fibres is interrupted and the nuclei lose their normal contour, shape, size and orientation (Figures 8, 9). The muscle fibres are separated from each other by wide spaces (Figure 9). Some cardiomyocytes show damage of sarcolemma and myofibrils. Others show vacuolation and interruption of the normal striation (Figures 8, 9). Some muscle fibres appear more acidophilic with ill defined myofibrils (Figure 8).

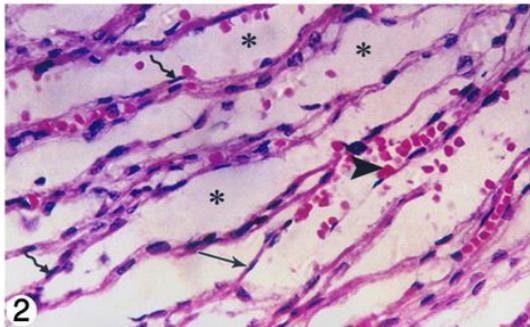
#### **B) Electron microscopic examination**

Examination of myocardial cell of the control pup shows regular arrangement of myofibrils and regular pattern of striations with well demarcated intercalated discs. Mitochondria are abundant (Figures 10, 11).

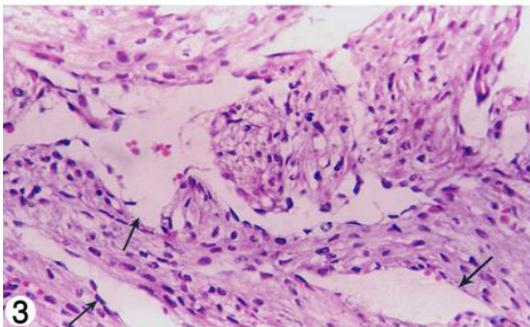
Examination of myocardial cell of undernourished pup shows perinuclear rarefaction of cytoplasm, massive loss of myofibril striations and a massive degeneration of mitochondria (Figure 12).



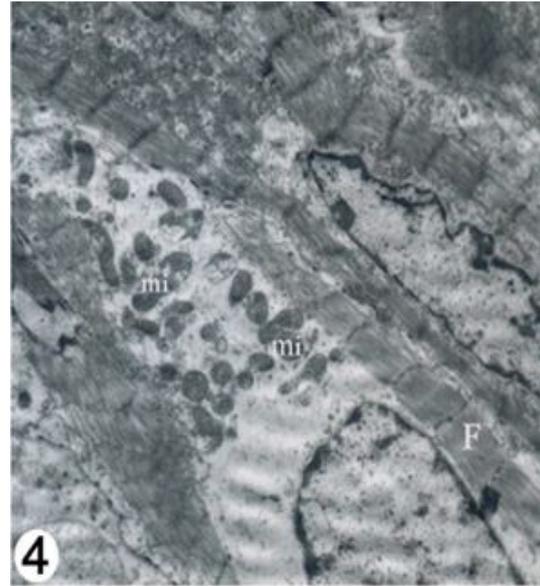
**Fig. 1:** A photomicrograph of a longitudinal section in a heart of a newly born control albino rat showing longitudinal orientation of cardiomyocytes with ovoid nuclei (arrow head). Numerous intact blood capillaries are found in the specimen (arrow). (Hx&E x400)



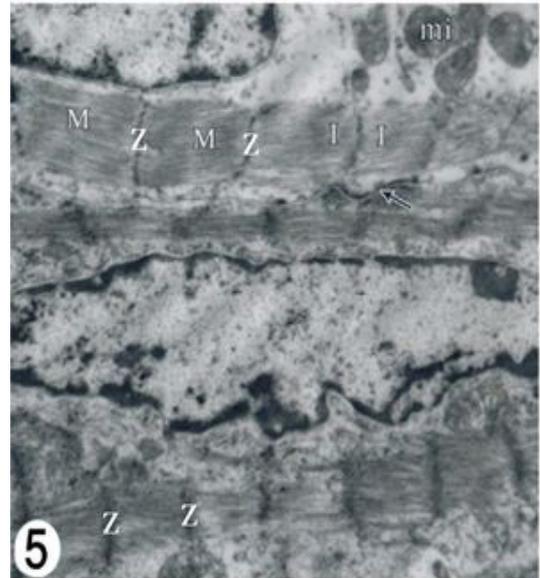
**Fig. 2:** A photomicrograph of a longitudinal section in a heart of a newly born undernourished offspring showing thinning of cardiomyocytes (arrow) with wide spacing among them (star). Notice the dense flattened cardiomyocytes' nuclei (corrugated arrow). Blood vessels are widened. Rupture of some blood vessels is observed (arrow head). (Hx&E x400)



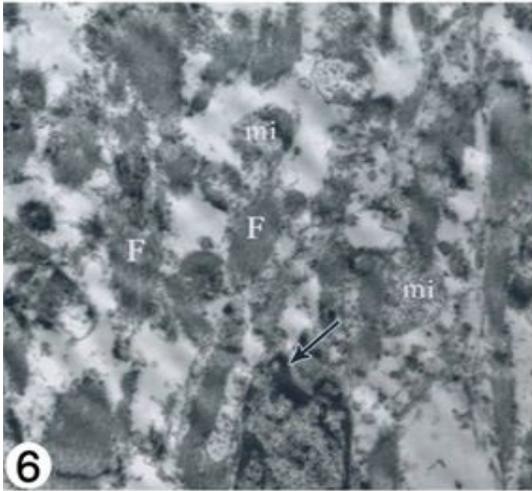
**Fig. 3:** A photomicrograph of a longitudinal section in a heart of a newly born undernourished pup showing abnormally dilated blood vessels (arrow). (Hx&E x400)



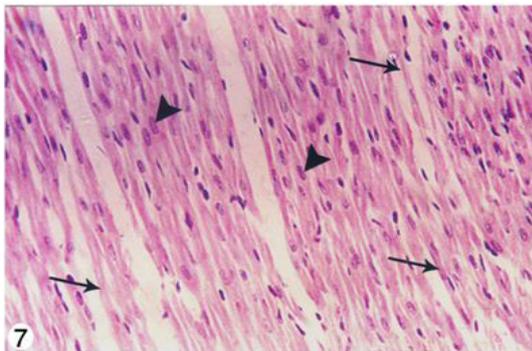
**Fig. 4:** An electron micrograph of cardiomyocyte in a newly born control animal showing that myofibrils (F) consist of sarcomeres with regular pattern of striations. Numerous mitochondria (mi) are seen (X 5800).



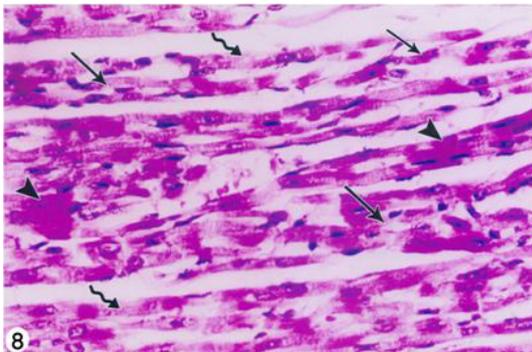
**Fig. 5:** A magnification of the previous section showing the regular pattern of sarcomeres with well demarcated Z line, I,M bands and intercalated disc (arrow). Intact mitochondria are seen (mi). (X 10000)



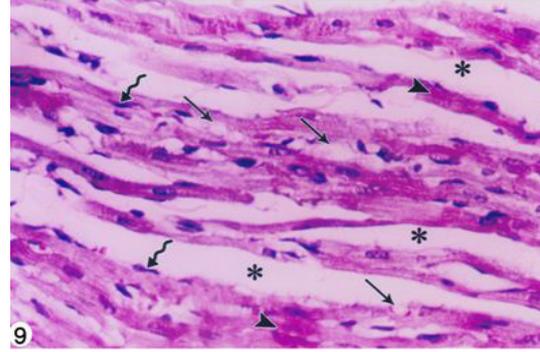
**Fig. 6:** A magnified electron micrograph of myocardial cell in a newly born undernourished rat offspring showing marked disruption of myofibrils with obscured striations and streaming of Z line (F). Degenerative changes in mitochondria (mi) are observed. Notice the indentation of cardiomyocyte nucleus (arrow). (X 10000).



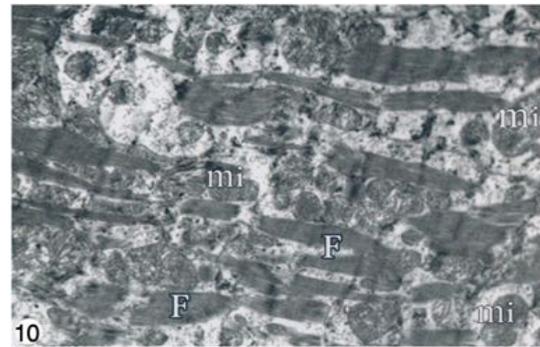
**Fig. 7:** A photomicrograph of a longitudinal section in a heart of a control offspring aged one month showing branching cardiomyocytes (arrow) with their central ovoid nuclei (arrow head). (Hx&E x400)



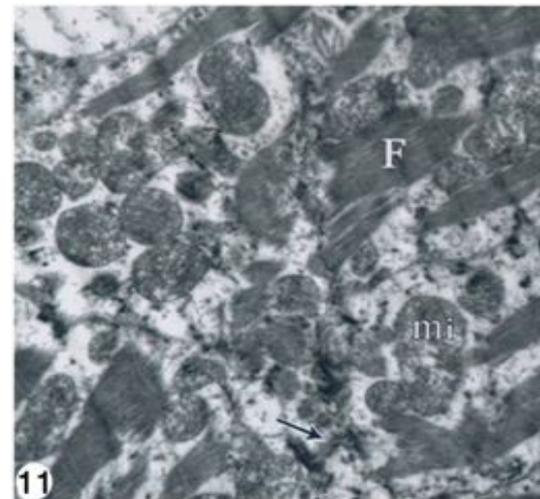
**Fig. 8:** A photomicrograph of a longitudinal section in a heart of an undernourished pup aged one month. Notice the interruption of myofibrils (corrugated arrow). Lack of cytoplasmic acidophilic hemogenicity (arrow head), cytoplasmic vacuolation (arrow) and disfigured striations are observed. (Hx&E x400)



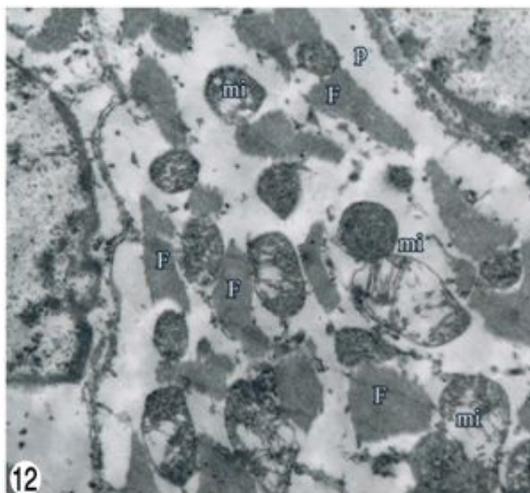
**Fig. 9:** A photomicrograph of a longitudinal section in a heart of an undernourished pup aged one month showing wide spaces among cardiomyocytes (star), cytoplasmic vacuoles (arrow), dense flattened nuclei (corrugated arrow) and lack of cytoplasmic acidophilic hemogenicity (arrow head). Notice the abnormal shape, size, contour and orientation of the nuclei of cardiomyocytes. (Hx&E x400)



**Fig. 10:** An electron micrograph of myocardial cell in a heart of a control rat offspring aged one month showing regular pattern of myofibrils (F) with abundance of mitochondria among them (mi). (X 5800)



**Fig. 11:** A magnification of the previous section showing regular pattern of striation of myofibrils (F) with abundance of mitochondria (mi). Notice the well demarcated intercalated disc (arrow). (X 10000)



**Fig. 12:** An electron micrograph of cardiomyocyte in a heart of an undernourished pup aged one month showing interruption of myofibrils (F) with loss of their striations. Degenerated mitochondria (mi) and perinuclear rarified cytoplasm (p) are observed. (X 10000)

## DISCUSSION

The studies on animal and human hearts in protein calorie malnutrition are few. Protein deficiency became a serious problem not only in underdeveloped areas but also in modern hospitals (*Masayo & Torao, 1999*).

The present work shows that the administration of low protein diet (LPD) induces microscopic alterations in albino rats' myocardium and that the severity of damage depends on the period of administration. These findings are in consistency with those of *Cleal et al. (2007)* who reported that the prevalence of cardiovascular diseases increased with the administration of LPD especially during the early fetal life.

In the present study, striking light and electron microscopic changes are detected as a result of the prenatal exposure to the protein energy under nutrition. By the light-microscopy, the current study reveals sarcoplasmic vacuolization of the cardiac muscle fibres, loss of cross striations, damage of myofibrils and small foci of necrosis. These findings are in agreement with the results of *Bayo et al. (1979)*, *Rossi and Zucoloto (1982)* and *Reed and fariss (1984)*. On the ultra structural level, the changes include myofibrillar degeneration, mitochondrial swelling, dehiscence of intercalated discs, and interstitial edema. These results are in harmony with the observations of *Rossi and Zucoloto (1982)*. Moreover, fracture of myofibril of sarcomere and streaming of Z-line are also observed. Similar to these findings, were

the observations of *Masayo and Torao (1999)*.

Protein derangement most probably results in loss of selective permeability of cell membrane which in turn leads to intracellular vacuolization and interstitial edema. This explanation was postulated by *Opie (1991)* who stated that cytoplasmic vacuolization could be due to disturbance in electrolyte exchange across the cardiac cell membrane.

Protein energy malnutrition disturbs mitochondria responsible for the energy production and storage that appear swollen and disfigured in cardiomyocytes as observed by *Junqueira and Carneiro (2003)*.

The present study shows an obvious fracture and a disruption of cardiomyocytes in LPD rats. Degradation of cytoskeleton is manifested as streaming of Z-line and dehiscence of intercalated disc. These findings agree with those of *Masayo and Torao (1999)*.

Streaming of Z-line is thought to be caused by degradation of cytoskeleton. Perhaps the degeneration of the desmin connecting the neighboring myofibrils in the cardiac muscles is responsible. Degradation of desmin may be due to an enhanced action of proteolytic enzymes which may explain dehiscence of intercalated disc as well as streaming of Z line (*Beclastro et al., 1988 and Masayo & Torao, 1999*). In addition, excess leaking of  $Ca^{2+}$  ion to cytosol may also be closely associated with streaming of Z-line (*Masayo & Torao, 1999*).

The presence of wide spaces among the muscle fibres could be attributed to interstitial edema resulting from histamine release from mast cells. This may lead to increase filtration rate and protein leaks out from capillary pores into interstitial spaces as explained by *Bayo et al. (1979)* and *Reed & fariss (1984)*.

Beginning from animal models, many studies concerning the early nutrition, epigenetic modifications and genes expression were carried out. Prenatal under nutrition, especially in pre-implantation period, not only caused prolonged growth retardation but also modified the programming of biochemical mechanisms related to endocrine-metabolic control and increased the risk of diseases later in life (*Desai et al., 1996; Secki, 1997; Barker's, 1998; Langley-Evans and Nwagwu, 1998; Henriksen, 1999; Petry and Hales, 2000; Roseboom et al., 2000; Rudolph, 2000; De Vries et al., 2002; Liotto et al., 2009;*

*Tappia et al., 2013; Nascimento et al., 2014 and Zohdi et al., 2015).*

*Desai et al. (1996) and Barker (1998)* reported that in humans, intrauterine growth retardation (IUGR) was linked to increased incidence of heart disease later in life. The pathways that led to the increased risk were unknown. IUGR induced reduction in the cardiomyocyte number. Because postnatal growth of the heart was predominantly due to cardiomyocyte hypertrophy, it was plausible that the reduced cardiomyocyte number would remain into the adulthood.

It can be deduced that, degenerated cardiac muscle cells may not be capable of normal contractile function. Thus the cardiac function may be impaired predisposing to the heart failure. This explains, at least in part, the occurrence of sudden unexpected deaths, probably of cardiac nature, during the treatment of children with protein-calorie malnutrition reported by *Smythe et al. (1962).*

#### **CONCLUSION AND RECOMMENDATION**

Management of protein energy malnutrition and its effects on the heart should be more preventive rather than curative. This is because of the early differentiation of cardiomyocytes and the difficult regeneration of damaged myocardial cells.

#### **CONFLICT OF INTERESTS**

There are no Conflicts of Interest.

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## تأثير نقص البروتين على نمو عضلة قلب الفأر الأبيض

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### ملخص البحث

**مقدمة:** لقد أصبح سوء التغذية – خاصة نقص البروتين – مشكلة خطيرة فى المناطق كثيفة السكان والنامية، ومن المعروف أن هذا النوع من سوء التغذية يمكن أن يؤثر على تركيب ووظيفة عضلة القلب؛ لذلك كان الهدف من هذه الدراسة هو توضيح تأثير نقص البروتين فى غذاء الام على تركيب عضلة القلب فى أجنة الفئران البيضاء .

**الوسائل والوسائل:** لقد تم استخدام عشرين أنثى فئران بيضاء بالغة، حيث تم تقسيم هذا العدد الى مجموعتين : ضابطة ومعالجة بحيث تشتمل كل مجموعة على عشرة فئران؛ المجموعة الاولى تم تغذيتها بغذاء يحتوى على النسبة العادية من البروتين (20%) ، بينما تم تغذية المجموعة الثانية بغذاء قليل البروتين (9%) لمدة أسبوعين قبل التزاوج واثناء فترة الحمل والارضاع لكلا المجموعتين، ثم تم فحص قلوب المواليد باستخدام المجهر الضوئى و الاليكترونى عند الولادة وبعد شهر.

**النتائج:** ولقد أظهرت النتائج أن تغذية الأمهات بغذاء قليل البروتين كان لها تأثيرا سلبيا على تركيب عضلة القلب فى المواليد؛ فقد تبين حدوث الأتي: ظهور مسافات واسعة بين خلايا عضلة القلب، وتكوين فجوات بالسيتوبلازم ، مع وجود تقطع فى ألياف العضلات وفقدان الخطوط المستعرضة وتهالك فى الميتوكوندريا.

**الاستنتاج والتوصية:** ان نقص البروتين يؤثر على تركيب وبالتالي على وظيفة عضلة القلب؛ لذا فإنه يوصى بمكافحة حدوثه خاصة فى مراحل التطور الأولى.