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Blood lipids in young and adult Watanabe Heritable Hyperlipidemic (WHHL) and adult normolipidemic rabbits – strain and sex differences

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Introduction

In 1980 Watanabe described a new rabbit strain which developed spontaneous atherosclerosis and exhibited heritable hyperlipidemia due to a recessive genetically conditioned deficiency in low density lipoprotein (LDL) receptor. The homozygous Watanabe heritable hyperlipidemic (WHHL) rabbits were reported to have 8 to 10-fold increased plasma cholesterol and triglyceridemia compared to normolipidemic rabbits. The grown up heterozygous WHHL rabbits were reported to have blood lipid levels as normolipidemic rabbits (*Esper et al. 1993*) or intermediate values between those in normolipidemic rabbits and WHHL homozygotes (*Goldstein et al. 1983*).

In 1989 a breeding colony of WHHL rabbits was established in our laboratory from 2 homozygous males and 6 heterozygous females obtained with the permission of Dr. Y. Watanabe from professor Jansen, University of Leiden, Holland. Since then the WHHL rabbits from our colony have been used to monitor the development of spontaneous atherosclerosis in homozygous and heterozygous animals (*Hansen et al. 1994*) and for dietary and drug intervention during the first 6 months of life (*Hansen et al. 1995, Mortensen et al. 1995 AB*).

The hyperlipidemia in homozygous WHHL rabbits was reported to diminished with age (*Watanabe 1980, Havel et al. 1982*). Also a sex difference in blood lipids were reported for spontaneously hyperlipidemic rabbits (*Lind et al. 1990*). The age related physiological decrease, if present, and possible sex difference in blood lipids might interfere with the interpretation of the effect of drug or dietary intervention on blood lipids. It has earlier been demonstrated for homozygous WHHL rabbits from our colony that at the age from 4 to 11 weeks no significant changes in total cholesterol occurred while triglycerides significantly decreased in females at the 11th week of life (*Mortensen & Frandsen 1996*). The aim of this study was 1) to further examine the possible blood lipid changes from the 11th to 23rd week of life in homozygous WHHL rabbits i.e. up to the age of the end of the intervention studies, 2) to compare the concentration of plasma cholesterol and triglycerides in lipoproteins of homozygous WHHL, heterozygous WHHL and NZW rabbits at least 6 months old and 3) to compare the physiological levels of total plasma cholesterol and triglycerides of adult homozygous WHHL, heterozygous WHHL from our colony and normolipidemic rabbits of different strains.

Materials & Methods

Housing, clinical observation of the rabbits and diet

All the rabbits kept in our laboratory animal unit were housed individually in steel cages under controlled environmental conditions (temperature 18 ± 2°C, relative humidity 55 ± 5%, 12/12 hrs light/dark cycle, air changed 10 times/hr). The rabbits were observed at least twice a day for any abnormalities in the clinical condition. The rabbits were fed either 100g/rabbit/day or *ad libitum* of a standard rabbit chow Altromin 2113 (Lage Germany) containing 3.5% fat. They had free access to tap water. The diet of the rabbits from which the plasma samples were provided by Charles River, Germany, contained 3.5% fat. The diet of the rabbits from which the plasma samples were provided by Møllegaard Breeding Center, Denmark contained 5% and 3% fat for females and males respectively.

Blood samples

All the rabbits were fasted overnight before blood sampling. The blood samples were taken from the marginal ear vein of unanesthetized animals in tubes containing potassium EDTA, and plasma was isolated after centrifugation at 2000g for 10 minutes. Repeated blood samples were collected from 31 female and 36 male homozygous WHHL rabbits from own breeding colony from 11 weeks until 23 weeks of age at intervals of 4 weeks. Single blood samples were obtained from 12 homozygous WHHL females 12 to 19 months old, 4 homozygous WHHL males 8.5 months old, 47 female and 23 male heterozygous WHHL rabbits 6 to 18 months old, 4 NZW breeding females (Thomae-Pharma-Biberach, Department of Laboratory Animal Service, Biberach, Germany) from own breeding colony, from 45 males Chinchilla (Chbb:CH) (Thomae-Pharma-Biberach, Department of Laboratory Animal Service, Biberach, Germany) approximately 6 months old, 16 female and 16 male NZW rabbits (Danish Serum Institute, Hvide Steen, Denmark) no less than 6 months old, and from 14 male Russian rabbits (Møllegaard Breeding & Research Centre A/S, LI. Skensved, Denmark) at mean age of 22 months. Additionally, plasma samples were provided by Charles River, Germany from 20 female and 20 male NZW breeders, 40 female and 40 male Chinchilla (Chbb:CH) breeders, 20 female and 20 male Hasen breeders and by Møllegaard Breeding & Research Centre A/S, LI. Skensved, Denmark, from 15 female and 31 male Russian rabbits from the breeding colony.

Assay of plasma lipids

The concentration of plasma cholesterol and plasma triglycerides was determined enzymatically (CHOD-PAP, GPO-PAP, Boehringer Mannheim and UNIMATE, Hoffman La-Roche respectively). Lipoproteins were separated by density gradient ultracentrifugation by the method of *Terpstra et al. (1981)*. The density ranges of the isolated fractions were: very low density lipoprotein (VLDL) $p<1.0063$ g/ml, low density lipoprotein (LDL) $1.019<p<1.063$, high density lipoprotein (HDL) $1.063<p$. The concentration of cholesterol in each fraction was determined as above.

Statistical procedures

The results on blood lipids in homozygous WHHL rabbits from 11 to 23 weeks of age, blood lipids in lipoproteins, strain and sex differences in blood lipid levels between the heterozygous WHHL, NZW, Chinchilla, Russian and Hasen rabbits and sex differences within the same strain were analyzed by analysis of variance followed by Duncan's test. The comparison of the blood lipid levels in homozygous WHHL rabbits with blood lipid levels in heterozygous WHHL and normolipidemic rabbits of different strains was performed using the analysis of variance followed by Dunnett's test. Sex differences in blood lipids in homozygous WHHL rabbits were analyzed by Student t-test. The effects were considered significant for p values less than 0.05. All statistical analysis were performed using Statistical Analysis System (SAS) software.

Table 1. Changes in blood lipids (mean ± SD) in homozygous WHHL rabbits from 11 to 23 weeks of age.

		Age in weeks			
Blood lipids	Number	11	15	19	23
Total cholesterol					
Males	36	23.5 ± 4.0	23.1 ± 4.5	24.9 ± 5.9	24.4 ± 5.2
Females	31	24.0 ± 2.9	25.7 ± 5.1†	28.0 ± 5.1★†	25.7 ± 5.0
Triglycerides					
Males	36	5.78 ± 2.78†	4.87 ± 1.81	5.61 ± 2.03	4.42 ± 1.77◊
Females	31	4.61 ± 1.49	4.49 ± 1.17	5.21 ± 1.27◆	3.94 ± 0.93

★ $p<0.05$ different from the level at 11 weeks of age within the same sex.
◊ $p<0.05$ different from the levels at 11 and 19 weeks of age within the same sex.
◆ $p<0.05$ different from the levels at other ages within the same sex.
† $p<0.05$ sex difference in the same age.

Table 2. Concentration of cholesterol and triglycerides (mmol/l ± SD) in lipoproteins in homozygous and heterozygous WHHL and NZW rabbits.

	Homozygous WHHL		Heterozygous WHHL		NZW	
	Females	Males	Females	Males	Females	Males
Total cholesterol	25.7 ± 5.0◆	24.5 ± 5.3 ◆	1.15 ± 0.35†	0.71 ± 0.28	1.34 ± 0.36†	0.88 ± 0.29
HDL	0.31 ± 0.16◆	0.32 ± 0.25	0.51 ± 0.11†	0.28 ± 0.12	0.66 ± 0.22	0.62 ± 0.11◆
LDL	12.4 ± 3.36◆	13.4 ± 4.83◆	0.41 ± 0.31†	0.12 ± 0.06	0.41 ± 0.32	0.20 ± 0.22
IDL	4.91 ± 1.73◆	4.50 ± 2.07◆	0.11 ± 0.09	0.11 ± 0.06	0.17 ± 0.06†	0.07 ± 0.07
VLDL	6.36 ± 2.14◆	5.67 ± 3.12◆	0.13 ± 0.18	0.20 ± 0.18	0.12 ± 0.09	0.05 ± 0.01
Number of rabbits	31	34	8	8	6	6
Triglycerides	3.95 ± 0.89◆	4.59 ± 1.82◆	0.73 ± 0.22	1.10 ± 0.58	0.47 ± 0.19	0.62 ± 0.10
HDL	0.21 ± 0.39	0.25 ± 0.45	0.27 ± 0.04	0.25 ± 0.12	0.28 ± 0.05	0.40 ± 0.10
LDL	1.71 ± 0.38◆	2.07 ± 0.42◆	0.19 ± 0.12	0.11 ± 0.04	0.08 ± 0.02	0.09 ± 0.07
IDL	0.76 ± 0.26◆	0.86 ± 0.42◆	0.05 ± 0.04	0.07 ± 0.02	0.05 ± 0.02	0.06 ± 0.04
VLDL	1.30 ± 0.69◆	1.54 ± 1.23◊	0.22 ± 0.09	0.66 ± 0.45	0.16 ± 0.25	0.09 ± 0.03
Number of rabbits	30	34	6	7	6	6

◆ $p<0.05$ different from values in two other strains.
† $p<0.05$ sex difference within the same strain.
◊ $p<0.05$ different from value in NZW.
Mean recovery of cholesterol (% ± SD): 97.50 ± 7.41.
Mean recovery of triglycerides (% ± SD): 102.95 ± 12.67.

Results

Plasma cholesterol did not change significantly in homozygous WHHL males from 11 to 23 weeks of age but their triglyceride at 23 weeks was significantly lower than at 11 and 19 weeks of age. No significant age related changes in plasma lipids were recorded for females from 11 to 23 weeks of age apart from a transient increase in plasma cholesterol and triglycerides at week 19 of age. Females had plasma cholesterol significantly higher than males at 15 and 19 weeks of age and triglycerides significantly lower than males at 11 week of age. No sex difference in blood lipids was recorded at 23 weeks of age (Table 1).

The concentration of plasma cholesterol and triglycerides in lipoproteins of these homozygous WHHLs, 4 females and 4 males 6 months old and 4 males and 4 females 18 months old heterozygous WHHLs and 6 females and 6 males NZWs are shown in Table 2. As no statistically significant difference in blood lipids and their concentrations in lipoproteins was found for heterozygous WHHL females and males of the two age groups their results were pooled. In homozygous WHHL rabbits no significant sex difference in plasma cholesterol and triglycerides in lipoproteins was recorded. About 50% of their total cholesterol and 44% of triglycerides were present in LDL. The homozygous WHHLs had significantly higher levels of plasma cholesterol and triglycerides compared to heterozygous WHHLs and NZWs due to significantly elevated levels of LDL, VLDL and IDL. The HDL cholesterol of homozygous females was significantly lower than in females from the two other strains which had comparable levels of this fraction. The HDL cholesterol of homozygous and heterozygous males was found significantly lower than in NZW males. The concentration of HDL triglycerides in the three rabbit strains were identical from statistical point of view. In heterozygous WHHL rabbits females had significantly higher total, HDL and LDL cholesterol compared to males. In NZW rabbits females had significantly higher total and IDL cholesterol compared to males. No sex difference was seen in concentration of triglycerides in heterozygous WHHL and NZW rabbits.

In Table 3 the results on blood lipids in adult homozygous and heterozygous WHHL and normolipidemic rabbits of different strains are compiled. The blood lipids of homozygous WHHLs were significantly higher than in heterozygous WHHLs and

Table 3. Total cholesterol and triglycerides (mmol/l ± SD) in grown up homozygous and heterozygous WHHL and normolipidemic rabbits.

Rabbit strain	Number	Total cholesterol	Triglycerides
WHHL			
Homozygous			
Females	43	25.30 ± 5.46★	3.91 ± 1.23★
Males	39	24.58 ± 5.02★	4.70 ± 1.96★†
Heterozygous[a]			
Females	47	1.38 ± 0.37†A	0.71 ± 0.47 AB
Males	23	0.68 ± 0.28 B	1.40 ± 0.58†A
NORMOLIPIDEMIC			
NZW			
Females	40	1.31 ± 0.55†A	0.79 ± 0.26 A
Males	36	0.97 ± 0.44 A	0.80 ± 0.43 B
Chinchilla (Chbb:CH)			
Females	40	1.30 ± 0.35†A	0.69 ± 0.27 AB
Males	84	0.96 ± 0.38 A	0.65 ± 0.35 B
Russian			
Females	15	1.32 ± 0.21†A	0.85 ± 0.44†A
Males	45	1.03 ± 0.31 A	0.60 ± 0.28 B
Hasen			
Females	20	1.05 ± 0.45†B	0.53 ± 0.22 B
Males	20	0.37 ± 0.10 C	1.19 ± 0.54 †A

★ $p<0.05$ different from values in heterozygous WHHL and normolipidemic strains within the same sex.
† $p<0.05$ sex difference within the same strain.
[a] triglycerides were measured in 34 females and 10 males only.
Means with the same letter are not statistically significantly different within the same sex.

normolipidemic rabbits. The blood lipids of heterozygous WHHL and normolipidemic rabbits were comparable. In heterozygous WHHLs and other normolipidemic rabbit strains females had significantly higher plasma cholesterol compared to males. Sex differences in plasma triglycerides were found for heterozygous WHHLs, Russian and Hasen strains.

Discussion

Changes in plasma cholesterol in homozygous WHHL rabbits

Previous reports have demonstrated an age dependent decrease in blood lipid levels in spontaneously hyperlipidemic rabbits (*Watanabe 1980, Lind et al. 1990*). However, the decrease either does not occur (*Watanabe 1980, nota bene* that the sex of animals was not stated) or is minimal in males (*Lind et al. 1990*) during the first 6 months of life. Thus, the lack of significant changes in plasma cholesterol of homozygous WHHL rabbits of both sexes from our breeding colony from 11 to 23 weeks of age is in accordance with the previous reports. Concerning the sex difference in plasma cholesterol, *Lind et al. (1990)* demonstrated in spontaneously hyperlipidemic rabbits a higher plasma cholesterol in females than in males from the 22nd to the 52nd week of life. In our colony, no sex difference in the plasma cholesterol levels was recorded in 23 weeks old and adult homozygous WHHL rabbits (Table 1 and 3). However, a higher female plasma cholesterol level was recorded at the 15th and 19th week of age. Thus, it may be concluded that the sex difference in plasma cholesterol was transient.

Changes in plasma triglycerides in homozygous WHHL rabbits

Watanabe (1980) demonstrated for homozygous WHHL rabbits that their plasma triglyceride values were markedly lower at 6 months of age than at 1 months of age. *Lind et al.* (1990) demonstrated a marked decrease in male triglyceride levels from 10 to 16 weeks of age and a lack of significant changes from 16 to 40 weeks of age. For female triglyceride levels, *Lind et al.* (1990) reported a slight decrease from 10 to 16 weeks of age followed at first by an increase with the maximum value at 28 weeks of age and then a decrease up to 40 weeks of age. In the present study, the female triglycerides significantly but transiently increased at 19 weeks of age while the triglycerides in the males significantly decreased at 23 weeks when the values are compared to those at 11 and 19 weeks of age. Thus our findings on triglyceride changes in males are comparable to those reported by *Watanabe* (1980).

Blood lipid levels in lipoproteins in homozygous WHHL rabbits

The concentration of blood lipids in the lipoproteins of homozygous WHHl rabbits from our colony are in accordance with previous reports (*Havel et al. 1982, Goldstein et al. 1983*). Our results confirmed that the hyperlipidemia is mainly associated with increased LDL levels when compared to normolipidemic NZW rabbits. Furthermore, the homozygous WHHL rabbits also have significantly increased levels of VLDL and IDL whereas the HDL levels are relatively reduced.

Comparison of data for homozygous WHHL animals with those of heterozygous WHHL and normolipidemic rabbits

The comparison of blood lipid levels in adult homozygous WHHL, heterozygous WHHL and normolipidemic rabbits of different strains revealed significantly higher plasma lipid values in homozygous WHHL animals. The values of plasma cholesterol were 20-fold and of triglycerides 3- to 8-fold higher than in heterozygous and normolipidemic rabbits. Thus the plasma cholesterol level in the homozygous WHHLs in our colony is higher than the reported by *Watanabe* (1980). Our data also showed lack of a statistically significant difference in blood lipids between heterozygous WHHL and normolipidemic rabbits. This is in accordance with the report of *Esper et al.* (1993) but in contrast with the report of *Goldstein et al.* (1983). The significantly higher plasma cholesterol in heterozygous WHHL and normolipidemic rabbit females than in males is furthermore, in accordance with the report of *Roberts et al.* (1974) for normolipidemic rabbits.

In conclusion, our determinations of blood lipids in homozygous WHHL rabbits from 11 week up to 23 week old rabbits demonstrated only transient changes in blood lipid levels. However, these transient changes should be kept in mind when designing intervention studies in the first 6 months of life of homozygous WHHL rabbits. The use of an equal number of females and males in all experimental groups and a higher number of animals per group is recommended as it may facilitate the interpretation of the effects of an intervention on blood lipid levels. This may prevent a possible misinterpretation of the obtained data. Furthermore, the present study confirmed that the major carrier of plasma lipids in homozygous WHHL rabbits from our colony was LDL, that plasma lipids of adult homozygous WHHL rabbits were significantly higher than those of heterozygous WHHL and normolipidemic rabbits. Finally, the blood lipid levels of heterozygous rabbits are comparable to those in normolipidemic rabbits.

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Summary

The aim of this study was to examine blood lipids in homozygous WHHL rabbits from 11 to 23 weeks of life and to examine blood lipids of adult WHHL and normolipidemic rabbits for strain and sex differences. In homozygous WHHL males plasma cholesterol did not change significantly from the 11th to 23rd week of age but their triglyceride level at 23 week of age was significantly lower ($p<0.05$) than at the 11th and 19th week of age. No significant changes with age were recorded for females apart from a transient increase in plasma cholesterol and triglycerides at the 19th week of age ($p<0.05$). The females had plasma

cholesterol significantly higher than males at the 15th and 19th weeks of age ($p<0.05$) and the female triglyceride level was significantly lower than in males at the 11th week of age ($p<0.05$). No sex difference was seen in blood lipids of 23 weeks old and adult homozygous WHHLs. The adult homozygous WHHL rabbits had significantly higher levels of plasma cholesterol and triglycerides compared to heterozygous WHHLs and normolipåidemic rabbits due to significantly elevated LDL, VLDL and IDL ($p<0.05$). Blood lipids of adult heterozygous WHHL and normolipidemic rabbits of different strains were comparable. The heterozygous WHHL females and females from the normolipidemic strains had a higher plasma cholesterol level than males.

Sammendrag

Formålet med dette forsøg var at monitorere blodlipiderne hos homozygote WHHL kaniner fra egen avlskoloni fra 11 til 23 ugers alderen, at sammenligne blodlipiderne hos voksne WHHL og normolipidemiske kaniner og at undersøge blodlipiderne hos disse stammer for eventuelle sex- og stammeforskelle. Plasmakolesterol hos homozygote WHHL hankaniner ændrede sig ikke signifikant fra 11 til 23 ugers alderen mens plasma triglycerid ved 23 ugers alderen var signifikant lavere ($p<0.05$) sammenlignet med niveauet ved 11 og 19 ugers alderen. Hos homozygote WHHL hunkaniner sås ingen signifikante ændringer i niveauerne af blodlipider undtagen en forbigående forhøjelse ved 19 ugers alderen ($p<0.05$). Plasmakolesterol hos de homozygote WHHL hunkaniner var signifikant højere ved 15 og 19 ugers alderen ($p<0.05$) og plasmatriglycerid signifikant lavere ved 11 ugers alderen ($p<0.05$) end hos de homozygote WHHL hankaniner. Der sås ingen kønsforskel i blodlipiderne ved 23 ugers alder og hos de voksne homozygote WHHL kaniner. Plasmakolesterol og triglycerid hos homozygote WHHL kaniner af begge køn var signifikant højere end hos heterozygote WHHL kaniner og normolipidemiske kaniner som følge af signifikant forhøjede niveauer af LDL, VLDL og IDL ($p<0.05$). Blodlipider hos de voksne heterozygote WHHL kaniner og voksne kaniner fra normolipidemiske stammer var sammenlignelige. De heterozygote WHHL og normolipidæmiske hunkaniner havde højere plasmakolesterol sammenlignet med hankanincrne.

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