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INTRODUCTION

Cardiovascular disease (CVD) is a global health issue, affecting not only the affluent societies but also the developing countries. Approximately 80 percent of CVD -related deaths worldwide now take place in low and middle-income countries, where about 30 percent of all deaths are attributed to CVD.¹ According to the official estimates in Pakistan, CVD results in more than 100,000 deaths every year.² Local surveys indicate a very high prevalence of CVD risk factors affecting 30% of population over 45 years.³

Hyperlipidaemia is one of the established risk factors for atherosclerotic CVD. Elevated levels of total cholesterol, triglycerides and low density lipoprotein cholesterol (LDL-c) increase the risk of atherogenesis. In contrast, the blood level of High

LIPID PROFILE;

EFFECT OF MATERNAL HIGH-FAT, HIGH-CHOLESTEROL DIET ON THE LIPID PROFILE OF POST-WEANING AND ADULT RAT OFFSPRING

Ambreen Javed¹, Shakir Khan², Faizania Shabbir³, Tausif Ahmed Rajput⁴, Abdul Khaliq Naveed⁵

ABSTRACT... Objectives: To study the effect of maternal high-fat, high-cholesterol diet on the lipid profiles of rat offspring. **Study Design:** Randomized control trial (RCT). **Place and duration of study:** Department of Biochemistry, Army Medical College, Rawalpindi in collaboration with Chemical Pathology Laboratory (CPL), Army Medical College, Rawalpindi and National Institute of Health (NIH), Islamabad. The study was completed in six months. **Material and Methods:** Sixty adult female albino rats of Sprague-Dawley strain were divided into two groups of 30 each. Male rats of the same strain were used for breeding. Ten days before expected mating experimental group was shifted to a high-fat, high-cholesterol diet, keeping the control group on normal rat diet. Lipid profiles of pregnant dams of both groups were done at day 20 of gestation. Offspring of both groups from weaning onwards were fed the normal rat diet. One male and one female offspring from each litter of both groups (30 male and 30 females from each group) were selected and their lipid profiles were evaluated at post-weaning and adult stage. **Results:** At day 20 of gestation, high-fat and cholesterol fed dams showed highly significant increase in TC, LDL-c, HDL-c and LDL-c / HDL-c ratio than the control dams ($p < 0.01$). Maternal high-fat, high- cholesterol diet was found to raise some parameters of lipid profiles of the offspring. **Conclusions:** Maternal high fat and cholesterol diet in pregnancy and lactation causes hyperlipidaemia in the rat offspring.

Key words: High- Fat Diet, Cholesterol, Maternal Nutrition, Lipid Profile, Offspring.

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density lipoprotein cholesterol (HDL-c) has an inverse relationship to the risk of atherosclerosis and coronary heart disease.⁴ One of the lipid ratios i.e., of LDL-c to HDL-c has also been recognized as a valuable indicator of CVD risk.⁵

Universally there is a change seen in the dietary trends, with an inclination towards higher intakes of fats with lower intakes of fibre. Changing activity patterns are also observed, swiftly changing towards reduced energy expenditure.⁶

Dr. David Barker and his colleagues were the first ones to implicate the “in utero” factor in the development of CVD.⁷ Some retrospective cohorts provided information about the effects of severe under-nutrition in pregnancy on the health of the adult offspring. Supporting evidence came

from the association of low birth weight and the occurrence of type II diabetes, hypertension, and other features of metabolic syndrome, later in life.⁸

Abundant epidemiological data is now available, suggesting that under-nutrition during early life can predispose an individual to disease later on. However, data concerning over-nutrition especially the consumption of excess calories from fat during pregnancy or during early life and its subsequent outcomes later in life is relatively scarce.⁹ Keeping in view, this study was planned using a rat model in order to see the effect of maternal high-fat, high-cholesterol diet during pregnancy and lactation, on the lipid profiles of the offspring.

MATERIAL AND METHODS

The study was a randomized control trial conducted in Army Medical College, Rawalpindi, in collaboration with National Institute of Health (NIH), Islamabad. The duration of study was six months and total number of rats used were 180. Optimum conditions were maintained throughout the study, since it was expected to take a long time. Rats were housed in steel cages using wood shavings as bedding. A 12 h light and dark cycle was maintained in a room at 22-24°C. Food and water was available to the rats ad libitum. Pelleted diet was prepared at NIH for both groups.

At the start of study, 60 healthy adult female Sprague-Dawley rats (Group I) having an age of 90-110 days were obtained from animal house of NIH for the purpose of mating and producing offspring. They were randomly divided into two groups;

Group Ia (Control dams)

(n=30) fed on normal control standard pelleted diet throughout pregnancy (and lactation)

Group Ib (Experimental dams)

(n=30) fed on experimental high-fat, high-cholesterol diet starting from 10 days before expected day of mating and continuing in gestational and lactational period. Experimental diet was prepared at NIH, Islamabad by the

addition of 40% butterfat, 5%cholesterol and 0.35% cholic acid in the normal control rat diet (10).

15 male rats of the same strain were used for breeding purpose only.

Lipid profiles of pregnant dams of both groups were done at day 20 of gestation. All pregnancies were allowed to continue. Within 24-48 hours after parturition, litters were reduced to six pups/dam. One male and one female from each litter (sixty of each group) were selected for the study, making a total of 120 offspring.

Group-Ila (Control offspring)

Sixty offspring (male n=30 female n=30) of group Ia rats were randomly included in this group and fed on control rat diet from weaning onwards.

Group-Ilb (Experimental offspring)

Sixty offspring (male n=30 female n=30) of group Ib were randomly included in this group and fed on control rat diet from weaning onwards.

The only difference between group Ila and Ilb rats was the nutrition of their mothers.

Lipid profile was done at 30 and 90 days of age of offspring. Serum total cholesterol (TC), TG and HDL were measured by using commercial kits, in accordance with the instructions of the manufacturers, by applying the enzymatic colorimetric principles. LDL was calculated by using Friedewald formula
$$LDL = TC - [HDL + TG/5]$$

The data was entered and analysed using SPSS version 15.0. The arithmetic mean and standard error of mean of TC, TG, LDL, HDL and LDL to HDL ratio were calculated. The statistical significance of difference between the groups was determined by applying independent sample's *t* test. The difference was considered significant if *p* value was found to be < 0.05.

RESULTS

Comparison of lipid profile at 20 days of gestation between control and experimental dams is

presented in Figure-1 and 2. Experimental dams showed highly significant increase in TC, LDL-c, HDL-c and LDL-c / HDL-c ratio than the control dams (p < 0.01).

Maternal high-fat, high- cholesterol diet was found to raise some lipid profile parameters of the offspring. Lipid profile of male offspring at day 30 and 90 is presented in Table 2. At day 30, post-weaning IIb male offspring exhibited highly significant rise in TC and TG (p < 0.01) and a significant increase in LDL-c(p < 0.05), as compared to IIa male offspring (Figure 3 and

5). At day 90, adult IIb male offspring had highly significantly increased TC, TG, and HDL-c than IIa male offspring (p <0.01).

Lipid profile of female offspring at day 30 and 90 is presented in Table 3. None of the parameters was significantly different between 30 days post-weaning IIb and IIa female offspring (p > 0.05). The 90 days post weaning lipid profile of female offspring showed that IIb rats exhibited highly significant rise in TC, TG, LDL-c and LDL-c/HDL- c ratio than IIa female offspring (p <0.01).

| Parameter | Age Post Weaning | | | | | |
|---------------------|------------------|-------|---------|---------|--------|---------|
| | 30 Days | | | 90 Days | | |
| | IIa | IIb | p value | IIa | IIb | p value |
| TC (mg/dl) | 108.4 | 120.0 | 0.004* | 90.27 | 105.17 | 0.001* |
| TG (mg/dl) | 68.90 | 79.90 | 0.001* | 67.40 | 96.90 | 0.000* |
| LDL-c (mg/dl) | 61.52 | 68.3 | 0.044* | 48.32 | 53.62 | 0.148 |
| HDL-c (mg/dl) | 33.10 | 33.73 | 0.061 | 28.47 | 32.17 | 0.004* |
| LDL-c/HDL-c (Ratio) | 1.89 | 1.97 | 0.545 | 1.76 | 1.68 | 0.550 |

Table-I. Comparison of lipid profile of control and experimental male offspring at 30 and 90 days post weaning. * p<0.05 (Significant difference)

| Parameter | Age Post Weaning | | | | | |
|---------------------|------------------|--------|---------|---------|--------|---------|
| | 30 Days | | | 90 Days | | |
| | IIa | IIb | p value | IIa | IIb | p value |
| TC (mg/dl) | 115.40 | 117.23 | 0.714 | 78.47 | 119.50 | 0.000* |
| TG (mg/dl) | 71.87 | 73.33 | 0.634 | 71.90 | 80.23 | 0.002* |
| LDL-c (mg/dl) | 65.42 | 68.06 | 0.504 | 32.45 | 69.72 | 0.000* |
| HDL-c (mg/dl) | 35.60 | 34.50 | 0.397 | 31.63 | 33.73 | 0.056 |
| LDL-c/HDL-c (Ratio) | 1.82 | 2.00 | 0.085 | 1.05 | 2.09 | 0.000* |

Table-II. Comparison of lipid profile of control and experimental female offspring at 30 and 90 days post weaning. * p<0.05 (Significant difference)

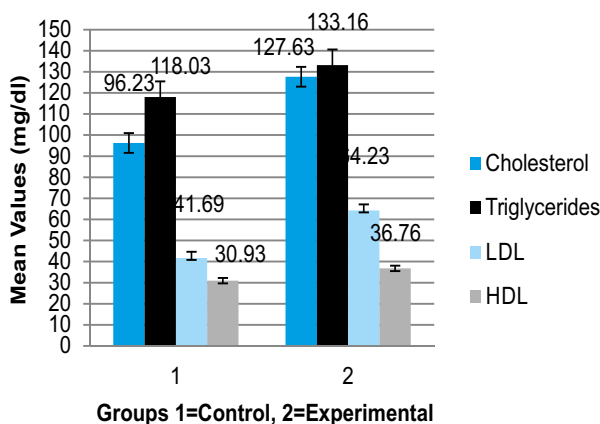


Figure-1. Comparison of lipid profile of Control and Experimental rat dams at day 20 of gestation.

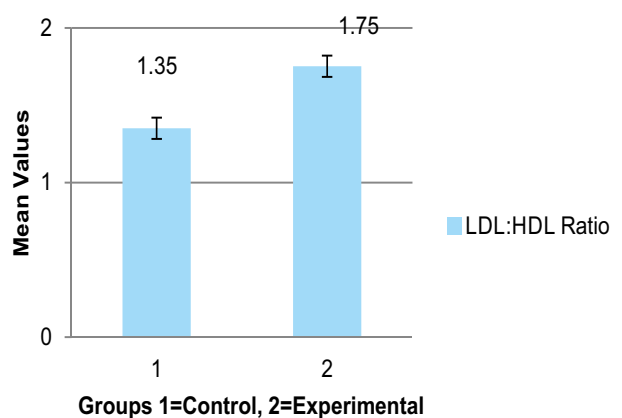


Figure-2. Comparison of LDL-c/HDL-c ratio of Control and Experimental rat dams at day 20 of gestation.

DISCUSSION

The present study was designed to investigate whether hyperlipidaemia finds its roots in the in-utero exposure to a high-fat, high-cholesterol diet, through maternal nutritional experience.

The two groups of rat dams were different in terms of their diets only, which affected them and their offspring as well. Comparison between dams was done at 20 days of gestation, whereas comparison between offspring was done at 30 and 90 days post weaning respectively. Our major focus was on the lipid levels of the offspring.

Post-weaning, at 30 days of age, male offspring of high fat, high-cholesterol fed (experimental) dams had highly significant raised TC, TG and significantly increased LDL-c, compared to control male offspring. Levels of HDL-c and the LDL-c to HDL-c ratio at this stage also rose in the experimental group but did not achieve statistical significance. None of the parameters was significantly different in the female offspring of experimental and control group at this stage. Raised lipids in 30 days old offspring of our study can be explained by considering the sustained effect of the milk, having elevated fat content in case of high-fat, high-cholesterol fed mothers. High-fat fed rats have greater milk lipid concentration than that of rats fed with a normal, high carbohydrate, low fat diet.¹⁰ Triglycerides were also raised in the study done by Guo F and Jen KL., who reported that weanlings of high-fat fed Wistar rat dams weighed more and had higher TG levels than controls.¹¹ Contrary to our results is the study by Koukkou et al., who have reported significantly lower total cholesterol and similar TG levels in 15 days old offspring of dams fed the high-fat diet.¹² In our study, we did not assess the lipids at day 15. The mechanisms behind the female offspring being unaffected at this stage remain unclear. The possibility of either gender being hyperphagic, at any stage, was not explored; it could have provided the answer to this gender specific difference in results.

Post-weaning, at 90 days of age, both male and female offspring were found to be affected. Experimental male offspring exhibited highly

significant increase in TC, TG and HDL-c, compared to control male offspring. However, LDL-c and the LDL-c/HDL-c ratios were not different. On the other hand, in the experimental female offspring, rise in TC, TG and LDL-c was highly significant, compared with control female offspring. Levels of HDL-c were not different. The LDL-c/HDL-c ratio also highly significantly rose in females of experimental group. Our study is in line with the already mentioned study by Koukkou et al., where plasma TG levels were significantly higher in the 60-day-old offspring of fat-fed rats.¹²

Srinivasan et al., have reported that 120 days old male progeny of high fat fed dams weaned on laboratory chow had increased plasma levels of triglycerides, which is in accordance with our study.¹³ Our results partially support those of Ghosh et al in which a high-saturated-fat diet in pregnant rats resulted in raised plasma TG and lower HDL-c in the adult female offspring. There was no difference in total cholesterol between the groups.¹⁴ Gender related differences in the biochemical parameters have also been seen in our study. The present study is also consistent with that of Khan et al., who reported that lard fed pregnant rats, brought about a gender-related cardiovascular dysfunction in offspring fed with normal diet. In male rats, none of the tested biochemical parameters was significantly different at 80 days or 180 days. In contrast at 360 days, the male offspring had reduced total cholesterol and HDL-c. In the female offspring, the biochemical parameters were in the same way unaffected at 80 and 180 days. However, at 360 days, the female offspring demonstrated a rise in fasting triglycerides and reduced HDL levels.¹⁵ The predisposition of only female offspring to hypertension in adult life concurs with our results from the 90 days old female offspring of fat and cholesterol fed dams. This group had raised serum lipids and had the higher ratio of LDL-c to HDL-c. Samuelsson et al., focused on the hypothesis that diet-induced-obesity during pregnancy can pass on a tendency for adiposity, glucose intolerance, and cardiovascular dysfunction to the offspring. The results at 3 months revealed higher plasma TG concentrations than the controls. At 6 months, experimental animals had higher cholesterol levels

though TGs were similar to controls. Experimental offspring also showed hyperphagia, increased adiposity, and an adult metabolic syndrome-like phenotype.¹⁶ One recent study by Oliveira et al., supports our results. They evaluated the long-term effects of a perinatal palatable high-fat diet on the food intake and cholesterol profile of adult Wistar rats. Serum total cholesterol, LDL-c, HDL-c, TG, VLDL-c were increased in the pups from mothers fed a palatable high-fat diet compared to the control group. They also concluded that an early life environment with a high-fat diet could contribute to metabolic disease in later life.¹⁷

This study along with others in this field provide a support for the fetal origin of adult disease though the underlying mechanisms largely remain unexplained.

CONCLUSION

Simple dietary adjustments during the crucial perinatal period can save the future generation from the burden of cardiovascular disease in the productive years of life. Pregnant women and nursing mothers should avoid the diets with high fat and cholesterol content. A balanced maternal diet with a right proportion of all essential nutrients should be advocated at all forums.

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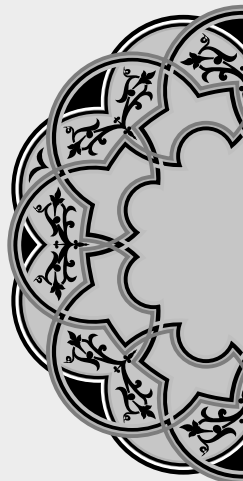
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



17. Oliveira TW, Leandro CG, de Jesus Deiró TC, dos Santos



*“All our knowledge has
its origin in our perceptions.”*

Leonardo Da Vinci

AUTHORSHIP AND CONTRIBUTION DECLARATION

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| 2 | Shakir Khan | Sample collection, Laboratory protocols. |  |
| 3 | Faizania Shabbir | Paper Writing, Lab Assays. |  |
| 4 | Tausif Ahmed Rajput | Lab Assays, Statistical Analysis and inference. |  |
| 5 | Abdul Khaliq Naveed | Project Supervision, Paper Writing |  |