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Effect of Food Inequality and Unstable Social Status on Myocardial Cells of Male Rabbits

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Abstract: The relationship between socioeconomic status (SES) and health is well defined. Individuals with lower SES experience higher rate of mortality. Although life expectancies affected by poverty but the precise pathways that link socioeconomic status and health remain unclear. Lipofuscin is highly oxidized cross-linked aggregate consisting of oxidized protein and lipid clusters. This eminent terminal oxidation outcome accumulates within cells during aging process. Fifty four rabbits were randomly assigned into six groups of eight each. The first group (control G.) had free access to diet without any deprivation and changed place or room-mate. The second group had food deprivation, but did not change place or room-mate. The third group had food deprivation and changed place or room mate. The fourth group had similar condition with third group only five weeks then, conditions were the same to control group. The fifth group had condition similar to the third group, but its room was separated from other Groups. The sixth group had free access to diet without any deprivation but changed place or room-mate. All hearts were removed for histopathologic evaluation. Cross-sections of hearts were examined by light and electron microscopy for the presence of yellow-brown lipofuscin pigment granules ($p<0.05$). We found that cardiac lipofuscin deposition increased under kind of stress that we applied. Our findings demonstrated that relation between environmental stresses such as inequality in food intake and social status instability with formations of lipofuscin pigments. Both stresses were probably created external source for oxidative stress in the myocardial muscle of left ventricular rabbits.

Key words: Food intake inequality · Unstable social status · Histopathologic change · Lipofuscin pigment · Food deprivation

INTRODUCTION

Social justice implementation is one of the ideal goals for human. Equanimity is a universal concept and concedes with temperament of human, thus all societies attempt to reach this goal.

Inequality in health is one of the controversial subjects that researchers pay attention.The relationship between socioeconomic status (SES) and health is well defined [1-3]. Individuals with lower SES experience higher rate of mortality and are more likely to undertake numerous health conditions. This so-called social "gradient" in health has been observed across different time periods and age groups using an extensive range of SES indicators, health measures and methodologies [2, 3]. Although life expectancy is affected by poverty but the precise pathways that link socioeconomic status and health remain unclear. There is increasing evidence that many known disease risk and protective factors influence the rate of cellular damage accumulation and hence biological aging and that the pathogenesis of some important diseases is related to biological aging [4]. Lipofuscin is highly oxidized cross-linked aggregate consisting of oxidized protein and lipid clusters. This eminent terminal oxidation outcome accumulates within cells during aging process. The rate at which cellular

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damage accumulates is determined by the balance between damage occurring and the action of defense and repair mechanisms [5]. Four key types of cellular damage had been described: mitochondrial changes; the accumulation of aberrant protein in the cytosol; oxidative stress, caused primarily by free radicals and somatic mutations [6, 7].

As early as 1886, histologists observed that the relative quantity of these lipofuscin pigment granules in the nerve cells appeared grossly correlated with the age of human and animal subjects [6,7]. These early morphologic observations were subsequently confirmed by numerous other studies [5-7]. Since it seems reasonable to assume that any extensive intracellular accumulation of these pigment bodies with age may result in significant changes in cellular physiology, several theories of aging have proposed that the deterioration and loss of non-dividing cells may represent one of the most fundamental aspects of biological aging [4, 8, 9]. Proteins within lipofuscin are linked by intramolecular and intermolecular cross-links. Many of these cross-links are caused by nonproteineous compounds including oxidation products [8, 9]. The intracellular formation of lipofuscin is a complex arrangement of reactions in involving numerous cellular compartments and enzymes. The intracellular rate of lipofuscin formation is negatively correlated with the life expectancy of a post-mitotic cell and enhances with age [9, 10]. It is understood that the higher the rate of intracellular lipofuscin accumulation over the time, the shorter the potential life time of the cell [11]. There is a considerable body of evidence indicating that oxidative stress is a causal factor both in lipofuscinogenesis as well as in aging [12]. We evaluated the effect of food intake inequality with or without unstable social status in white Newslandian Rabbits and showed that food intake inequality with or without unstable social status had affected histopathologic changes and accumulation of lipofuscin pigments in myocardial muscles in groups of rabbits that we designed.

MATERIALS AND METHODS

Fifty four Newslandian rabbits with a mean weigh of 2.200 kg were randomly assigned into six groups. Each group had eight or nine rabbits. Before study, rabbits assimilate new home four two weeks. In this time, rabbits had no limit for food and water. Food intake was weighed daily for determined. Six groups set in two rooms, five groups in one room and one group in another room. Each group set in the cage with three stories. After two weeks we applied four kinds of stresses in some of groups during ten weeks.

- Food deprivation (To one third of whole food per day)
- Changed place or room-mate every two weeks (unstable social status)
- Encountered with groups that had free access to diet without any deprivation.(suffered from inequality)
- Isolated situation

Table 1 shows number of groups and kind of stress that we applied for rabbits.

Table 1: Experimental groups

Stress Group	food deprivation	Stable social status	Live with other groups suffered from inequality))	Isolated situation
1	—	—	+	—
2	+	—	+	—
3	+	+	+	—
4	Only five weeks			
+	Only five weeks			
+	Only five weeks			
+	—			
5	+	+	—	+
6	—	+	+	—

First group (*control G.*) had free access to diet without any deprivation and without changed place or room-mate. This group set in the room with other groups.

Second group had food deprivation (food intake one third of whole food per day) but did not change place or room-mate. This group was set in the room with other groups.

Third group had food deprivation (food intake one third of whole food per day) and changed place or room-mate. This group was set in the room with other groups.

Fourth group had similar condition with third group only five weeks and then received free access to food and stable social status similar to first group. This group was set in the room with other groups.

Fifth group had food deprivation (food intake one third of whole food per day) and changed place or room-mate. This group was set in another room and was isolated.

Sixth group had free access to diet without any deprivation but changed place or room-mate.

This Group Was Set in the Room with Other Groups: We checked serum glucose, triglyceride, cholesterol, VLDL and weigh three times, start of period, middle of period and end of period. After ten weeks five rabbits in each group were randomly selected. After tissue perfusion with 0.9% saline and 0.1 M phosphate buffer under anesthetic condition, all hearts were removed for histopathologic evaluation. Cross-sections of hearts were examined by light and electronic microscopy for the presence of lipofuscin pigment granules or early stage of lipofuscin formation. We perpetrated pictures of the histopathologic changes in myocardium muscle by light and electronic microscopy.

RESULTS

Statistical analysis by Kruskal-Wallis test showed significant differences among all groups ($p<0.05$). Further analysis using Mann-Whitney test showed significant differences between two groups which we selected ($p<0.01$). This is a remarkable increase in the lipofuscin pigment accumulation and more significant damage in the animal in five groups compared with control group.

First Group (Control G.): Histopathologic evaluation showed normal structure and none of the histopathologic changes were recorded, which was significantly different from other groups (Figure 1: A, B).

Second Group: Histopathologic evaluation showed more edematous than other groups. The mean of lipofuscin pigmentation accumulation was 0.8 more than lipofuscin in the control group as well as had moderate histopathologic changed in the myocardium sample of left ventricle (Figure 1: C, D).

Third Group: The group had intensive pathological changes; particularly in the lipofuscin pigmentation accumulation also the group had number of focal apoptosis. Third group is only group with focal apoptosis (Figure 2: E, F).

Fourth Group: The group had little histopathologic changes because we applied stresses for five weeks but the short period was affected in the animals of group (Figure 2: G, H).

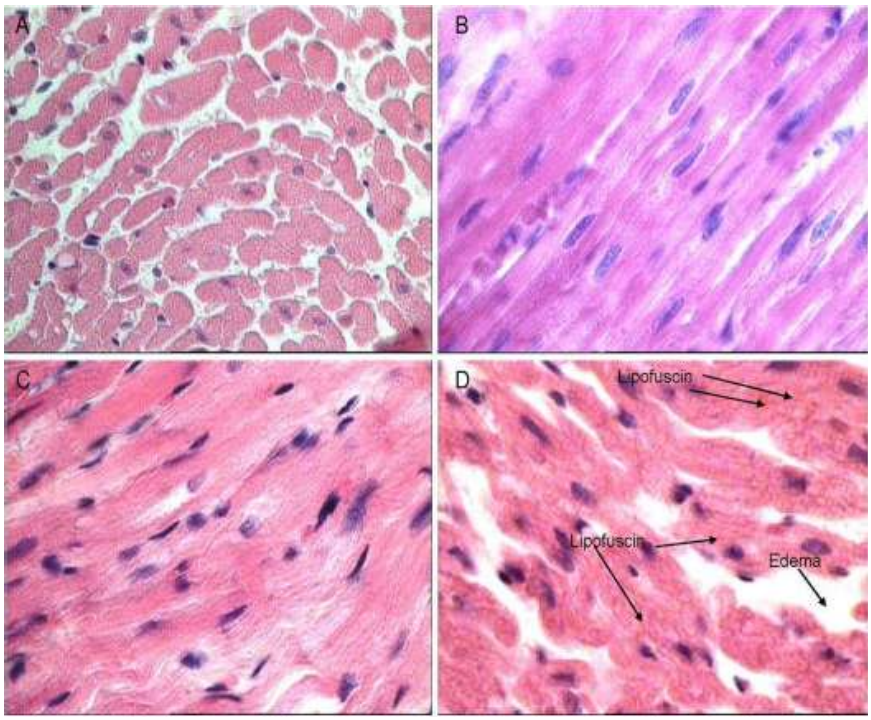

Fig. 1: Transverse section through the heart of a rabbit showing normal structure of cardiac muscle in the control group(A).Longitudinal section through the heart of a rabbit showing normal structure of cardiac muscle in the control group (B).Longitudinal section through the heart of a rabbit in the second group (C).Transverse sections through the heart of a rabbit showing lipofuscin pigment and edema in the second group (D).(Prepared by light microscope and Stained by hematoxylin and eosin stain × 100)

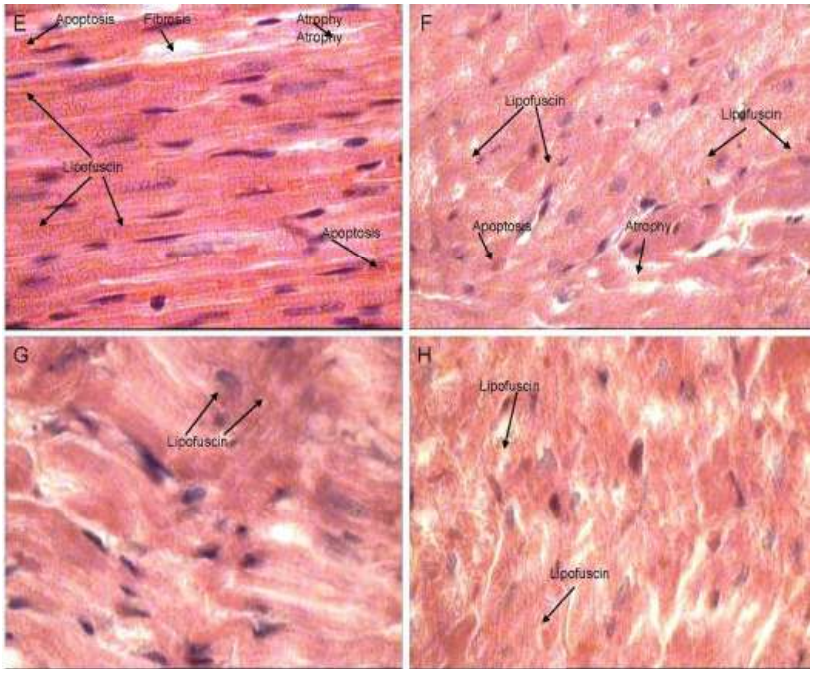

Fig. 2: Longitudinal section through the heart of a rabbit showing lipofuscin pigment, apoptosis, atrophy and fibrosis in the third group (E).Transverse section through the heart of a rabbit showing lipofuscin pigment, apoptosis and atrophy in the third group (F).Longitudinal section through the heart of a rabbit showing lipofuscin pigment in the fourth group (G).Transverse section through the heart of a rabbit showing lipofuscin pigment in the fourth group (H). (Prepared by light microscope andStained by hematoxylin and eosin stain × 100)

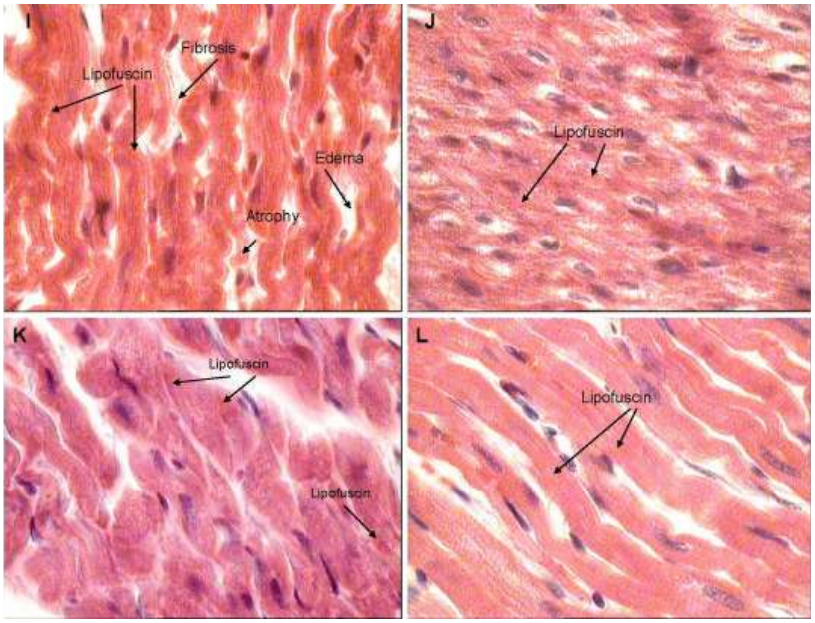

Fig. 3: Longitudinal section through the heart of a rabbit showing lipofuscin pigment, edema, atrophy and fibrosis in the fifth group (I).Transverse section through the heart of a rabbit showing lipofuscin in the fifth group (J).Longitudinal section through the heart of a rabbit showing lipofuscin pigment in the sixth group (k).Transverse section through the heart of a rabbit showing lipofuscin pigment in the sixth group (L). (Prepared by light microscope and Stained by hematoxylin and eosin stain × 100)

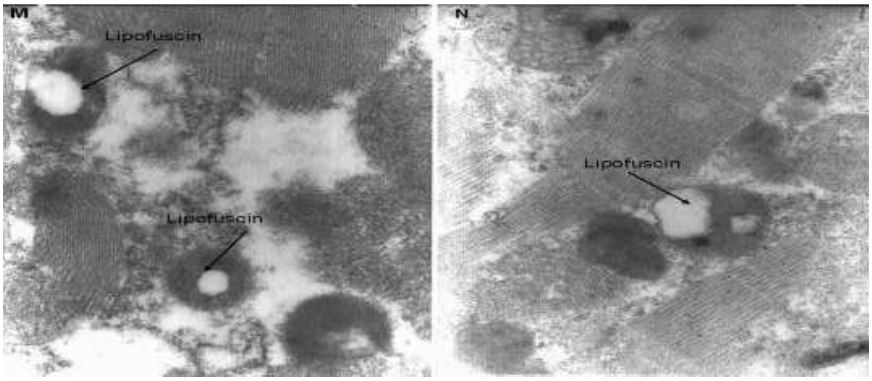

Fig. 4: Electronic images of ventricular myocardium from third group showing lipofuscin pigmentation (M, N). (Prepared by transmission electronic microscope × 30000)

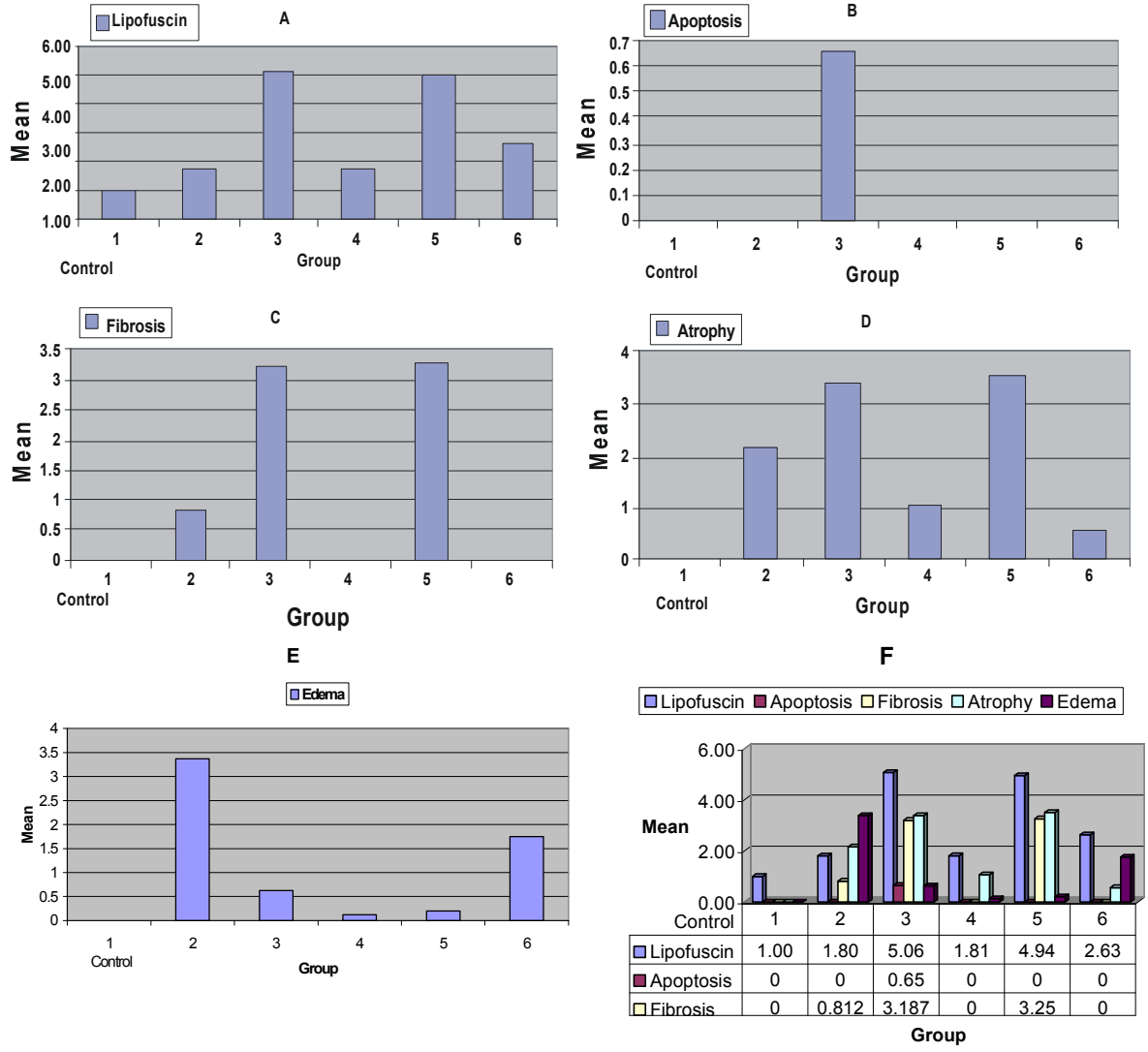

Fig. 5: Mean of lipofuscin accumulation between groups (A). Mean of focal apoptosis between groups (B). Mean of fibrosis between groups(C). Mean of Atrophy between groups (D). Mean of edema between groups (E). Mean of all histopathologic changes in each group

Fifth Group: Histopathologic evaluation showed almost similarity between the groups five and three but two groups had differences in the accumulation of focal apoptosis (Figures 3: I, J).

Sixth Group: The group had the lipofuscin pigmentation accumulation. The mean of lipofuscin pigmentation accumulation in the group was 2.625 more than lipofuscin in the control group. Other histopathologic changes such as atrophy and edematous were found in the group (Figure 3: K, L).

Figure 5: A-E shows mean of each histopathologic change in animals of six groups and Figure 5: F compared mean of all histopathologic changes in each groups. As well as, we did histopathologic evaluation by TEM (Transmission Electron microscopy) and detected lipofuscin pigment in the cell of myocardium muscle in left ventricle of rabbits (Figures 4: M, N).

DISCUSSION

Both epidemiological and controlled studies have demonstrated relationships between psychosocial stressors and disease. Animal models provide an important tool for helping to understand the specific influences of stressors on disease processes. Perhaps the best-known animal model relating stress to atherosclerosis was developed by Kaplan. Their study was carried out on male cynomolgus monkeys, who normally live in social groups. The investigators stressed half the animals by reorganizing five-member social groups at one- to three-month intervals on a schedule that ensured that each monkey would be housed with several new animals during each reorganization. The other half of the animals lived in stable social groups. All animals were maintained on a moderately atherogenic diet for 22 months. Animals were also assessed for their social status (i.e., relative dominance) within each group. The major findings were that (*a*) socially dominant animals living inunstable groups had significantly more atherosclerosis than did less dominant animals living in unstable groups; and (*b*) socially dominant male animals living in unstable groups had significantly more atherosclerosis than did socially dominant animals living in stable groups [13,14].

Whereas the studies in cynomolgus monkeys indicate that emotionally stressful behavior can accelerate the progression of atherosclerosis, McCabe *et al*. [15] have provided evidence that affiliative social behavior can slow the progression of atherosclerosis in the Watanabe heritable hyperlipidemic rabbit. This rabbit model has a genetic defect in lipoprotein clearance such that it exhibits hypercholesterolemia and severe atherosclerosis. The rabbits were assigned to one of three social or behavioral groups: (*a*) an unstable group in which unfamiliar rabbits were paired daily, with the pairing switched each week; (*b*) a stable group, in which littermates were paired daily for the entire study; and (*c*) an individually caged group. The stable group exhibited more affiliative behavior and less agonistic behavior than the unstable group and significantly less atherosclerosis than each of the other two groups. The study emphasizes the importance of behavioral factors in atherogenesis, even in a model of disease with extremely strong genetic determinants [14, 15].

We demonstrated that animal in this model, similar to last search [16], like people; respond negatively to inequality and social unstable condition. Our most important findings of this study are that the unstable social statues accelerated the lipofuscin formation in the left ventricular muscle heart of rabbits.

We compared third group with fifth group. Although exertion of stress was similar but we received histological differences. Fifth group had isolated situation but third group set in the room with other groups therefore encountered with groups that had free access to diet without any deprivation (suffered from inequality). comparison between them showed that two groups have approximately similar changes in lipofuscin aggregation but focal apoptosis was only in the third group (Figure 5: B, F). The Results showed that this group had been under more stress than fifth group.

Histopatolgical evaluation showed that the second group had more edematous than other groups but the mean of lipofuscin pigment accumulation was 0.8 more than lipofuscin in the control group and less than quintuple in the third group (Figures 5: A, E and F).

Animals in the second group had constant place or room-mate therefore had not sever contact between them. Adverse effect of food deprivation and suffered from inequality were probably decreased by presence of social status stability within them. This point is proved by comparison between the second with sixth groups. Our data showed that the sixth group had quantitative changes such as atrophy and edematous in the sample of were prepared from left ventricular myocardium but had the mean of lipofuscin pigmentation accumulation 2.65 more than control group and near twice than the second group and half than the third group (Figure 5: A, F). Animals in the sixth group had transitory place or room-mate therefore had sever contact between

them. This point showed that the instability in social status had major effect in animals of sixth group; however the stability in social status decreased adverse effect of food inequality in animals of the second group.

However three important points were probably proved by experimental models that we designed.

- Food deprivation with unstable social statues accelerated the lipofuscin formation in the left ventricular muscle heart of all rabbits.
- Inequalities in food intake with unstable social statues lead to severity of tissue lesions and formation of focal apoptosis (Comparison between the third and fifth groups. Figure 5: B, F).
- Social status stability decreased adverse effect of inequality in food intake (Comparison between the second and third groups. (Figure 5: A, B).
- Unstable social statues without food deprivation accelerated the lipofuscin formation (comparison between the controls with sixth groups. (Figure 5: A, D, E).

We demonstrated relation between environmental stresses such as inequality in food intake and social status instability with formations of lipofuscin pigments. Both stresses were probably created external source for oxidative stress in the myocardial muscle of left ventricular rabbits. The study must be followed by more researches in other tissues of rabbits.

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