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Effect of dietary cholesterol on spermatogenesis

R. S. Gupta and V. P. Dixit

Reproductive Physiology Section, Department of Zoology, University of Rajasthan, Jaipur, India

Summary: Cholesterol fed to male rats and rabbits for 120 days impaired testicular function. The production of secondary spermatocytes in rats and rabbits was reduced by 26.1 % and 46.9 %, respectively. There was significant reduction in spermatid cell population, seminiferous tubules, and Leydig's cell nuclear dimensions.

In rats aortic endothelium showed some alterations while in rabbits full-blown atherosclerotic plaque containing a necrotic core and a proliferative fibrous cap was found. Total cholesterol, triglycerides and phospholipids of the testes were increased whereas, testicular glycogen was significantly reduced.

Three- and sevenfold increases were noticed in the serum cholesterol levels of rats and rabbits. The LDL cholesterol values were increased while the HDL cholesterol/Total cholesterol ratio was significantly decreased ($P < 0.001$).

It is concluded that hypercholesteromia affects the testicular function and aortic endothelium.

Zusammenfassung: Cholesterinfütterung über 120 Tage beeinträchtigt bei männlichen Ratten und Kaninchen die Testikularfunktion. Die Produktion von sekundären Spermatozyten wird bei Ratten und Kaninchen um 26,1 % bzw. 46,9 % reduziert. Ferner kommt es zu einer signifikanten Reduktion der Spermatid-Population, der Samenkanälchen und der Dimension der Zellkerne der Leydig-Zellen.

Im Aortenendothel finden sich bei der Ratte einige Alterationen, beim Kaninchen dagegen voll ausgeprägte atherosklerotische Plaques. Gesamtcholesterin, Triglyceride und Phospholipide in den Testes nehmen zu, während der Gehalt an Glykogen in den Testes abnimmt. Die Cholesterinkonzentrationen im Serum sind bei Ratten dreifach, bei Kaninchen siebenfach erhöht, wobei LDL-Cholesterin ansteigt, der Quotient HDL-Cholesterin/Gesamtcholesterin dagegen signifikant abfällt.

Als Schlußfolgerung ergibt sich, daß Hypercholesterinämie neben dem Aortenepithelium auch die Hodenfunktion beeinträchtigt.

Key words: cholesterol feeding, spermatogenesis, Leydig's cell, VLDL/LDL-cholesterol

Schlüsselwörter: Cholesterinfütterung, Spermatogenese, Leydig-Zellen, VLDL/HDL-Cholesterin

Introduction

Among the numerous recognized risk factors for the development of atherosclerosis, one of the best documented is the association between the concentration of lipids in blood and the development of coronary heart

Table 1. Changes in body and organ weights along with sperm population, seminiferous tubule and Leydig's cell nuclear diameter of cholesterol-fed rats and rabbits.

	Body weight	Testis	Heart	Liver	Kidney	Sperm density	Seminiferous tubule diameter	Leydig's cell nuclear diameter
	(gms)	mg/100 gm body weight				million/ml	μm	
Rat								
Control	180 ± 10	1345 ± 65	319 ± 7	3210 ± 31	605 ± 26	4.39 ± 0.22	275 ± 10	6.89 ± 0.18
Cholesterol fed 100 mg/day oral in olive oil for 120 days	191 ± 17^{ns}	1164 ± 40^{a}	388 ± 9^{c}	3830 ± 102^{b}	816 ± 37^{b}	0.65 ± 0.15^{c}	146 ± 13^{c}	3.31 ± 0.12^{c}
Rabbit								
Control	1680 ± 90	150 ± 5.7	161.4 ± 1.8	2603 ± 26	640.5 ± 19.3	3.92 ± 0.4	192 ± 6.0	7.1 ± 0.2
Cholesterol fed 500 mg/day oral in 5 ml olive oil for 120 days	1980 ± 107^{ns}	145.6 ± 4.5^{ns}	213.8 ± 4.4^{c}	3743 ± 64^{c}	715.6 ± 14.7^{a}	0.650 ± 0.04^{c}	156 ± 2.1^{b}	5.9 ± 0.25^{a}

Levels of significance ^{a} $P < 0.05$; ^{b} $P < 0.01$; ^{c} $P < 0.001$; ^{ns} P – non significant.

disease (10). Human and animal experiments show dietary cholesterol levels to be a key factor in inducing atherosclerosis leading to organ damage (11, 12). This study was designed to determine the possible relations between hypercholesteromia and spermatogenesis.

Materials and Methods

A total of 20 albino rats and 10 rabbits were used. The route and regimen of treatment were as outlined in Table 1. After 120 days of daily cholesterol feeding, the testes, liver, kidney, heart plus aorta were excised, weighed, fixed in Bouin's fluid, dehydrated and embedded in paraffin. 5 µm sections were cut and stained with hematoxylin and eosin. The density of sperm from the testes was also determined (19).

Total cholesterol (16), phospholipid (21), triglycerides (7), and glycogen (15) of the testes, liver, and heart were determined. Similarly, serum was analyzed for total cholesterol (22), HDL-cholesterol (2), phospholipids (21), triglycerides (7), LDL-cholesterol, and VLDL-cholesterol (4). Mean tubular diameter was determined by measuring and tracing an average of 100 selected seminiferous tubules. Diameters of Leydig's cell nuclei were measured at ×100. The results were analyzed using Student's *t*-test. The evaluation of cell population dynamics is based on the calculations made for each cell type per cross tubular section. All "raw" counts were transformed to nuclear points by an adaptation of Abercrombie's formula (1).

Results

There was no significant change in the body weights of rats and rabbits after cholesterol feeding. The testicular weights of rats were reduced significantly. However, there was a significant increase in the weights of liver, kidney, and heart after cholesterol feeding (Table 1).

Testicular histology

Rats and rabbits showed impaired testicular function. Cholesterol feeding produced a marked diminution in the spermatogenic cell population. The production of secondary spermatocytes in rats and rabbits was reduced by 26.1 % and 46.9 %, respectively (Table 4). A significant reduction in spermatid cell population ($P < 0.001$) occurred in both animal species. Reduction in testicular sperm count was noticed after cholesterol feeding (Table 1). Seminiferous and Leydig's cell nuclear diameters were significantly reduced (Table 1). Cholesterol feeding for 120 days produced definite lesions in the aortic wall of both animal species. In rabbits these lesions developed into a full-blown atherosclerotic plaque containing a necrotic core and a proliferative fibrous cap. Total cholesterol, triglycerides, and phospholipids of the testes, liver, and heart were increased, whereas testicular glycogen content was significantly reduced ($P < 0.001$) (Table 2).

Serum biochemistry

Three- and sevenfold increases were noticed in the serum cholesterol levels of rats and rabbits. The LDL-cholesterol values were increased while the HDL-cholesterol/total cholesterol ratio was significantly

Table 2. Effect of cholesterol feeding on lipid profile of liver, testis, and heart.

	Rat											
	Cholesterol			Triglycerides			Phospholipids			Glycogen		
	Liver	Testis	Heart	Liver	Testis	Heart	Liver	Testis	Heart	Liver	Testis	Heart
Control	8.9 ± 0.38	7.2 ± 0.59	5.96 ± 0.2	2.88 ± 0.2	2.38 ± 0.15	5.18 ± 0.07	9.38 ± 0.4	9.4 ± 0.27	8.42 ± 0.2	8.68 ± 0.69	1.39 ± 0.03	0.76 ± 0.2
Cholesterol fed for 120 days	15.11 ± 0.26^{c}	11.11 ± 0.15^{b}	13.44 ± 0.33^{c}	5.21 ± 0.4^{b}	3.05 ± 0.3^{ns}	7.64 ± 0.25^{c}	15.09 ± 0.2^{c}	11.23 ± 0.8^{ns}	12.37 ± 0.1^{c}	12.65 ± 0.48^{a}	0.9 ± 0.03^{c}	1.9 ± 0.1^{b}
	Rabbit											
Control	10.92 ± 0.19	8.4 ± 0.5	9.15 ± 0.35	3.95 ± 0.06	4.7 ± 0.09	4.2 ± 0.10	8.33 ± 0.09	7.8 ± 0.8	10.03 ± 0.10	5.71 ± 0.04	2.23 ± 0.2	1.1 ± 0.04
Cholesterol fed for 120 days	15.8 ± 0.3^{c}	16.0 ± 0.9^{c}	17.5 ± 0.25^{c}	5.7 ± 0.2^{c}	6.01 ± 0.38^{a}	6.2 ± 0.2^{b}	13.1 ± 0.1^{c}	11.37 ± 0.6^{a}	14.7 ± 0.2^{b}	5.6 ± 0.1^{ns}	1.5 ± 0.1^{b}	3.0 ± 0.1^{c}

Levels of significance: ^{a} $P < 0.05$; ^{b} $P < 0.01$; ^{c} $P < 0.001$; ^{ns} P – non significant.

Table 3. Changes in serum lipids and lipoproteins in cholesterol-fed rats and rabbits.

	Cholesterol	Trigylcerides	VLDL-Cholesterol	LDL-Cholesterol	HDL-Cholesterol	HDL Chol/Total cholesterol ratio
			mg/dl			
Rat						
Control	91.4 ± 4.0	69.9 ± 4.6	15.1 ± 0.28	30.5 ± 0.31	46.5 ± 0.83	0.508 ± 0.03
Cholesterol fed for 120 days	275 ± 12[c]	161 ± 13.6[b]	18.6 ± 1.2[a]	64.4 ± 4.3[b]	74.9 ± 3.27[c]	0.272 ± 0.02[b]
Rabbit						
Control	100.6 ± 6.73	49.18 ± 1.93	10.46 ± 0.86	51.1 ± 4.75	47.48 ± 0.05	0.472 ± 0.007
Cholesterol fed for 120 days	768 ± 35.5[c]	179.16 ± 10.2[c]	35.83 ± 1.45[c]	609.17 ± 21.7[c]	122.88 ± 1.77[c]	0.160 ± 0.05[c]

Levels of significance: [a] $P < 0.05$; [b] $P < 0.01$; [c] $P < 0.001$.

Table 4. Testicular cell population dynamics following cholesterol feeding (m ± S.E.M.).

	Rat					Rabbit				
	Germinal cell					Types				
Treatment	Spermato-gonia	Primary spermato-cytes	Secondary spermato-cyte	Sperma-tids	Sperma-tozoa	Spermato-gonia	Primary spermato-cytes	Secondary spermato-cyte	Spermatids	Sper-mato-zoa
Control	5.62 ± 0.8	35.5 ± 2.6	51.92 ± 2.5	58.9 ± 3.4	(+++)	5.77 ± 0.6	44.32 ± 2.01	51.27 ± 3.7	49.19 ± 4.0	(+++)
Cholesterol fed for 120 days	5.2 ± 0.9^{ns}	33.63 ± 1.8^{ns}	37.95 ± 3.0^{a}	13.8 ± 2.29^{a}	(−)	4.41 ± 0.3^{ns}	26.9 ± 1.9^{a}	2.75 ± 0.25^{a}	1.0 ± 0^{a}	(−)
Percentage deviation	−7.5 %	−5.3 %	−26.1 %	−76.5 %		−23.4 %	−33.3 %	−46.9 %	−98 %	

Levels of significance: ^{a} $P < 0.001$; ^{ns} P – non significant; (+++) numerous; (−) NIL.

decreased ($P < 0.001$). Elevation of VLDL-cholesterol and triglycerides was noticed (Table 3).

Discussion

Cholesterol is known to produce various grades of aortic damage in different animals (5). In rats aortic endothelium showed some alterations while in rabbits full-blown atherosclerotic plaque containing a necrotic core and a proliferative fibrous cap were found. It may be pertinent that the rat is relatively less prone to atherosclerotic changes (17).

Present investigations show that cholesterol feeding induced infertility in rats and rabbits by inhibiting the process of spermatogenesis at the spermatocyte and spermatid levels. Cholesterol feeding may be either directly acting on the sperm itself within the testis or decreasing the circulating levels of testosterone. Philips (18) observed that plasma testosterone levels are inversely related to plasma cholesterol, triglycerides, and beta lipoprotein levels. Gutai et al. (8) suggested the negative correlation between total cholesterol and VLDL-cholesterol and triglycerides. There is some evidence that testosterone is a key factor in regulating HDL levels and composition (6). In the present study, significant elevation in the concentration of total cholesterol and triglycerides indirectly prove the reduced level of testosterone. However, the early stages of spermatogenic cycle remain unaffected; they are independent of serum concentration of FSH, LH, and testosterone (20). The total volume of Leydig's cells is positively correlated with the onset of spermatogenesis. Therefore, shrinkage in Leydig's cell nuclei of rats and rabbits further reflects the reduced levels of testosterone (14). Reduced glycogen content of the testis after cholesterol feeding to rats and rabbits could be due to impaired glycolysis. The increased level of cholesterol in the testes of cholesterol fed rats and rabbits may be associated with impaired spermatogenesis (9). Davis and Conglio (3) showed an increase in the concentration of cholesterol after cryptorchidism.

In conclusion, increased serum cholesterol is alarming as it affects the process of spermatogenesis, in addition to the specific indications of myocardial infarction.

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Author's address:

Dr. R. S. Gupta, Reproductive Physiology Section, Dept. of Zoology, University of Rajasthan, Jaipur 302004, India