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Histological impact of nutritional style alteration in mice

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Objectives It is well established that diet and lifestyle are important in the maintenance of health. Transition from a plant-based diet mostly to a high-calorie diet of animal products might raise the chronic diseases which is called "degenerative". This work aimed to study the histopathological effect of transition from complete plant-based diet to 10% animal products (sheep's brain) on various body organs of mice.

Methods Eight-week-old Balb/c male mice were divided into two groups ($n = 8$); the first is restricted group in which mice were fed on restricted diet containing 10% of sheep's brain homogenate, while the second is the control group in which fed on *ad libitum* on the diet for 7 days. During the duration of experiment, body weight and the amount of food intake were recorded daily, then at the end of experiment, all mice were sacrificed and various organs were obtained and processed for histopathological study.

Results The results showed that food intake by each mouse of restricted group are significantly lower than in control group. Although the mean of body weight in both groups revealed non-significant difference, the relative weight of various organs showed significant differences. On the other hand, sever histological changes were detected in all studied organs sections of restricted group.

Conclusion It can be concluded that changing in nutritional style rather than conventional diet play a crucial role in modifying the architectural aspects of different organs at tissue level. Therefore, these findings need further investigation at cellular, physiological, and molecular levels.

Keywords nutritional style, conventional diet, vegetarian diets

Introduction

It is well established that the diet and lifestyle are important in the maintenance of health.[1] A good nutritional status maintaining has important implications for health and well-being, leading to delay and reduced risk of disease, maintaining functional autonomy and thus promoting the continuation of independent living.[2]

Improper nutrition may play a role in the progressive degradation of many body functions[3] and caused diseases. Diabetes causes by nutrient sparse foods which contained concentrated sugars or refined flour products that contribute to impaired glucose metabolism[4] while cancer risk causes by the low fiber intake,[5,6] consumption of red meat,[7,8] and imbalance of omega-3 and omega-6 fats.[9] Colorectal cancer development, a major global health concern, is affected by various lifestyle factors and red meat consumption. The processed meat has been associated with increased risk for this cancer.[10–12]

During human evaluation, the changes in availability of food and composition of dietary have created strong selective pressures on multiple biological processes such as identifying the genetic loci and biological pathways for common diseases.[13]

Vegetarian diets for cats and dogs have health benefits range,[14] while the transition from a plant-based diet mostly to a high-calorie diet of animal products; which is high in saturated fats, salt, and sugar, and low in fiber; has been identified as a notable contributor to the rise in chronic diseases which is called "degenerative" diseases such as cardiovascular disease, cancer, obesity and diabetes.[15,16]

This study aimed to study the effect of transition from complete plant-based diet to 10% animal products (sheep's brain), contain high fat, on various peripheral body organs of mice.

Materials and Methods

Eight-week old Balb/c male mice were obtained from preventive research center, Baghdad, Iraq. After 1 week for adaptation on local condition, they are divided into two groups. One group is restricted group (R) which consumed 10% sheep brain, and second group consumed the normal diet *ad libitum* and served as control group (C). Every group contains eight mice. The weight of consumed feed and the weight of body mice were measured. The mice were scarified by cervical dislocation after 7 days. Spleen, kidney, liver, heart, small intestine, testes were obtained extracted. After weight recording, these organs were put in 10% formalin solution and processed by standard procedures. Sections of paraffin-embedded tissues were stained with hematoxylin and eosin and examined by light microscopy.[17] All experiments are carried out in Department of Biology, College of Science, Mustansiriyah University.

Results are expressed as mean ± standard deviation (M ± SD) or mean ± standard error (M ± SE). Data were anas lyzed by one-way analysis of variance[8] followed by Fisher's test for multiple comparisons, using Statview version 5.0. Differences considered significant at P-value <0.05.

Results

The food intake by each mouse in restricted group (R) decreased from 2.8 g in the first day down to 0.8 g at the seventh day and with an average of (1.4 ± 0.4 g/day). This result is significantly different from control group 2.9, 3.8 g, 3.0 ± 0.6 g/day respectively as shown in Fig. 1.

The body and organs weight of mice in both groups showed no significant differences between them except the weight of heart in restricted group (0.250 ± 0.03 g) was significantly higher compared with that in control group (0.206 ± 0.01 g) as shown in Table 1.

However, calculation of the relative change% in the organ weight of restricted mice in respect to their corresponding organs in control group showed significant decreasing in (spleen, liver, and testis) and increasing in (kidney and heart) as shown in Fig. 2.

Concerning with the histological study of the internal organs in both groups, the results demonstrated several pathological changes in the liver (Fig. 3), kidney (Fig. 4), spleen (Fig. 5), intestine (Fig. 6), testis (Fig. 7), and heart (Fig. 8) of restricted mice.

Discussion

It is well established that most biological membranes have higher proteins/lipids ratio while the membrane of neurons in CNS and PNS is characterized by a high proportion of lipid and low of protein.[18] Jennifer et al.[19] reported that the myelin had about 78–41% lipid containing of dry weight, white matter had about 49–66% and gray matter had about 36–40%. Many studies proved that the amount of fatty in tissues and biofluid are highly valuable in detecting disorderly regulation in biochemical pathways. The circulating fat is derived from both diet and internal metabolism. These fats are interactive biological molecules have highly dynamic. These form most cellular components and signal molecules, dictate energy and control the intake of food.[20] Eating fatty foods may lead to indigestion and cause gastrointestinal distress because the rate

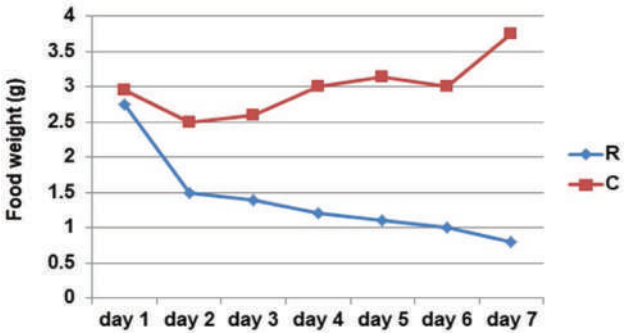

Fig. 1 **Food intake in control and restricted groups.**

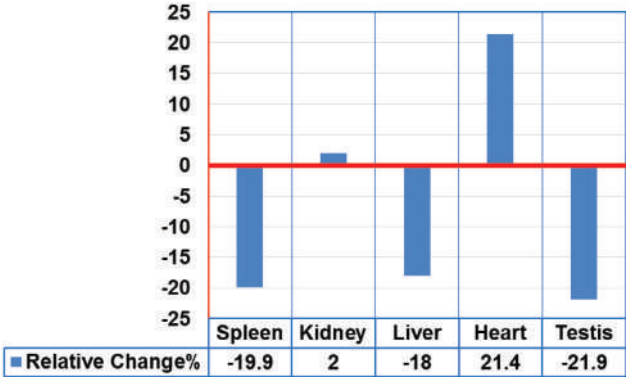

Fig. 2 **Relative change% of organs in restricted from control group**

of inhibition of gastric emptying by the inhibitory receptors is most likely found in the duodenum and proximal jejunum. Studies conducted by Hunt and Stubbs (1975)[19] indicated that dietary density (kilocalories per milliliter) determines the rate of stomach emptying. This inhibitory activity is mediated by the neural and hormonal mechanisms and their interactions.[21] All these evidences can explain the lower food intake in treated group but without significant difference in body weight of this study. This reduction in food intake may be due to disruption in digestion or absorption processes, or negative neural reflexes, or even immunological response in the gut because our results demonstrated infiltration of inflammatory cells in lamina propria of villi with high mucin secretion in the lumen of the intestine.

The relationship between liver disease and specific foods is not well understood.[22] Some researchers suggest that the insulin resistance and an increase in fatty acids after ingestion high fat and sugar-diet contribute to livers inflammation and irreversible scarring.[23–25] The various pathological changes in the liver of restricted mice demonstrated in this study agree with several studies that have reported that over-consumption of carbohydrates especially refined carbohydrates, fats (particularly saturated fats), and the protein of meat can cause liver disease.[26–28]

In compatible with our result concerning with testicular changes, several studies confirmed that environmental factors, diseases and various nutrients may affect the composition of sperm directly or indirectly, thus consuming of high-fat diet by male mice alters global methylation and microRNA content in their mature sperm, and transcriptional profiles in their testes.[29] Another study demonstrated decreasing in the testis/body weight, seminiferous tubule diameter, spermatogenetic cells and interstitial cells.[30] Furthermore, the seminiferous tubule diameter also decreased in high-fat diet group which could be related to a disturbance of spermatogenesis.[31]

There is growing evidence suggest that rich-fat-diet intake affects the heart failure development and discontinuation.

Table 1. **Body and organs weight in control and restricted groups**

Groups	The absolute weight (in g) (M ± SD)					
	Body	Spleen	Kidney	Liver	Heart	Testis
Control	28.34 ± 2.1	0.286 ± 0.03	0.246 ± 0.02	1.83 ± 0.16	0.206 ± 0.01	0.105 ± 0.0008
Restricted	29.95 ± 2.8	0.229 ± 0.04	0.251 ± 0.03	1.50 ± 0.23	0.25 ± 0.03*	0.082 ± 0.01

*Significant difference at $P < 0.05$.

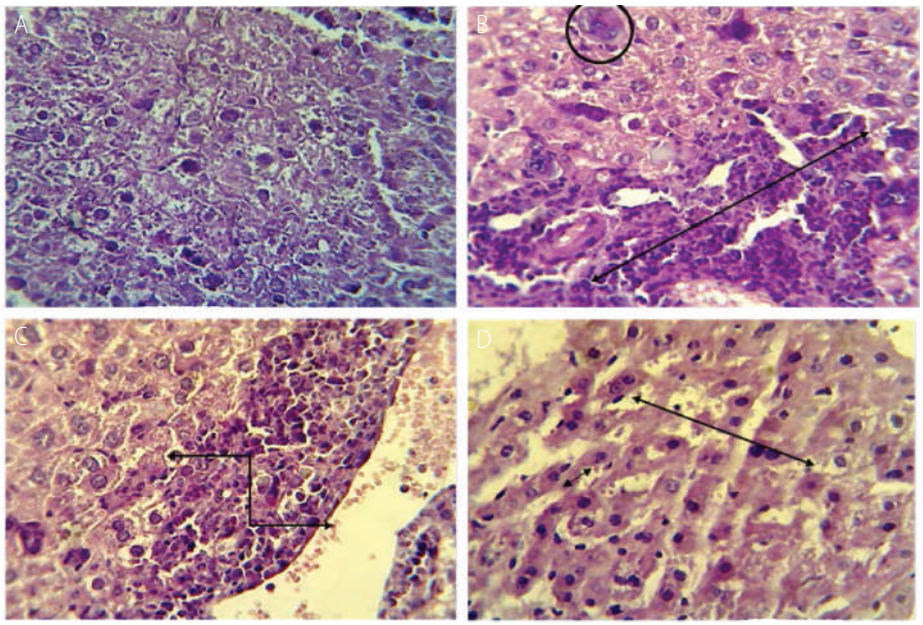

Fig. 3 **Pathohistological section in liver of control (A) and restricted (B–D) group. (A) Histopathological section in the liver of normal animal shows no clear lesions. (B) Inflammatory cells particularly neutrophils and mononuclear cells aggregate in liver parenchyma ↕ with megakaryocytes ○ in the sinusoids. (C) Inflammatory cells particularly neutrophils and mononuclear cells aggregate around congested blood vessels with vacuolar degeneration of hepatocytes. (D) This shows dilatation of sinusoids, necrosis, and vacuolar degeneration in the hepatocytes.**

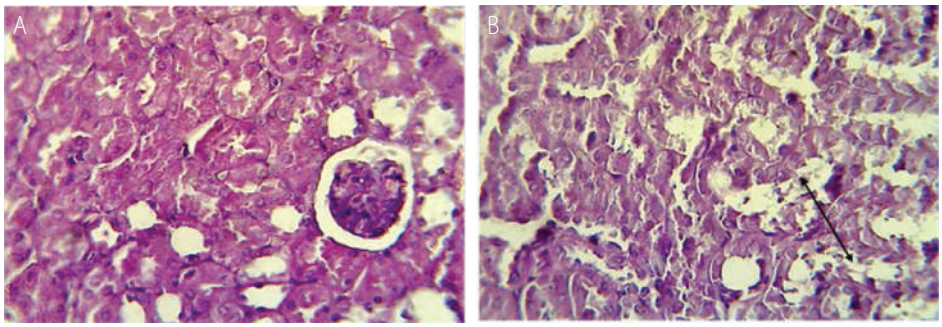

Fig. 4 **Pathohistological section in kidney of control group (A), and restricted group (B and C). (A) Histopathological section in the kidney of control group shows no clear lesions. (B) Histopathological section in the kidney of treated group shows acute cellular degeneration characterized by vacuolation of epithelial cells.**

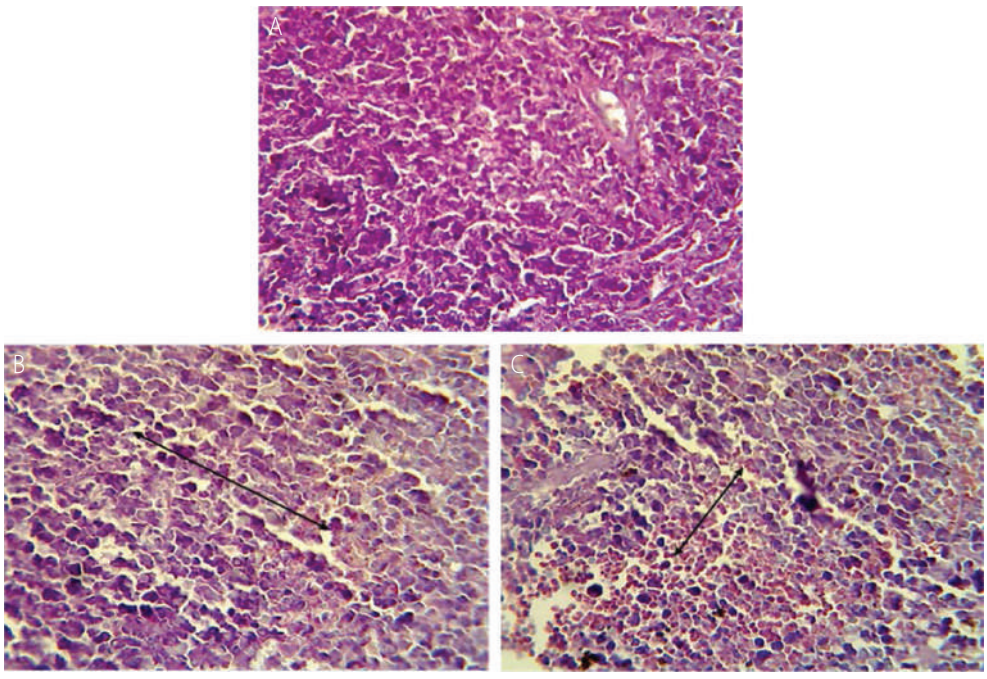

Fig. 5 **Pathohistological section in spleen of control group (A), and restricted group (B and C). (A) Normal spleen shows no clear lesions. (B) This shows moderate depletion of white pulp. (C) This shows severe congested of red pulp.**

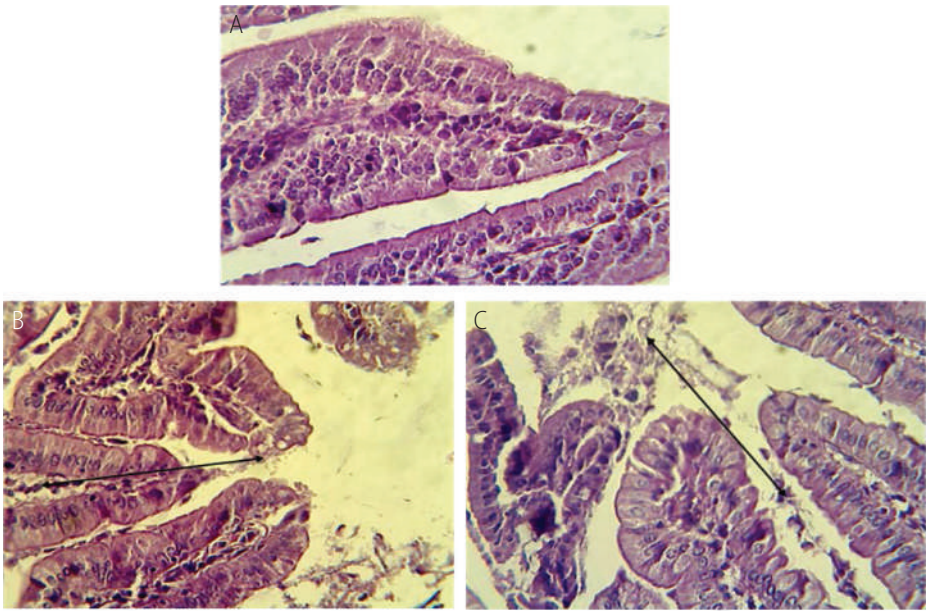

Fig. 6 **Pathohistological section in intestine of control group (A), and restricted group (B and C). (A) Histopathological section in the intestine of normal animal shows normal structure. (B) This shows mild mononuclear cells infiltration in lamina propria with mucin secretion. (C) This shows inflammatory cells between villi and lamina propria with mucin in their lumin.**

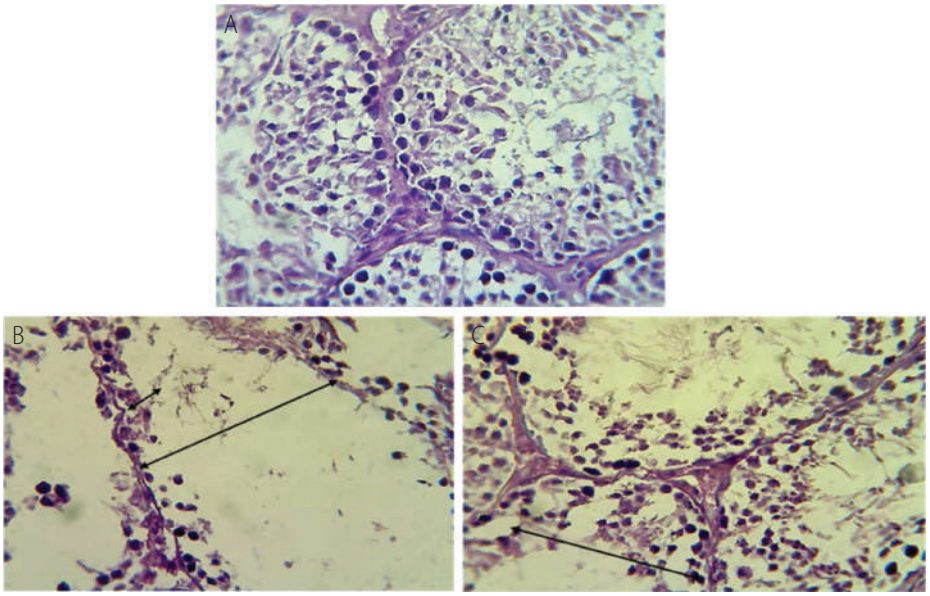

Fig. 7 **Pathohistological section in testis of control group (A), and restricted group (B and C). (A) Histopathological section in the testis of control group shows normal structure. (B) This shows complete disappearance of epithelial layer and only single cell lining of basement membrane of seminiferous tubules. (C) This shows incomplete spermatogenesis.**

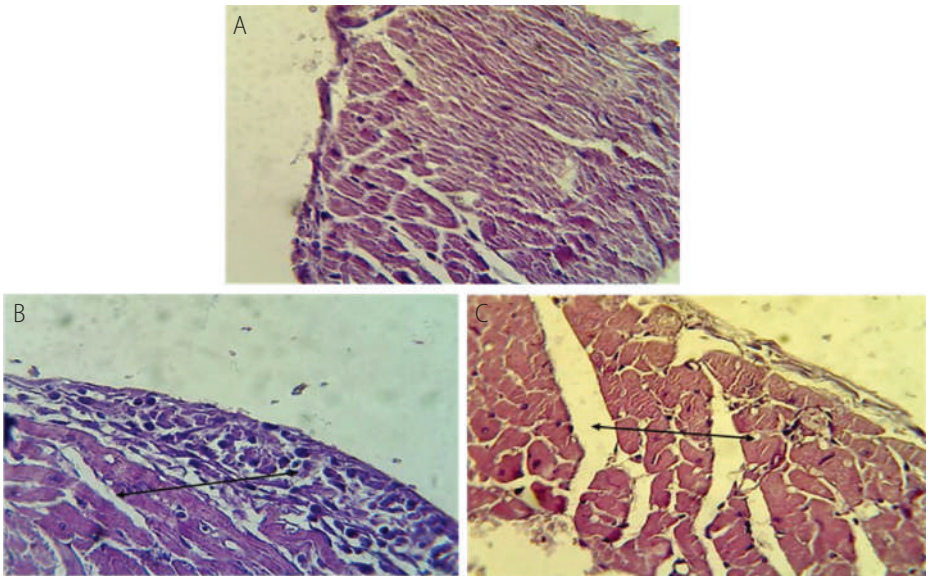

Fig. 8 **Pathohistological section in heart of control group (A), and restricted group (B and C). (A) Histopathological section in the heart of control group shows no clear lesions. (B) This shows mononuclear cells infiltration in the pericardium. (C) This shows fatty changes in cardiac muscle with edema between cardiac muscle.**

Studies in rodents show that in the absence of obesity, the fat replacement of refined carbohydrates can weaken or inhibit ventricular expansion and contractile dysfunction in response to high blood pressure, myocardial infarction or myocardial genetic.[32] Moreover, Sahraoui et al.[33] showed that a relative short period of high fat diet results in sever alterations of cardiac structure. In our results, many cardiac alterations detected such as mononuclear cells infiltration in the pericardium and fatty changes in cardiac muscle with edema between cardiac muscle, so we can suggest that edema is responsible for the increase in the weight of heart.

On the other hand, the result of our study demonstrated an increase in the weight of kidney that is compatible with those obtained by other studies. For instances, Altunkaynak et al.[34] found that fatty diet-consuming animals may lead to increase in the volume of the kidneys and renal deformities due to edema resulted from mononuclear cell infiltrations among the renal tubules.[34]

In concern with spleen, our results found moderate depletion in white pulp in agreement with other studies. Many researchers found that rodents fed with high fat-diet (fish oil) caused many immunological and histological effects in the white pulp of spleen.[35–37] A diet rich in saturated fatty acids is thought to stimulate low-grade chronic inflammation, and may contribute to increased serum inflammatory mediators levels.[38] The exact mechanism of negative effect of fat diet on white pulp is still not clear.[39,40] However, Sahraoui et al.[33] suggested that the sort period of high fat diet resulting activation not only of inflammatory but also apoptotic processes.[33] All these evidences may explain the inflammatory responses demonstrated in this study after feeding mice 10% brain sheep saturated with lipid.

Conflicts of Interest

None.

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