Physiological and Anthropometric changes in the Offspring of Albino Rats fed on Dietary cholesterol in the presence of *Plasmodium malariae.*

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ABSTRACT

The present study has examined the physiological and physical changes in young albino rats fed cholesterol supplemented diet in presence of plasmodia. *Plasmodium malariae* was established intrauterine in offspring of animal infected with the parasites and fed on 2% cholesterol supplement in diet. Mean serum cholesterol level obtained from control animals (Group A) was 121 ± 1.3 (rng/100ml of blood). The mean serum cholesterol obtained from animals removed from cholesterol diet (Group B) on the day of litter was 123 ± 1.4(mg/100ml of blood). Mean serum cholesterol obtained from animals on cholesterol supplemented diet in the presence of plasmodium parasites (Group C) was 519 ± 1.5 (rng/100ml of blood). Animals on cholesterol supplemented diet in the presence of parasites showed significantly higher (P<0.000l) level of serum cholesterol than the other two groups.

The weight of animals in groups A and B was significantly higher (P<0.000l) than the weight of animals' in-group C. The group B animals died six weeks after birth probably due to development of early atherosclerosis.

Cholesterol diet evidently elevated serum level of cholesterol. Plasmodium parasites put down completely the fledging immunity of the animals. The study further highlighted the danger of elevated serum cholesterol level in the presence of malaria.

Keyword: Cholesterol, Atherosclerosis Intrauterine and Parasitemia,

Numerous Angiographic trials have shown that atherosclerotic process can be modified by reducing levels of cholesterol (Frick *e al,* 1987; Kanet *et al.,* 1990; Gaziano *et al* 1992). A variety of factors can help bring about elevated plasma levels of cholesterol and the accompanying well documented increased risk for coronary artery diseases. The most obvious vehicle is a high fat or cholesterol diet (Gaziano *et al* 1992; Jones, 1998).

The occurrence of atherosclerosis world - wide has assumed a disturbing dimension. Elevated cholesterol level (above 200mg/dl) is a major factor related to the pathophysiological changes associated with the disease (Rajaratnam *et al* 2000). It has been shown that in Rabbit, a period of cholesterol feeding followed by a period of normal diet was found to cause important functional and metabolic changes in the aorta (Ragazzi *et al.,* 1990). Indeed, vasodilatation response to acetylcholine was almost totally impaired. ATP - induced relaxation was reduced and high-energy metabolites were greatly dirninished (Jiang *et* al 1999). Although at the time of the experiments, blood lipid level was normal, these alterations were accompanied by cholesterol infiltration in the vessel wall. It is well known that atherosclerosis may progress even after the end of the cholesterol diet.

Dietary cholesterol is absorbed from the intestinal lumen, while dietary cholesterol esters must be hydrolysed by pancreatic cholesterol esterase before they are absorbed (Goodman, 1965). The capacity of cholesterol absorption mechanism is limited and dietary cholesterol must compete with 1 to 3 gm of cholesterol excreted in bile daily.

It was shown (that fat supplemented diet increased serum total cholesterol concentration, but the rise was lower in animal receiving endotoxin infusions. The concentration of esterified cholesterol in the aortic intima and inner media was increased

in the fat supplemented group of animals.

In the present study, it was shown that *P. malariae* can infect Albino rats and the hypothesis that intrauterine infection of offspring is possible, is further reinforced. The study also highlighted the pathophysiological effects of dietary cholesterol as it may be relevant in malaria.

MATERIALS AND METHODS

Experiments Al Animals

Adult Albino rats, both male and female, 12weeks old at onset of studies (weight 114 -120g) were obtained. In all there were 15 adult rats. The animals were shared into 3 groups-

A - Control animal not fed with extra cholesterol and not infused with parasites.

B - Animals on 2% cholesterol supplemented diet, but stopped as soon as one littered.

C - Animals fed with 2% cholesterol supplement in diet and injected with parasites.

Parasites

Plasmodium parasites were in an animal host as reservoir (Black, 1945; Hawking 1945). Experimental animals were inoculated from this reservoir.

In the present study, attempts were made to inoculate laboratory bred albino rats with herparinised blood from human patient known to carry malarial parasites. The patients had their blood examined after staining to confirm the infection at the Irrua Specialist Teaching Hospital Pathology Laboratory. Of the three rats inoculated with a patient blood, one of them became heavily infected with malarial parasites, one slightly and one remained negative until the end of the investigations 14days after. The heavily infected rat was confirmed to be with *P. malariae* and it was from this that the experimental- rats were infected.

Parasitized blood (60% parasitaemia) was obtained from the reservoir. This was diluted with 0.5m1 saline (0.9% NaC1). The Experimental rats were infected by injecting this suspension of infected blood and saline intraperitonially and subcutaneously.

Feeding

Each group of five animals were given 117. 5g of corn meal per day for 12 weeks of the experimental period. .

Each experimental animal excluding control group had 2% cholesterol (cholesterol gepulvert, Darmstadt Germany) added to their feed (corn meal) each morning through out the experimental period. Each group except group A had 2.66g of cholesterol added to their feed each day. In addition clean pure water was provided for the animals in each of the cages.

Cholesterol

The cholesterol used was ordered from a scientific supplier in Benin city (cholesterin gepulvert, Merk Darmstadt, Germany). For each animal at the onset of the experiments blood fasting cholesterol levels were analysed using standard cholesterol kit (Randox cholesterol kit UK). Subsequently during the course of the experiments, cholesterol levels in blood were analyzed at weekly intervals. Randox cholesterol kit (UK) was used for serum cholesterol analysis.

Statistical Analyses

The results were expressed in text tables as mean values $\pm$ SEM Statistical analyses were made using student's paired T - test.

RESULTS

Mean Total Serum Cholesterol In Mg/100ml Of Blood

Analysis of variance was used to look at the effects of cholesterol on all three groups studied. Significant variations occurred at week 6 of the studies. This is shown in table 1. Cholesterol level in the control (n=5) animals remained consistently low, only increasing slightly over the 6-week experimental period.

Group A was made up of normal young animals (n =5). Normal serum cholesterol levels were established when they were 4 weeks old. Group B animals (n =5) were offspring of animals who were on 2% cholesterol supplement in diet but cholesterol was removed from their diet on the day of litter; cholesterol levels consistently remained the same. Group C

animals (n = 5) these animals were at the onset littered 13. These animals were offspring of animals who remained on the 2% cholesterol supplement and were also injected with protozoan parasites. These animals were also found to have *Plasmodium* parasites in their blood as shown in the film. The level of total serum cholesterol was fatally high. All the animals died at the 6th week after birth.

The Group C animals (n=5) were littered by an albino rat on 2% cholesterol supplement in diet and injected with plasmodium parasites. This animal littered a record (13). The cholesterol supplement was however, not discontinued. Growth of the offspring was heavily retarded and they remained stunted. Recorded weight ranged between 5.64±0.lg and 12.34±0.3g over a six week period. Plasmodium parasites were seen in the blood film of these animals. The difference in weight between group A and C was very signmc811t (P<0.0001). This is shown in table 2

Table 1: Mean (±SEM) Total serum cholesterol (mg/100ml of blood) during the study

Age	Week 4	Week 5	Week 6
Normal Offspring (n=5) Group A	120.2±1.1	120.2±1.2	120.2±1.3
Offspring of anima removed from cholesterol on the day of litter (n=5) Group B	120.2±l.2	120.2±1.2	120.2±l.4
Offspring of animal with 2% cholesterol and injected with parasites (Group C)	449.4±0.75	449.4±0.9	519±1.5

Table 2: Mean (±SEM) weight (g) of Experimental Animal over a period of 6 weeks.

Age	Week 4	Week 5	Week 6
Normal Offspring (n=5) Group A	18.44±0.2	27.15 ±0.7	43.75±0.5
Offspring of animal removed from cholesterol on the day of litter (n=5) Group B	17.6±0.4	27.12±1.1	44.52±0.6
Offspring of animal with 2% cholesterol and injected with Plasmodium parasites (Group C)	5.64± 0.1	8.29± 0.4	12.34±t 0.3

DISCUSSION

The present study revealed that treatment of Albino rats with a high fat and cholesterol diet over a six week period resulted in an increase in total plasma cholesterol (and by implication J3-lipoprotein, free fatty acid, VLDL, LDL) and caused development of early atherosclerotic signs-(Rajaratnamet *et al.*, 2002). There was an insignificant build up of serum cholesterol in the control animals. This supports the belief that cholesterol is synthesised normally in animals, but a supplement in diet no doubt significantly builds up serum cholesterol in some cases to pathological levels (Jones, 1998). In a previous study Jayakodi *et al.*, (1985) showed that in rabbits that were fed a 2% cholesterol diet for 6 weeks and then reverted to normal diet for 32 weeks thence, aortic vasodilatation responses were impaired. This shows slow metabolic rate of cholesterol and damage to body tissues results in a short time as soon as pathophysiological level are achieved. This is consistent with our findings in the present studies. These young animals were believed to have shown poor metabolic capabilities. This situation as shown in their diminished feeding is believed to be as a result of the very high concentration of serum cholesterol observed in group C.

The offspring fed on normal diet showed higher weight gain than the atherosclerotic animal. The group B animals who were offspring of animal on cholesterol diet but discontinued on their litter also showed normal growth and their weight compared with those entirely on normal diet. The group C animals had high level of cholesterol (5 1 9mg/lOOrnl of serum).

Interestingly this same group of animals had intrauterine infection of *Plasmodium* parasites. A combination of the level of serum cholesterol and parasites possibly stunted the growth of these young animals. It has been established that high level of cholesterol decrease the level of circulating leucocytesa (Majarian *et al.*, 1984). This obviously affected the immune system of the animals. It has been shown in rodent malaria model13 that, monoclonal antibodies to the surface proteins of the different species of plasmodium parasites confer protection of the malaria parasites following passive transfer. This is capable of sustaining the parasites within the animals for a longer time. Being that the animals were quite young and incapable of preventing the parasites from proliferating; the result is significantly decreased level of metabolism, thus a massive impairment of growth. The animals were observed to have dropped in level of feeding, hence there was a significant weight loss.

Because of the attendant difficulty in culturing plasmodia parasites, the use of asexual stages derived from patients suffering from malaria proved to be successful. It has also been shown (Majarian *et al.*, 1984) that in rodent malaria model monoclonal antibodies to the surface glycoproteins of the different species of plasmodia parasites confer protection following passive transfer. Blood film from the young uninfected animal clearly showed no sign of plasmodia infection. In the present study there was no direct injection of parasites into young animals. The young animals were infected through intrauterine means. An animal with 2% cholesterol supplement in diet and injected with plasmodia parasites littered thirteen (13) and all had plasmodia parasites in their blood. The animals were not treated for malaria, and they had rather undeveloped immune system This in complement with the rather high serum cholesterol may have been responsible for their stunted growth. Hence these animals could not survive for more than a few weeks. There was a rapid loss of weight and, ultimately they died as a result of atherosclerosis.

The present study highlights the danger of hypercholesterolemia especially in younger individuals. It also shows the possibility of congenital infection of offspring from individuals infected with Plasmodia parasites. This is particularly important in women of childbearing age who inherently have relatively higher level of serum cholesterol in malaria endemic area.

REFERENCES

1. Black, RH. A preliminary note on the cultivation in-vitro of new Guinea strains of human malaria parasites. *Med. J. Australia* 1945. 2: 500-501.

2. FRICK, MR, Elo, 0., Haapa, K, Heinonen, O.P., Heinsalmi, P., Helo, P., Huttunen, J.K, Kaitaniemi, P., Koskinen, V., Maenpaa, H., Malkonen, M,Manttari, M, Norola, S., Pasternack, A, Pikkaramen, J., Romo, M.,Sjoblom, T. and Nikkila, E.AHelsinki heart study: Primary - prevention trial with gemfibrozil in middle-aged men with dyslipidea. Safety treatment, changes in risk factors and incedence of coronary heart disease. *N Engl.* J: *Med.*. 1987, 317:1237-1245.

3. GAZIANO, J.M, Manson, J.E., Branch L.G., LaMotte, F., Coblitz, G.A, Buring, J. E. and Hennekens, C.H. Dietary beta carotene and decreased cardiovascular mortality in an elderly cohort. *J. Am. Coli. Cardio* 1992: 62: *31B-36B.*

4. GOODMAN, D.S. The metabolism of chylomicron cholesterol ester in the rat. *J. Clin. Invest* 1965, 41: 1886 - 1896.

5. HAWKING, F. Growth of protozoa in tissueculture.I *Plasmodium gallinaceum* exo-erythrocytic forms. *Trans Roy Soc. Trop. Med. & Hyg.* 1945, 39 : 245-263.

6. JAYAKODY, D.L., Senaratne, MP.J., Thomas, A.B.R.and Kappagoda, S.T. Cholesterol feeding impairs endothelium - dependent relaxation of rabbit aorta. *Can. J. Physiol. Phaemacol,* 1985 63: 1206 - 1209.

7. JONES, PH. Clinical Diagnosis of Lipid Disorders. Clin-Cornerstone. 1998 1(1): 15-30

8. Jiang, Y, Kohara, K, Hiwada, K Low wall Shear Stress Contributes to Atherosclerosis of the Carotid Artery in Hypertensive Patients. Hypertens-Res. 1999: 22(3):203-7.

9. KANE, J.P., Malloy, MJ., Ports, T.A, Philips, N.R, Diehl, J.C. and Havel RJ. Regression of coronary atherosclerosis during treatment of familial hypercholesterolemia with combined drugs regimens. *JAMA.* 1990, 264: 3007 - 3012.Parasitol. 28: 17 - 22.

10. MAJARIAN, W.R, Daly, T.M, Weidanz, W.P. and Long, C.A, Passive immunization against murine malaria with an IgG3 monoclonal antibody. *Journal of Immunology* 1984132 (6) : 3131 - 3137. 9.

YLA - HERTTUALA, S., Pesonen, E., Kaprio, E., Rapola, J.. Soveri, T., Viikari,J., Savilahti, E. Oksanen, H. and Nikkari, T. Effect of repeated endotoxin treatment and hypercholesterolemia on preatheroscleotic lesion in weaned pigs. *Atherosclerosis,* 1988 ,72 : 173 - 181.

11. RAGAZZI, E, Pondolfo, L., Chinellato, A, De Bias~ M., Caparrotta, E.,zambonin. M and Prosdocimi, M. Cholesterol diet followed by normal diet alters functional and metabolic responses in rabbit oarta. *Arteries* 1990, j 7 (2) : 17 - 83.

12. RAJARATNAM, RA, Gylling, H; Mettinen, TA. Independent Association of Serum Squalene and non Cholesterol Sterols with Coronary Artery Disease in Postmenopausal women. J-Am-Coll-Cardiol. 2000: 35 (5) : 1185-91.

13. YLA - HERTTUALA, S., Pesonen, E., Kaprio, E., Rapola, J.. Soveri, T., Viikari,J., Savilahti, E. Oksanen, H. and Nikkari, T. Effect of repeated endotoxin treatment and hypercholesterolemia on preatheroscleotic lesion in weaned pigs. *Atherosclerosis,* 1988 ,72 : 173 - 181.