Effects of Oxidised Dietary Cod Liver Oil on the Reproductive Functions of Wistar Rat

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Abstract

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Weanling Wistar rats, males and females, were fed for 185 days with diets containing 15% of dietary fat in the form of a mixture of lard and partially oxidised cod liver oil. The proportion of cod liver oil in the dietary fat ranged from 0 to 100%, and the content of malondialdehyde from 0.3 to 19.6 mg/kg of the fat used. Animals fed with diets containing higher proportions of oxidised cod liver oil had higher concentrations of malondialdehyde in their livers. Serum lipid levels were lower in animals fed with higher proportions of cod liver oil than in animals fed control diets (milk fat or lard). The lowest concentration of serum lipid was found in the rats fed the diet containing half of its fat as fish oil. Increased intakes of cod liver oil resulted in lower body weight gains, weights of livers, kidneys, and weights of the reproductive organs. The relative weights of livers and kidneys/body weight were higher in the groups with higher intakes of cod liver oil. High intakes of cod liver oil led to a drastically impaired fertility of females, a decreased litter size, a higher postnatal mortality, and an increased incidence of morphologically abnormal spermatozoa in males.

Keywords: oxidised cod liver oil; rat; fertility; reproduction; organ weights

In recent years, there has been a rapid expansion of interest in the effects of cod liver oil on various physiological functions of animals (Abayasekara & Wathes 1999). Many studies in humans and experimental animals have focused on the effects of cod liver oil on serum lipids (Saynor *et al*. 1984), cholesterol, and lipoproteins, and on its role in atherogenesis and thrombogenesis (Ross 1986). These studies show that cod liver oil influences lipid metabolism in several ways that may be of value in the treatment and prevention of cardiovascular disease and inflammatory disorders (Simopoulos 1989). The mechanism by which cod liver oils confer their benefits are not fully understood (Din *et al*. 2004).

Polyunsaturated fatty acids (PUFA) found in marine cod liver oil, mainly eicosapentaenoic acid (20:5 3; EPA) and docosahexaenoic acid (22:6 3; DHA), proved their desirable effects, however, they are also extremely prone to autooxidation. It is well known that lipid peroxidation generates a variety of aldehydic compounds (Esterbauer *et al*. 1988) which are biologically very active and which can promote multiple effects on the cell function and viability (Esterbauer 1985; Benedetti & Comporti 1987).

The autooxidation of polyunsaturated fats occurs at ambient and even subambient temperatures; consequently, stored cod liver oil without a proper antioxidant contains certain amounts of its oxida-

tion products (Fritsche & Johnston 1988) which may have adverse effects on the consumers. A high consumption of rancid cod liver oil may result in yellow fat disease which is characterised by steatitis, hemolytic anemia, and myocardial fibrosis (Sanders 1986). Biological effects of low intakes of oxidised cod fish oil are not so well described but malondialdehyde, one of the many carbonyl compounds derived from oxidation of PUFA which is found in the diet as well as in the tissues, has been reported to be mutagenic in bacterial and mammalian cells. Malondialdehyde is also known to inactivate enzymes by the formation of intra- and inter-molecular protein crosslinks and to exhibit a range of nucleotoxic effects. Therefore, adverse effects of even a low consumption should be expected.

The reproductive performance of an animal is one of the most sensitive indicators of the effects of toxic compounds but the causes of the adverse effects are multiple and not fully understood. It is generally accepted that mutagens can adversely affect reproduction (McBee & Bickham 1990).

The effects resulting from the exposure to exogenous agents differ with the period of the reproductive cycle which may be divided for this purpose into three periods: the formation of gametes, gestation, and postnatal life. Adverse effects on gametogenesis may result in reduced fertility or even sterility due to morphological, biochemical, or physiological disturbances. The gestation period may be lengthened, shortened, or interrupted, thus leading to stillbirth or abortion due to intoxication. Exogenous toxic compounds may influence maternal behaviour, hormonal balance, and nutrition, which may then affect the survival and the physical and functional development of the newborn (Baeder *et al*. 1985).

In the present multigeneration study, we evaluated the effects of partially oxidised cod fish liver oil on pubertal development and on the reproductive function in the male and female Wistar rat.

MATERIALS AND METHOD

Animal experiments and diets. Seventy two male and female weanling Wistar rats (Ipcv; WlST) weighing 45–68 g were housed in groups of 3 animals of the same sex in plastic cages (BEM, Prague), in a temperature and light controlled room (21–23°C; 12 h light-dark cycle). These animals were randomly assigned to one of six experimental groups and fed a control natural diet DOS 2b (BEM, Prague) based on cereals, or a semipurified experimental diet derived from AIN-76 diet (American Institute of Nutrition, 1977) based on sucrose. The control diet contained 5% fat. Experimental diets contained 15% fat which had been prepared as a mixture of cod liver fish oil (FO) and lard (LA) in the ratios of LA:FO 100:0 (diet A), 75:25 (B), 50:50 (C), 25:75 (D),

Table 1. Composition of the diets

Component	Content (g/kg)
Ground wheat	600
Milk	130
Casein	130
Wheat bran	90
DOS-1-komplex-stabil	50
Sucrose	200
Casein	200
Starch	350
Cellulose	50
Fat	150
Vit. mineral mix	50

– fat content 28%-extracted (fat and vitamin free)
– potato starch
– mixture of fish oil (FO) and lard (LA) in a ratio FO:LA 0:100 (diet A), 25:75 (B), 50:50 (C), 75:25 (D), 100:0 (E)
– vitamin content in 1 kg: thiamin 240 mg, riboflavin 160 mg, pyridoxin 80 mg, cyanocobalamin 0.6 mg, E 2 g, A 30 mg, D 2.5 mg, folic acid 120 mg, Ca-pantothenate 160 mg, inositol 2 g. Mineral content in 1 kg: calcium carbonate 84 g, calcium phosphate 221 g, sodium chloride 150 g, ferrous sulfate 32 g, cupric sulfate 392 mg, zinc sulfate 440 mg, manganous carbonate 2 g, potassium iodide 26 mg (Velaz, Prague. Czech)
– vitamin content in l kg of diet: thiamin 370 mg, riboflavin 282 mg, pyridoxin 200 mg, cyanocobalamin 0.8 mg, E 2.5 g, A 36 mg, D 2.5 mg, K 1.5 mg, folic acid 131 mg, Ca-pantothenate 1.0 g, inositol 4.3 g, biotin 5 mg, nicotinic acid 1.3 g, choline chloride 23 g, L-methionine 72 g. Mineral content in 1 kg: calcium carbonate 92 g, calcium phosphate dibasic 340 g, sodium chloride 164 g, ferrous sulfate 35 g, cuprous sulfate 427 mg, zinc sulfate 479 mg, manganous carbonate 207 ng, potassium iodide 29 mg, magnesium sulfate heptahydrate 196 g, potassium chloride 166 g, sodium selenite 8 mg, sodium fluoride 22 mg, potassium chromate 109 mg

and 0:100 (E). These diets were prepared twice a month, packed as daily portions in polyethylene bags, and stored at –28°C. The composition of the diets is given in Table 1.

The animals were fed the diets for 60 days and then were mated. During the mating period, each male was housed with one female of the same dietary group for maximum of 7 days. Mated female rats were removed and placed in individual cages to allow for the normal delivery and rearing of offspring. The young animals were weaned at 30 days of age and then placed in groups of three of the same sex and diet into the plastic cages as described above. Food intakes were measured daily on a cage (three rats) basis and the body weights were monitored throughout the experimental period. At the end of the experiment (at the age of 185 days for the parental generation and at the age of 65 and 95 days for progeny), the animals were left to fast for 12 h and then anaesthetised with Thiopental (Spofa, Prague: 50 mg/kg animal weight intraperitoneally). Blood samples for the lipid analyses were taken from the inferior vena cava, dotted (1 h glass tubes at 4°C) and centrifuged at 2900 rev/min for 15 min. The contents of total lipids, triacylglycerols, and cholesterol were determined immediately.

Table 2. Main fatty acid composition of dietary fats (weight % total fatty acids)

Fatty acid	DOS	Fish oil	Lard
14:0	8.7	8.6	1.9
16:0	27.5	20.4	26.3
16:1	3.0	10.2	3.2
18:0	9.7	3.3	12.4
c 18:1	24.3	12.8	43.2
t 18:1	1.3	–	–
c 18:2	18.1	14.1	12.8
18:3	3.4	–	–
20:0	–	2.3	–
c 20:1	0.6	3.1	–
20:2	3.7	–	–
20:5	–	13.8	–
22:1	–	1.6	–
22:6	–	9.8	–

c – cis isomer; t – trans isomer; – not detected

Diet analysis. The diets were analysed for their fatty acid profiles and for the contents of peroxides and aldehydes. Fatty acids were determined by reversed phase high performance liquid chromatography (RP-HPLC) with UV detection (Hanis *et al*. 1988) after lipid extraction by the method of Folch *et al*. (1957) and derivatisation of fatty acids with phenacyl bromide (Wood & Lee 1983). Fatty acid profiles are shown in Table 2.

Peroxide value (PV) of the dietary fats was determined by iodometry with 0.01N $Na_2S_2O_3$ (AOCS 1973); the content of malondialdehyde was determined by spectrophotometry with thiobarbituric acid (TBA) (Davídek *et al*. 1977).

Biochemical analysis. Total serum lipids, triacylglycerols, and cholesterol were determined using the prepacked kits Bio-La-Test (Lachema, Brno). Total lipids were analysed spectrophotometrically (after hydrolysis with H_2SO_4 followed by the reaction with phosphovanilic acid) at 530 nm (Chromy *et al*. 1975). Triacylglycerols were determined by spectrophotometry at 410 nm after saponification, oxidation to formaldehyde, and the reaction with acetylacetone (Chromy *et al*. 1977). Total cholesterol was determined by the reaction with 2,5-dibenzensulfonic acid followed by spectrophotometry at 580 nm (Hornakova *et al*. 1974).

Body and organ weights. Livers, kidneys and testicles, epididymis and seminal vesicles or uteris, ovaries and oviducts were removed, washed in cooled saline (9 g sodium chloride per l) and wet weighed.

Determination of malondialdehyde in rat liver. Livers were homogenised in a glass homogeniser at 4°C. Two-gram samples were then thoroughly mixed with 5 ml of 5% trichloracetic acid (TCA) (Lachema, Brno) and centrifuged at 2500 rev/min in a Janetzki K 235 centrifuge (Janetzki, Jena, Germany) for 10 min. An aliquot of the supernatant was mixed with an equal volume of 1% TBA solution in a screw-cap tube and heated in a boiling water bath for 30 min. After cooling to room temperature, the absorbance of the pink supernatant was immediately measured at 532 and 450 nm on Specol spectrophotometer (Zeiss, Jena). A standard curve was prepared with the use of malondialdehyde bis (diethyl acetal) (Aldrige, Milwaukee. Wl) (Davídek *et al*. 1977).

Reproduction tests. The percentage of pregnant females (after maximum of 7 days of mating period) and the litter size and survival of weanlings at day 30 were recorded for each nest and dietary

group. In the offspring, the age of vaginal opening was determined by daily observations. The age of the first estrus and regularity or the estrous cycle were determined from daily vaginal lavages. Rats with three consecutive regular, 4 d, estrous cycles were considered as regular-estrous cycle rats. The length of pregnancy was calculated from the first day of spermatozoa presence in the vaginal lavage (day 0).

The morphological abnormality of spermatozoa, taken from seminal vesicles, was determined in males of parental generation at the age of 185 days and in the offspring at the age of 65 and 95 days by staining with (Giemsa-Romanowski reagent (Washington *et al*. 1983); 400 sperms were used for the mean value calculations.

Statistical analysis. The Student-Newman-Keuls multiple-range test (Miller 1981) was used for the evaluation of the significance of the differences between the experimental groups.

RESULTS

Diets

Significant differences were observed between the dietary fats in the peroxide values and the concentrations of malondialdehyde. The highest values were found in diet E (100% FO), and the lowest values were in diet A (100% LA) Table 3.

Food intakes

Food intakes differed significantly between rats fed diets D and E (75% and 100% of cod liver oil, oxidised at temperature 20°C under normal storage conditions in the dietary fat) and the rats fed either control DOS diet or diets containing only lard (produced by classical smelting technology (Pipek 1994) (A), or 25% (B) or 50% (C) of cod liver oil in their dietary fat. The food intake combined for males and females in DOS, A, B, and C groups was about 17.3 g/day (SE 1.3), in diets D and E it was about 12.6 g/day (SE 1.6). The calculated daily cod liver oil intake based on the feed consumption was thus about 0.64 g in group B, 1.28 g in group C, 1.46 g in group D, and 1.95 g in group E, respectively.

Mean body and organ weights

Mean body weights at the end of 185-day experimental period were significantly lower in animals fed with the diets containing higher proportions of cod liver oil than in animals fed DOS diet or diets with lard or lower proportions of cod liver oil. Similarly, the weights of hearts, kidneys, and livers were lower in animals fed higher concentrations of cod liver oil. Significantly lower weights were observed in animals fed diets D and E, i.e. with 75% and 100% of cod liver oil in the dietary fat, as compared with animals fed the other diets. The diferences between the body organs weights of animals fed DOS diet, diet A (100% lard) and diets B and C (25% and 50% of cod liver oil in dietary fat) were not significant.

The weights of livers and kidneys in rats fed diets D and E were significantly higher than in rats on diets DOS, A, B, and C. The weights of hearts in the dietary group E were also significantly higher then those of rats in the other groups (Table 4).

Serum levels of lipids

Significantly lower concentrations were found of serum total lipids (TL), triacylglycerols (TG), and cholesterol (CH) in the rats fed diets D and E, containing 75% and 100% cod liver oil (FO) in their dietary fat, as compared with the rats on diets DOS, A (0% FO) and B (25% FO). The rats in the dietary group C (50% FO) exhibited significantly

Table 3. Autoxidation indicators of dietary fats (mean values with their standard errors)

		Dietary group					
		DOS	A	B	C	D	E
PV	Mean	13.6	1.0	53	202	310	352
	SE	1.27	0.16	2.4	8.0	11.4	10.0
TBA	Mean	1.1	0.3	3.2	10.8	15.4	19.6
	SE	0.1	0.1	0.1	0.5	0.6	0.9

PV – peroxide value (meqv/kg); TBA – concentration of malondialdehyde (mg/kg); mean values of 18 analyses

Table 4. Body and organ weights (g) of male and famale Wistar rats. Parental generation 185 days (mean values with their standard errors)

		Dietary group					
		DOS	A	B	C	D	E
	n	12	12	12	11	12	9
Male							
Bodyweight	Mean	475	525*	484	415*	259*	152*
	SE	3.3	4.2	4.0	3.6	3.8	3.5
Heart	Mean	1.46	1.52*	1.43	1.45	0.88*	0.76*
	SE	0.03	0.03	0.04	0.02	0.02	0.03
	% wt	0.31	0.29	0.30	0.35	0.34	0.50
Liver	Mean	18.2	19.4	18.5	16.4*	12.6*	10.2*
	SE	0.4	0.4	0.2	0.3	0.2	0.4
	% wt	3.83	3.70	3.82	3.95	4.86	6.71
Kidney	Mean	3.2	3.3	3.0	3.1	2.0*	1.5*
	SE	0.05	0.05	0.06	0.06	0.03	0.05
	% wt	0.67	0.63	0.62	0.75	0.77	0.99
Female							
Body weight	Mean	321	313	296	267	180*	123*
	SE	3.8	3.3	4.2	3.6	3.1	3.0
Heart	Mean	0.95	0.91	3.03	0.81	0.63*	0.56*
	SE	0.02	0.03	0.03	0.02	0.02	0.02
	% wt	0.30	0.29	0.26	0.30	0.35	0.46
Liver	Mean	15.8	15.4	14.3	14.9	10.3*	9.8*
	SE	0.3	0.4	0.3	0.3	0.2	0.2
	% wt	4.92	4.92	4.83	5.58	5.72	7.97
Kidney	Mean	2.6	2.4	2.5	2.4	2.0*	1.7*
	SE	0.06	0.05	0.07	0.06	0.07	0.06
	% wt	0.80	0.77	0.84	0.90	1.11	1.38

n – number of animals; wt – relative organ/body weight

*Mean values in the same horizontal row were significantly different from other values ($P < 0.01$)

lower concentrations of total cholesterol than the rats in any other group (Table 5).

Content of malondialdehyde in rat liver

There were significant diferences in malondialdehyde content in rat livers between the rats fed diet A (100% lard in dietary fat), the rats fed diet DOS or B (25% cod liver oil in dietary fat), the rats fed diets C and D (with 50% and 75% of cod liver oil) and diet E (100% of cod liver oil). The lowest concentration of malondialdehyde was found in the livers of the rats fed the experimental diet A, the highest one in the rats fed diet E. The differences between the rats on diets DOS vs. B and C vs. D were not significant (Table 6).

Reproduction parameters

Sperm was detected in the vaginal lavage of most of the females in all dietary groups, even though many of the parental generation females in the dietary group E were acyclic. In dietary groups DOS, A, B, C, and D, 85–100% females became pregnant

Table 5. Serum levels of total lipids (TL), triacylglycerols (TA) and cholesterol (CH) in Wistar rats at the age of 185 d (mean values with their standard errors)

			Dietary group					
			DOS	A	B	C	D	E
Total lipids (mg/dl)	Males	*n*	12	10	11	12	7	8
		Mean	238	255	212	167*	139*	142*
		SE	13.7	9.9	13.5	8.8	6.9	7.3
	Females	*n*	10	11	12	12	11	10
		Mean	202	224	210	133*	123*	120*
		SE	8.3	10.9	7.3	7.4	11.4	11.0
Triacylglycerols (mg/dl)	Males	*n*	12	12	12	11	12	11
		Mean	83	96	80	43*	35*	47*
		SE	2.2	1.8	3.3	3.6	3.7	3.3
	Females	*n*	11	12	12	10	8	9
		Mean	65	73	67	53	31*	38*
		SE	2.8	2.8	2.6	1.4	2.9	2.3
Cholesterol (mg/dl)	Males	*n*	10	12	9	11	8	9
		Mean	79.3	62.7	54.1	29.5*	34.3*	39.2*
		SE	2.3	2.6	2.4	3.1	3.1	2.7
	Females	*n*	9	9	12	11	7	8
		Mean	73.1	58.6	49.0	28.9**	35.4*	43.1*
		SE	2.1	1.9	1.7	1.5	3.0	2.7

n – number of samples

*Mean values in the same horizontal row were significantly different from values without ($P < 0.01$)

Table 6. Content of malondialdehyde (μg/g) in rat livers; parental generation 185 days (mean values with their standard errors)

		Dietary group					
		DOS	A	B	C	D	E
Males	*n*	12	12	12	11	12	9
	Mean	2.3	1.1	1.8	4.3*	4.3*	6.2*
	SE	0.4	0.2	0.2	0.7	0.8	1.1
Females	*n*	12	12	11	12	10	8
	Mean	2.5	0.9	1.3	4.6*	5.1*	6.0*
	SE	0.4	0.3	0.4	1.1	1.3	1.5

n – numbers of animals

*Mean values in the same horizontal row were significantly different from values in groups DOS, A and B ($P < 0.01$)

during one estrous cycle (4 days). A significantly lower percentage of pregnancy (60%) was observed in the females fed diet D containing 75% cod liver oil in its dietary fat. In the group E in which all the dietary fat was cod liver oil, no female became pregnant.

The length of pregnancy of females in the parental generation was 22–23.5 days with no difference in any of the group. An increase of the proportion of cod liver oil in dietary fat resulted in a decreased litter size. While no significant dif-

Table 7. Reproductive parameters of female Wistar rats (mean values with their standard errors)

		Dietary group					
		DOS	A	B	C	D	E
Litter size	*n*	12	12	11	10	7	–
	Mean	9.2	9.8	9.0	8.7	6.3*	0
	SE	1.1	0.5	0.8	1.1	1.1	
% of pregnant dams	Mean	100	100	90	85	60*	0
Number of weanlings/ damonday 30	*n*	12	12	11	10	7	–
	Mean	8.2	8.3	7.6	5.8*	3.2*	0
	SE	0.7	0.6	0.6	0.8	0.7	–
	in %	89	85	84	67	51	0
Age of vaginal opening (days) – progeny	*n*	30	33	33	24	13	
	Mean	32	32	34	39*	40*	–
	SE	0.3	0.2	0.3	0.4	0.4	

n – number of animals in a group

*Mean values in the same horizontal row were significantly different from values in groups DOS, A and B ($P < 0.01$)

ferences existed between the dietary groups DOS, A, B, and C in the number of live born young, an increase in the cod liver oil content in the diets D and E (75% and 100% FO) led to a significant decrease in the number of live born offspaings, a decrease in female fertility, and in the group E to complete infertility.

The number of the young born/nest was significantly lower for the females fed diet D (6.3 pups per nest) and diet E (0 pups/nest) in comparison with all the other groups (8.7–9.8). In the females born to mothers in dietary groups D and E, the vaginal opening was significantly delayed (d 39–40) as compared with the females in the control DOS

Table 8. Male reproductive organs weights and sperm morphology (parental generation) (mean values with their standard errors)

		Dietary group					
		DOS	A	B	C	D	E
	n	12	12	12	11	12	9
Testis (g)	Mean	3.2	3.6	3.5	3.5	3.3	2.0*
	SE	0.3	0.1	0.1	0.1	0.2	0.2
Epididymis (g)	Mean	1.55	1.67	1.49	1.38	1.42	0.79*
	SE	0.23	0.10	0.14	0.14	0.18	0.12
Seminal vesicles (g)	Mean	1.86	1.91	1.98	1.70	1.78	0.92*
	SE	0.26	0.13	0.20	0.22	0.18	0.22
Abnormal sperm head morphology (%)	Mean	4.9	4.6	4.6	4.8	8.3*	24.5*
	SE	0.8	1.1	0.9	0.9	1.6	1.8

n – number of animals in a group

*Mean values in the same horizontal row were significantly different from other values ($P < 0.01$)

and diet A groups (d 32) and C dietary group (d 34) (Table 7).

The mean litter weight of the young at birth was not affected by the diets. However, a significant depression of neonate body weight was noted after 30 days of lactation in groups of animals on diets with the higher proportion of cod liver oil. An increased intake of cod liver oil resulted also in an increase in postnatal mortality. Most of the progeny deaths occurred within several days after birth and very few occurred later than two weeks after the birth. The highest mortality rate in the groups D (49%) and C (33%) occurred between the 5^{th}–14^{th} day postpartum.

A high content of cod liver oil in diet E (100% FO) caused a significant reduction in the weight of testicles, epididymis, and seminal vesicles in the males of the parental generation. In addition, the qualitative analysis of spermatozoa from cauda epididymis revealed major abnormalities (24.5%) in the sperm head morphology in the males fed this diet as compared with the males fed DOS diet (4.9% abnormal spermatozoa) or experimental diets A (4.6%), B (4.6%), and C (4.8%). In the males on diet containing 75% of cod liver oil in dietary fat, morphologically abnormal sperm heads were observed in 8.3% (Table 8).

The reproductive organs in the progeny rats were weighed on the 65^{th} and 95^{th} day of age. In all the progeny fed experimental diets B, C, D (with 25–75% FO in the dietary fat), the weights of the reproductive organs of the males and the females were significantly lower than the weights of the respective organs in the animals fed diet DOS and diet A (100% lard as the only dietary fat). The reproductive organ weights decreased with increasing proportion of cod liver oil in the experimental diets as shown in Tables 9 and 10.

Table 9. Progeny male reproductive organs weights and sperm morphology (mean values with their standard errors)

		Dietary group					
		DOS	A	B	C	D	E
	n	12	10	12	10	6	0
Testis (g)	Mean 65 d	3.0	2.9	1.5*	1.4*	0.4*	–
	SE	0.2	0.1	0.2	0.2	0.1	
	Mean 95 d	3.3	3.1	2.4*	2.0*	1.0*	–
	SE	0.2	0.1	0.2	0.2	0.1	
Epididymis (g)	Mean 65 d	0.93	0.87	0.49*	0.40*	0.12*	–
	SE	0.10	0.09	0.05	0.05	0.02	
	Mean 95 d	1.28	1.32	0.96*	0.53*	0.28*	–
	SE	0.22	0.18	0.18	0.10	0.05	
Seminal vesicles (g)	Mean 65 d	1.12	1.14	0.70*	0.27*	0.10*	–
	SE	0.08	0.06	0.06	0.03	0.02	
	Mean 95 d	1.39	1.41	1.22	0.56*	0.12*	–
	SE	0.23	0.26	0.18	0.12	0.02	
Abnormal sperm head morphology (%)	Mean 65 d	4.8	4.9	8.6*	#	#	–
	SE	0.7	0.6	1.3			
	Mean 95 d	4.0	3.9	5.7	5.6	#	
	Se	0.9	0.7	1.1	1.3		

n – number of animals in a group; d – age of the animals in days; # – more than 80% of spermatozoa morphologically abnormal

*Mean values in the same horizontal row were significantly different from other values ($P < 0.01$)

Table 10. Progeny female reproductive organs weights (mean values with their standard errors)

		Dietary group					
		DOS	A	B	C	D	E
	n	12	12	12	10	6	–
Uterus (mg)	Mean 65 d	625	634	270*	197*	85*	–
	SE	43	58	18	16	13	
	Mean 95 d	647	642	344*	340*	120*	–
	SE	53	52	54	39	26	
Oviductus (mg)	Mean 65 d	28.3	30.3	15.0*	15.3*	10.2*	–
	SE	2.6	3.1	1.0	1.4	0.9	
	Mean 95 d	29.3	29.9	27.3	20.0*	12.5*	–
	SE	2.8	4.2	1.3	2.0	1.1	
Ovary (mg)	Mean 65 d	136	138	90*	42*	17*	–
	SE	17	18	16	3	2	
	Mean 95 d	141	136	93	56*	33*	
	SE	22	23	17	8	4	

n – number of animals in a group; d – age of the animals in days

*Mean values in the same horizontal row were significantly different from other values ($P < 0.01$)

In the male offspring fed diets C and D, very little sperm was found in epididymis at 65 days of age, and about 80–90% of it was morphologically abnormal. In the males of the same age in the groups fed diets DOS or A (100% LA), spermatogenesis was fully developed and morphological abnormalities occurred only in 4.8–4.9% of sperm. Males in the group fed with diet D (75% FO) at the age of 95 days were infertile.

DISCUSSION

Decreased body weight gains of Wistar rats fed diets with high proportions of oxidised cod liver oil (C, D, and E) demonstrate well that the oral intake of lipid autooxidation products is deleterious for the animal body (Kanazawa *et al*. 1989). Lower body weight gains in animals fed oxidised oils are usually accompanied by lower feed intakes (Yoshida & Kajimoto 1989). The animals in the groups D and E consumed about 73% of the diet on a daily basis compared to the groups A, B, and C, and this could also account for the lower weight gains in these groups. Fish oil intakes due to the differences in the feed consumption of animals in dietary groups C and D (1.28 and 1.46 g/d) were very close, which seems to be also confirmed by similar concentrations of malondialdehyde found in the livers of rats in these two groups. Different body weight gains of animals in groups C and D, could thus be ascribed rather to different energy/nutrient intakes than to the difference in oxidised cod liver oil consumption. The differences in the body weight gains between the groups D and E consuming about the same amounts of the diets could be ascribed to different intakes of toxic lipid peroxidation products derived from cod liver oil.

It is well documented that fish oil influences lipid metabolism (Sanders 1986; Haug & Hostmark 1987) and lipid serum levels (Suzuki *et al*. 1985), but it is not clear which effects partially oxidised fish oil may have on serum lipids (Heinecke 1987). In this experiment, feeding higher proportions of fish oil generally decreased the serum lipid level but differences between the animals fed diets D and E (75% and 100% FO) were not significant and the lowest serum cholesterol was not observed in the animals with the highest intakes of fish oil (D and E) but in the group C (50% FO). This finding correlates with the reports of Jessup *et al*. (1986) and Hoff *et al*. (1988) on a prolonged life span of aldehyde modified lipoproteins which carry cholesterol in the blood stream.

The effect of the exposure to exogenous agents differs with the period of the reproductive cycle. In reproductive toxicology studies, changes in

the formation of the gametes, in gestation, and in postnatal life are most often evaluated (Baeder *et al*. 1985). Animals in this experiment were exposed to oxidised fish oil during all three periods.

The females on all diets exhibited regular estrous cycles with the exception of the females on diet E (100% FO) where all were acyclic. This observation is similar to the report of Saigo (1990) who found acyclicity in female rats after high doses of chlorpromazine. Toxicity of aldehydes derived from fish oil peroxidation affected estral regularity only at the highest intake, so this function does not seem sensitive enough to be considered a suitable biological indicator.

The exposure of female rats to toxic compounds may generally lead to a decreased fertility (Nachtomi & Alumot 1989; Saigo 1990). In this experiment, we observed an impairment of female fertility in the dietary group D (75% FO), and the females in the group E (100% FO) were totaly infertile. These observations document a higher sensitivity of female rat fertility parameters to oxidised dietary lipids than the regularity of estrous cycles.

Exogenous agents may influence maternal behaviour, hormonal balance, and/or nutrition, which may then affect survival and physical functional development of the newborn (Baeder *et al*. 1985). Neonatal toxicity, characterised by decreased weight gains and the young mortality during lactation, was observed in the offspring in the groups C and D. Diet B (25% FO) caused only an insignificant increase in neonatal mortality compared to the dietary groups DOS and A. The observed neonatal toxicity could be related to the postnatal exposure to toxic agents derived from oxidised lipids through maternal milk and not to in utero exposure, because no significant differences existed in newborn weights between the dietary groups. The newborns in all groups were vital after the delivery and started to suck immediately. No differences in the maternal behaviour of the females in different dietary groups were observed throughout the lactation period.

The exposure of females to toxic agents may lead to a decrease of the newborn weight and an increase in postnatal mortality (Nachtomi & Alumot 1989). Our results show that oxidised cod liver oil may increase postnatal mortality even if the weight at birth is not apparently decreased. This observation is similar to the findings of (Scow *et al*. 1964) with various other toxic agents. The reason may be in increased utilisation of the depot fat during the latest stage of the gestastion period and during lactation which may lead to increased concentrations of toxic aldehydes in maternal milk (Scow *et al*. 1964).

The period of the enhanced neonatal toxicity in rats (up to approximately postpartum day 10) correlates with the completion of the blood-brain barrier in this species which, unlike that of humans, is formed postnatally (Bohr & Wolgard 1974; Betz & Goldstein 1981; Lancas *et al*. 1989). Therefore, higher toxicity of the diets with higher proportions of oxidised cod liver oil (C and D) in neonatal rats during the first postnatal week may be a direct consequence of their higher sensitivity to toxic agents in this period of development.

The age of vaginal opening in the rat is often used in developmental studies as the sole index of puberty. As the results of some studies (Gray *et al*. 1989a, b) clearly show, vaginal opening is not necessarily linked with the onset of cyclicity. In the F1 females, vaginal opening in the groups C and D was delayed 7–8 days as compared with the females in the dietary groups DOS and A. In the females on diets with 50% and 75% of the dietary fat as cod liver oil (the groups C and D), we also observed a dilayed onset of estrous cyclicity and frequent infertility caused by impaired ovulation.

A high intake of dietary cod liver oil led to a significantly decreased litter size in the dietary group D (6.3 pups/nest) as compared with the groups DOS, A, B, C, and to total infertility of the females in the group E. In the females of the parental generation on diet E, minimal level of ovulation, corpus luteum formation, and a high irregularity of estrous cycle were observed. These events depend on hypothalamic-pituitary function, however, none of the females fed more than 75% of cod liver oil became pregnant. That was probably due to a failure in fertilisation or implantation. The question still remains if those effects were a direct consequence of the inhibition of ovulation or fertilisation.

Ovarian weight was reduced in the progeny females of all groups fed diets containing any amount of cod liver oil as compared with those in groups DOS and A (100% lard). This is probably a result of the exposure of young female offspring to toxic aldehydes in the most critical period of their postnatal life (first 10 days) because in the parental females the toxic effects of high concentrations of cod liver oil in the diet were observed only in the group E fed the diet with 100% cod liver oil in dietary fat.

In males the reproductive toxicity is usually demonstrated by decreased reproductive organ weights, impairment of spermatogenesis and sexual behaviour (McBee & Bickham 1990; Ragan & Mast 1990). There is a strong correlation between testicular weight and spermatogenesis (Seethalakshmi *et al*. 1990) and excellent evidence that toxic agents decrease spermatogenesis well before decreasing testicular weight (Van Kroonenburgh *et al*. 1986; Ratnasooriya & Sharpe 1986). In our experiments, we found a significant reduction of the testicle weight in the parental generation only in the dietary group fed diet E (100% FO), which was also accompanied by a significant increase in the occurrence of morphologically abnormal sperm heads. In the males of the progeny, we found significantly lower weights of testicles, epididymis, and seminal vesicles, above all in the groups C and D. These findings correlate well with the work of Gray *et al*. (1989b) who reported similar effects in the males whose mothers were exposed to a different toxic agent (methoxychlor). The males in these groups had delayed puberty. Decreased spermatogenesis observed in the progeny males (65 d) on diets C and D is in a good agreement with the work of Krause *et al*. (1975), Sobhon *et al*. (1979) and Bint *et al*. (1985) who reported a correlation between the decreased body weight and the inhibition of spermatogenesis.

These results demonstrate a wide range of deleterious effects of partially oxidised cod liver oil on the reproductive performance of laboratory Wistar rats. The reproductive failure, above all the sperm head morphology (SHM) and the decreased viability of the young seem to be suitable bioindicators for the detection of the exposure of animals to lipid peroxidation aldehydes. It is also clear that careful precautions must be taken to prevent these effects from interfering with experimental designs in studies focused on the consumption of cod liver oil and thus to avoid misinterpretation of the experimental results.

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Souhrn

Zídková J., Sajdok J., Kontrová K., Kotrbová-Kozak A., Hanis T., Zídek V., Fučíková A. (2004): **Vliv oxidovaného rybího tuku na reprodukční vlastnosti krys (Wystar Kyoto)**. Czech J. Food Sci., **22**: 108–120.

Samci a samice krys (Wistar Kyoto) byly krmeny po odstavení po dobu 185 dnů dietou obsahující 15 % vepřového sádla a částečně oxidovaného rybího tuku. Obsah rybího tuku v dietě byl měněn v rozmezí od 0 do 100 % a obsah malondialdehydu byl v rozmezí 0,3 až 19,6 mg/kg tuku. Se zvyšujícím se obsahem oxidovaného tuku v dietě se zvyšovalo množství malondialdehydu v játrech laboratorních krys. Koncentrace serových lipidů byla nižší u zvířat krmených dietou s obsahem rybího tuku než u kontrol krmených mléčným tukem nebo vepřovým sádlem. Nejnižší koncentrace sérových lipidů byla naměřena u krys, u kterých polovinu diety tvořil rybí tuk. Zvýšený podíl rybího tuku má za následek snížení celkové hmotnosti, hmotnosti jater, ledvin a hmotnosti reprodukčních orgánů. Relativní hmotnost jater a ledvin byla vyšší u skupiny s vyšším příjmem rybího oleje. Vysoký příjem rybího oleje má za následek drastické zhoršení plodnosti samic, snížení velikosti vrhu, a zvýšení úmrtnosti po porodu a zvýšení množství morfologických změn u samců.

Klíčová slova: oxidovaný rybí tuk; krysa; plodnost; hmotnost orgánů; úmrtnost; abnormální spermie

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