

# NUTRITION OF



308 Lasani Town, Sargodha Road, Faisalabad - Pakistan Mob: +92 300 3008585, Fax: +92 41 8815544 E-mail: editorpjn@gmail.com

# The Essential Fatty Acids and the Diet of Polar Bears

Kaduce, Terry L.¹ and G. Edgar Folk, Jr.²\*

¹Department of Biochemistry, University of Iowa, College of Medicine

²\*Department of Physiology and Biophysics, College of Medicine, University of Iowa,
Iowa City, Iowa 52242, 319-335-7783, USA

e-mail: g-folk@uiowa.edu

Abstract: Plasma lipids of polar bears are significant because these bears prefer to consume high quantities of fat; furthermore one population fasts each year for over four months. In this paper plasma lipids of fed polar bears were compared to fasted bears. Fasted bears were hyperlipidemic to fed bears; both were hyperlipidemic to normal human plasma, in respect to cholesterol and triglycerides. In lipoproteins, the HDL (High Density Lipoproteins) triglyceride was very low as in human subjects in both fed and fasted animals. The other two, LDL (Low Density Lipoproteins) and VLDL (Very Low Density Lipoproteins) were consistently higher in fasted bears than in fed bears, and these fasted bears had much higher cholesterol and triglycerides than the fed bears. Since the fed bears seem to be protected against hyperlipidemia, the fatty acid composition of serum lipids was analyzed. The n-3 fatty acids not the n-6 type dominated in fed bears. These n-3 fatty acids (which were not available to fasted bears) seem to protect against high serum lipids. These results seem to support the concept of using fish oil capsules in the human clinic.

Key words: Plasma lipids, hyperlipidemia, omega-3 fatty acids, polar bear diet, fasted polar bears, lipoproteins of polar bears

### Introduction

In human subjects, elevated plasma lipid concentrations appear to predispose to the premature development of atherosclerosis (Lees and Karel, 1990). The approach of comparative physiology elucidates our understanding of this relationship. When plasma lipid is elevated artificially in the pig (Lees and Karel, 1990), the goat (Richard, 1990) and the rabbit (Bolton-Smith et al., 1988), fatty lesions develop in the aorta. It seemed reasonable to us to study a species, which in the free environment, and in our laboratory, selects a diet of nearly 100% fat (Folk, 1996). This species is the polar bear, a hibernator to a limited extent (Folk, 1981). This unusual diet is clearly associated with the fact that the polar bear is one of the most cold tolerant of mammals. By comparison, typical human diets are: Chinese 15% fat, U.S.37%. cold-weather natives vary from 42 to 50% (Story and Weaver, 1990). It is helpful to match the percent of fat in the diets of mammals with their susceptibility to atherosclerosis.

It seemed valuable to study polar bears under two circumstances: (1) when they were selecting nearly pure fat as a diet, and (2) when they had been fasted for at least one month but probably four months. In this way the effect of diet on the plasma lipids of this unusual species could be determined. Another facet of a study of polar bear diet is the fact that the fat selected by polar bears is from seals and whales. This food has a high content of those fatty acids which are called essential (Andersen et al., 1984). The first part of this plan has been completed and published, i.e. a report on two polar bears selecting a diet of nearly 100% fat (Kaduce et al., 1981). The present paper compares the former results with data from two fasted bears.

# Materials and Methods

Collection of Plasma: In the previous published study, venous blood samples were obtained from a 450 kg male polar bear, captive for 6 years, and a 205 kg female brought in from the Arctic icepack for the experiment and then released. They were fed dead seals from which they ate the blubber and a small amount of skin

In the second experiment, venous blood samples were obtained from two polar bears, 176 kg male and a 124 kg female. These two bears were both captured in traps near Churchill, Manitoba, Canada on October 1, 1990. They were initially ice-transported bears because the prevailing wind is south in Hudson Bay. When the ice melted about July 1, they were deposited on the shore. They had probably spent the summer walking up the west shore

of Hudson Bay to Churchill. The survey of the Canadian Department of Natural Resources indicated that apparently there was no available food for these bears, such as dead animals.

They were maintained in captivity during the entire month of October, and the blood samples were taken on November 6, 1990. While in captivity, the bears were not fed, were given only water, and were almost totally prevented from even the sight of human beings. Although some polar bears eat kelp and berries while walking to Churchill, their feces can reveal this diet later in captivity (Deroucher et al., 1993). Our two bears had not eaten kelp or berries and had probably fasted the previous three months as well as one month in captivity. The male (#3335) was six years old and had not been trapped before; the female (#9056) was nine years old and had been trapped on two other occasions. She was not lactating and she had given birth to a cub in 1981, and another in 1988. After the blood samples were obtained, the two bears were flown by helicopter and released by the Canadian Department of Natural Resources over 100 miles north and beyond Fort Prince of Wales. These bears had been present at Churchill waiting for sea ice to form. This takes place on approximately November 15, although there is a trend now for the ice to form later (Stirling et al., 1999).

The venous samples were obtained while the polar bears were under the influence of the immobilizer Telazol. The procedure was completed in approximately 20 minutes, and there were no convulsions associated with this drug.

The blood was drawn into tubes containing 1 mg of ethylene diamine tetra-acetate (EDTA) per ml of blood and immediately centrifuged for 10 minutes, and then transferred into screwcapped vials. The plasma was transported in a frozen condition to lowa City. After three days it was thawed for analysis.

Isolation of the Lipoproteins: Lipoproteins were isolated from the blood plasma by ultra centrifugal flotation using the procedure of Havel et al. (1955) as modified by Brenneman and Spector (1974). The VLDL\* fraction was isolated at a density of 1.006 g/ml, LDL at 1.063 g/ml, and HDL at 1.21 g/ml. The VLDL was washed once, and the LDL and HDL fractions were washed twice by ultracentrifugal flotation through KBr-NaCl solutions of appropriate densities. The washed lipoprotein fractions, each having a volume of approximately 3 ml, were dialyzed for 72 hours against one liter of 0.154 M NaCl which contained 0.02 % NaN<sub>3</sub>. The dialysis was changed three times.

Each of the lipoprotein fractions migrated as a single band on

agarose gel electrophoresis (DHEW publication No. 75-628, 1974).

Lipid Analyses: The triglycerides, cholesterol, cholesterol ester, and phospholipid content were measured in the intact plasma; the three isolated lipoprotein fractions, and the residual plasma of density were greater than 1.21 g/ml. Triglyceride and total cholesterol content were determined using an automated colorimetric and fluorometric analysis (DHEW Publication No. 75-628, 1974). The phospholipid content of the plasma and isolated lipoprotein fractions were estimated from measurements of organic phosphorus. Lipids were extracted from the samples by the method of Folch (1957). Aliquots of the lipid extract contained in the washed CHCl<sub>3</sub> phase of the extract were dried under N and assayed for organic phosphorus by the method of Raheja *et al.* (1973). The total and free cholesterol contents of the samples were measured using the method of Searcy and Bergquist (1960).

Fatty Acid Composition of Serum Components: The fatty acid composition of the phospholipids, cholesteryl esters, and triglycerides in the serum was determined using a combination of column and gas liquid chromatography (GLC). Silicic acid columns containing 2g of Unisil (Clarkson Chemical Company, Williamsport, PA) were employed to separate the lipids into classes. The lipid mixture was added to the column in hexane. After the column was washed with hexane, cholesteryl esters were eluted with diethyl ether-hexane (1:50 v/v) (Carroll and Serdarevich, 1967), neutral lipids were eluted with chloroform, and the phospholipids were eluted with methanol (Christie, 1973). The fatty acids contained in each of the lipid classes were hydrolyzed by saponification with 5 ml of 95% ethanol and 0.12 ml of the 33% KOH for 45 minutes at 70° C. Nonsaponifiable material was removed by extraction into n-hexane. The aqueous phase was acidified, and the fatty acids then were extracted into n-hexane. Following removal of the organic solvent by evaporation under N2, methylation of the fatty acids was carried out for 10 minutes at  $95^{\circ}$  C using 14% anhydrous boron trifluoride in methanol (Applied Science Inc., State College, PA). Fatty acid methyl esters then were extracted from the reaction mixture with three 3ml portions of n-hexane. The hexane was evaporated under N, and the fatty acid methyl esters were taken up in 25 I of carbon disulfide and separated by gas-liquid chromatography. Glass columns, 4mm x 6ft packed with 10% Apolar 10 C on gas Chrom Q (Applied Science, Inc. State College, PA) and a Hewlett-Packard model 5710A gas chromatograph equipped with a flame ionization detector were employed to separate and quantify the methyl esters. Peak areas were measured using an Infotronics model CRS 100 digital integrator. Peaks were identified by comparing their retention times with those of known fatty acid methyl ester standards (Supelco Inc., Belefonte, PA, and Nu-Check Prep, Elγsian, NM).

### Results

All results from the fasted animals will be compared with determinations from the male and female polar bears that had previously been maintained for several months on a fat diet. The results from fed bears have been published, but some of them are included to be compared in this paper. In all tables, the description "fasted" applies to the two bears that probably did not eat for four months.

Lipid Composition of Serum: Both the male and female fasted bears had approximately the same concentration of triglycerides but a larger difference in cholesterol (fasted male 333 mg/dl; fasted female 429 mg/dl). This same difference between sexes was found in the animals that had been eating fat.

When the sexes were combined, the cholesterol of the fed bears (mean 298 mg/dl) and the fasted animals (mean 381 mg/dl) represented values that were so high that in the human clinic, they would be considered seriously pathological (Table 1). The

Table 1: Plasma Lipid Analyses of Fed and Fasted Polar Bears

	Fed (mg/dl)	Fasted (mg/dl)	
	Avg ± Standard Erro	r of the Determination (n=3)	
Cholesterol Triglycerides	298 ± 24 199 ± 15	381 ± 36 292 ± 7	

Table 2: A Comparison of Plasma Lipid in Greenland and Danish Residents (From Bang et al., 1971)

	Greenland Inuits	Danes
	(mg/dl)	(mg/dl)
	n = 61 ♂	n = 61 ♂
Cholesterol	233	273*
Triglycerides	57	129*

\*P > 0.05

Fatty Acid

Table 3: Polar Bear Plasma Phosholipid Fatty Acid Composition

Fasted

Fed

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% Composition	(Avg $\pm$ Standard E	rror of the Determination. n = 3)
< 16:0	$\textbf{0.6} \pm \textbf{0.1}$	1.5 $\pm$ 0.1
16:0	$11.6 \pm 0.3$	$\textbf{22.4} \pm \textbf{4.7}$
18:0	$37.7 \pm 0.8$	$35.0 \pm 0.5$
16:1	$5.5\pm0.4$	$2.2 \pm 0.6$
18:1	$27.2 \pm 0.1$	$11.9 \pm 2.4$
18:2n-6	$2.5 \pm 0.2$	$6.7 \pm 1.1$
18:3n-3	$3.4 \pm 0.2$	$ extsf{0.5} \pm  extsf{0.2}$
20:4n-6	$3.4 \pm 0.1$	9.1 $\pm$ 0.1
20:5n-3	$\textbf{4.4} \pm \textbf{0.4}$	$3.5 \pm 1.4$
22:5n-3	$0.8 \pm 0.3$	$0.7 \pm 0.3$
22:6n-3	$\textbf{2.5} \pm \textbf{0.4}$	$2.0\pm1.0$
Others	$\textbf{2.5} \pm \textbf{0.4}$	$3.2 \pm 0.2$

Fatty Acid Classes (Avg. of the % Distribution)

Saturates	49.9	57.4	
Monounsaturates	32.7	14.0	
Polyunsaturates	14.9	23.9	
n-6 Polyunsaturates	5.9	15.8	
n-3 Polyunsaturates	9.0	6.2	

free/total cholesterol ratio was very similar in both the fed and the fasted animals.

Our hypothesis had been that the fasted bears would have lower lipid values than the fed bears. Note instead that the values for fasted bears were relatively high (Table 1). It was possible that some material in their diet was protecting the "fed" bears. We looked for guidance in testing this idea by turning to another case of a high fat diet; this example involves human subjects. Bang and coworkers studied Greenland Inuit eating a 50% fat diet of sea mammals and cold-water fish. They too seemed to be "protected" from high plasma lipid by their diet that was similar to our fateating polar bears (Table 2). A detailed analysis of the fatty acid (FA) composition of the polar bear serum lipids might give a clue to what this "protection" is.

Fatty Acid Composition of Serum Lipids: Results of the gas-lipid chromatographic analysis of the fatty acids in the plasma lipids are shown in Table 3, 4, and 5. In the fasted male and female, most of the total fatty acids contained in the phospholipids were either saturated (57.4%) or polyunsaturated fatty acids (PUFA). Only 14% were monoenoic. The trend was different in the triglycerides: 36% saturated FA and 29% monoenoic FA. In the cholesterol esters there were even lower saturated FA (25%) with 33% monoenoic.

Some specific selected triglyceride and cholesterol ester fractions of fasted bears contained high amounts of saturated acids, for example 27% (16:0) and 21% (16:0) respectively. However, the most striking results for these fasted bears were in the n-6 and n-3 series: for triglycerides, the combined percents were 14% (n-6)

Table 4: Polar Bear Plasma Triglyceride Fatty Acid Composition

Fatty Acid	d Fed	Fasted
% Compos	ition (Avg ± Standard	Error of the Determination. $n = 3$ )
< 16:0	$\textbf{0.6} \pm \textbf{0.1}$	$0.5 \pm 0.5$
16:0	$\textbf{6.4} \pm \textbf{0.4}$	$26.6 \pm 9.8$
18:0	$\textbf{4.7} \pm \textbf{0.6}$	$9.4 \pm 1.9$
16:1	$10.9 \pm 1.6$	$8.1 \pm 2.3$
18:1	$33.8 \pm 2.8$	$20.9 \pm 2.6$
18:2n-6	$3.1 \pm 0.4$	9.3 ± 0.2
18:3n-3	$\textbf{4.6} \pm \textbf{1.0}$	$0.6 \pm 0.2$
20:4n-6	$2.3\pm0.1$	$6.0 \pm 1.4$
20:5n-3	$12.7 \pm 0.9$	$3.8 \pm 1.9$
22:5n-3	$3.6 \pm 0.4$	$1.1 \pm 0.1$
22:6n-3	$13.7 \pm 3.2$	$6.0 \pm 0.7$
Others	$3.1 \pm 0.6$	$4.5 \pm 2.4$
	Fatty Acid Classes	(Avg. of the % Distribution)
Saturates	12.3	35.9

Table 5: Polar Bear Plasma Cholesterol Ester FaM Acid Composition

29 1

25.9

14.3

10.9

44 7

40.0

5.4

34.6

Monounsaturates

n-6 Polyunsaturates

n-3 Polyunsaturates

Polyunsaturates

Fatty acid	Fed	Fasted	
$\%$ Composition (Avg $\pm$ Standard Error of the Determination. n=3)			
< 16:0	$1.4 \pm 0.1$	$2.1 \pm 0.9$	
16:0	$7.8 \pm 0.2$	$21.4 \pm 0.7$	
18:0	$\textbf{1.6} \pm \textbf{0.2}$	$3.8 \pm 0.9$	
16:1	$\textbf{14.6} \pm \textbf{0.2}$	$8.5 \pm 2.6$	
18:1	$\textbf{25.7} \pm \textbf{0.9}$	$24.1 \pm 2.2$	
18:2n-6	$\textbf{5.0} \pm \textbf{0.4}$	$20.8 \pm 3.7$	
18:3n-3	$\textbf{0.9} \pm \textbf{0.2}$	$0.3 \pm 0.1$	
20:4n-6	$11.4 \pm 0.7$	$9.7 \pm 1.0$	
20:5n-3	$\textbf{29.3} \pm \textbf{1.4}$	$3.2 \pm 0.1$	
22:5n-3	$0.4 \pm 0.3$	$0.7 \pm 0.2$	
22:6n-3	$\textbf{0.8} \pm \textbf{0.1}$	$3.0\pm0.5$	
Others	$1.0 \pm 0.2$	1.8 ± 0.3	

Fatty Acid Classes (Avg of the % Distribution)

Saturates	10.8	25.2
Monounsaturates	40.3	32.6
Polyunsaturates	47.8	38.3
n-6 Polyunsaturates	16.4	30.5
n-3 Polyunsaturates	31.4	6.8

and 11% (n-3) and for cholesterol ester, 31 and 7%. The high value for n-6 is especially noticeable when considering the relative importance of the n-6 and n-3 FAs in this outdoor situation.

Now comparing fed and fasted bears, the phospholipid values were nearly the same in most cases (fed 396 mg/dl, fasted 395 mg/dl). Concerning triglycerides, saturated fatty acids (16:0 and 18:0) were very much higher in fasted bears; this finding was reversed for nearly all unsaturated fatty acids. There are eight specific selected FA's to consider (Table 4). In six of these cases there was a much larger percent in the serum lipid of the fed compared to fasted bears. Comparing the cholesterol ester figures, saturated fatty acids (16:0 and 18:0) in the fasted bears were up to three times the amount of saturated acid compared to the fed bears. Comparing unsaturated examples, there were eight cases of pairs to be assessed in Table 5. In five cases the differences were in favor of the fed animals.

Looking at the FAs as a whole, the n-6 FAs were not conspicuously larger in the fed animals; the large differences were found in the n-3 series. For example, in cholesterol ester (Table 4) the 20:5 n-3 was 29.3% in the fed animals and 3.2% in the fasted. Summarizing triglycerides (Table 4), total n-3 values were 34.6% fed, compared with 10.9% fasted; for cholesterol ester (Table 5)

the total n-3 values were 31.4% fed and 6.8% fasted.

Lipid Analyses of Lipoprotein Fractions: The lipoproteins of all classes were higher in most cases in the fasted bears than fed bears (Table 6). An analysis of the phospholipid alone in the lipoproteins of all bears showed that the majority was contained in the HDL fraction and as expected a small amount in LDL and VLDL. This picture was the same for total cholesterol: there was a substantial amount in HDL, a somewhat larger amount in LDL, and the expected low value of VLDL. The triglycerides for the fasted bears showed a reverse relationship with very small fractions of HDL, much larger fractions of LDL compared to the phospholipids, and an unusually large figure of 49 mg/dl for VLDL. We now consider the ratio between HDL and LDL for fasted bears only. Based upon information from human subjects, we can call the ratio for the phospholipids a healthy one (421 mg/dl HDL vs. 92 mg/dl). This ratio was reversed as expected in the triglycerides (16 mg/dl vs. 145 mg/dl). The expected ratio was not found in total cholesterol, 165 mg/dl HDL vs.194 mg/dl.

Of greater interest is the comparison of the lipoproteins in fed bears compared with fasted. Once again we are looking for a possible protection which benefits the bears eating 100% fat. First consider the VLDL. It is lower in all cases in fed bears; in our later discussions this will be considered an advantage for fed bears.

Next, consider the HDL. There is no trend favoring the fed bears except in cholesterol; the benefit seems to be in favor of the fasted bears with their higher HDL in phospholipids and triglycerides.

Of lesser importance are the LDL determinations; the three relatively low values (70, 121, 51  $\,$  mg/dl) for fed bears are important in later discussion.

The final analysis compares fed bears with fed human subjects (Table 7). The low VLDL figure for fed bears is 9  $\pm$  2 mg/dl, consistent with healthy human subjects (Pruzanski *et al.*, 2000). The HDL figure of 170  $\pm$  6 for the bears is extraordinarily high.

### Discussion

Why do polar bears eat high fat?: What is the species' advantage of eating nearly 100% fat? Polar bears must do this because of their habitat that consists of the ice pack of the Arctic region. They often are forced to tolerate a long fast sometimes in continuous darkness that may last over 90 days. Perhaps eating 100% fat is the most efficient way to lay down the necessary abundant depot fat when food is available. The longest fast known of any mammal (8 mos.) is found in the female polar bear of Hudson Bay.

Result of high fat diet: The mean cholesterol reported in this paper range from 306 mg/dl to 381 mg/dl. Such high values would be fatal to the domestic dog and rabbit (Ferguson and Folk, 1971). Note that the first of these values was obtained from the bears eating nearly 100% fat while the second value was obtained while the bears were living on abundant depot fat. In an earlier article, we had published high values of cholesterol and triglycerides from polar bears, but the samples were obtained from running bears immobilized from a helicopter. The values in mg/dl were: cholesterol 370, 257, and 353; the results for triglycerides were 249, 352, and 342 (Nelson et al., 1983). The results are consistent with our measurements from captive bears.

How does the bear tolerate high cholesterol?: This experiment was designed with two fasted bears to try to determine how they can tolerate high cholesterol. The fasted bears had considerably higher cholesterol esters in the plasma than the bears eating high fat. In Results, we reported high saturated fatty acids (16:0 and 18:0) in both cholesterol and triglycerides of the fasted bears. Since saturated FAs would tend to raise both substances in serum (Huang and Nassar, 1990), their presence helps to explain the high values of cholesterol and triglycerides in the fasted bears. When

Table 6: Composition of Polar Bear Lipoproteins

	VLDL		LDL	LDL		HDL	
	Fed	Fasted	 Fed	Fasted	 Fed	Fasted	
	(mg/dl)						
Phospholipid	7 ± 1	10± 3	70 ± 5	92 ± 12	308 ± 19	421 ± 15	
Triglycerides	$30\pm7$	$49\pm10$	$167\pm13$	$\textbf{145} \pm \textbf{22}$	5 ± 1	16± 6	
Cholesterol							
Total	$9\pm2$	$27\pm12$	$121\pm19$	$194 \pm 30$	170 ± 6	$165\pm18$	
Ester	$0\pm0$	2 ± 1	51 ± 3	77± 3	78 ± 1	70 ± 2	

Avg. ± Standard Error of the Determination (n = 3)

Table 7: Human and Fed-Bear Plasma Lipids

	Normal Human*	Fed Polar Bears <sup>∉</sup>
	(mg/dl)	
Triglycerides	35-160	199 ± 15
Total Cholesterol	< 200	306 + 22
VLDL	6-40	9 ± 2
LDL	< 130	121 ± 9
HDL	> 35	170 ± 6

<sup>\* (</sup>Havel et al., 1955)

we examine the eight specific selected unsaturated fatty acids in pairs (fed vs. fasted) we find that the n-3 series was much higher in the fed bears relative to the fasted bears; however, the n-6 fed values were not high.

It is reasonable to suppose that the high level of n-3 fatty acids protected the bears eating high fat. Other species have been protected by a high level of these n-3 fatty acids (Lees and Karel, 1990; Ando et al., 2000; Angerer and von Schacky, 2000) as well. Our fed bears ate seal blubber and some cold-water fish. The most abundant fatty acid in these two foods is the n-3 type (Kuhnlein, 1993, Huang and Nassar, 1990). No mammals can synthesize the essential fatty acids n-3 and n-6 (Uauy and Valenzuela, 2000). They are synthesized by phytoplankton in the ocean that is eaten by small crustacea (krill) which are eaten by fish and whales. Seals eat the fish. The polar bears are at the top of the food chain and eat both seal and whale. Thus the protective material of n-3 fatty acids reaches the polar bear as well as human subjects in their diet.

One might ask how does the polar bear eating mostly blubber obtain essential amino acids. We have observed our polar bears to eat a small amount of skin which would provide some of this necessary ingredient, and apparently blubber contains 3% protein. The most important point, however, is that relative to the controls (the fasted polar bears) the bears eating high fat had much lower cholesterol and triglycerides. We must presume that this lowering is under the action of the n-3 fatty acids which were not in the diet of the fasted bears.

How do cold-weather natives tolerate high cholesterol?: Greenland natives live with low cholesterol and triglycerides (Table 2). These natives were eating a 50% fat diet. Since they were eating a diet similar to that of our fed polar bears, it must be presumed that they also were protected by n-3 fatty acids (von Schacky, 2000). We estimate that there are some 90,000 cold-weather natives of the Inuit type; undoubtedly more than half of these live on native foods especially sea mammals and cold-water fish and therefore are obtaining n-3 fatty acids in their diet (Aubrey, 1990; Caulfield, 1993; Kuhnlein, 1993; Malcom, 1993). The n-3 quantity in the adipose tissue of Greenland natives has been analyzed (Boudreau, 1993) and was found to be over double the amount in the adipose tissue of Alaska natives and triple the amount in non-native Alaska residents.

Do the lipoproteins also protect fed bears?: The HDL values for

total cholesterol were higher in the fed bears compared to the fasted bears. Also for all determinations, the ratio between HDL and LDL were favorable for both fed and fasted polar bears. The lipid content of the isolated lipporoteins was high compared to human readings. However, since the total lipid concentrations in all bears were unusually high, one would expect the lipoproteins also to be high. As pointed out in Results, this was the case (HDL was 170 mg/dl, Table 7).

The total lipid concentrations in fasted animals were highest; and as expected, isolated lipoproteins are highest. The HDL in both fed and fasted animals were much higher than both VLDL and LDL in phospholipids and total cholesterol as in healthy human subjects. The HDL triglyceride was very low as in human subjects. The bears eating n-3 fatty acids (fed bears) tended to have lower values of lipoprotein lipids than fasted bears that were eating no n-3 fatty acids.

VLDL was usually much lower than the other lipoproteins regardless of diet. The n-3 fatty acids appear to raise HDL cholesterol to an unusually high level. VLDL is relatively low in polar bears compared to human subjects. LDL, based on cholesterol content, in fed bears is similar to human, but fasted bears are high compared to human subjects. HDL-cholesterol is very high as if to protect, compared to human subjects. The lipoprotein lipid content of fasted bears was much higher (with two exceptions) than fed bears. A plausible explanation is the presence of n-3 FA in the diet of fed bears eating nearly 100% fat; these FA's are very low in fasted bears.

What is the effect of "protection" on cardiovascular disease?: The habitual diet of polar bears contains a high percent of fat. Do they show a high incidence of cardiovascular disease? We surveyed the records of the Armed Forces Institute of Pathology and of the necropsies in three zoos and in two wildlife national service organizations. There is absolutely no record of any cardiovascular disease in polar bears, some of which had been maintained for as long as 33 years in captivity. One of our polar bears that had levels of 180 mg/dl triglycerides and 270 mg/dl cholesterol was studied until he reached the age of 21 years. At that time after many years of being on a high fat diet from sea mammals and marine fish, at necropsy this animal had no cardiovascular disease or atherosclerosis.

Since polar bears habitually eat a diet high in n-3 FAs it would seem that they are protected from cardiovascular disease. Does this protection also apply to the large population of cold-weather natives who habitually live on a preponderance of sea mammals and occasionally caribou? According to Caulfield (1993) the Greenland Inuit as a whole eat no more than 16% imported storebought food. A recent survey by Mulvad et al. (1996) showed an extremely low rate of ischaemic heart disease in Greenland. A recent study by Choiniere (1992) compared causes of death in 8,000 Baffin Inuit natives with non-native Canadians as a whole. He tabulated seven causes of which we present three here: neoplasms Inuit 23%, Canadians 26%; respiratory system disease Inuit 19%, Canadians 8%; circulatory system disease Inuit 11%, Canadians 43%. Once again it appears that a diet of n-3 FAs protects these natives from cardio-disease in spite of the fact that their habits include heavy smoking. In his study Bang et al. (1971) showed that the male Inuit in Greenland had significantly higher

<sup>#</sup> Avg  $\pm$  Standard Error of the Determination (n = 3)

levels of HDL cholesterol; this apparently reduces the risk of cardiovascular disease (Pederson *et al.*, 1996). Presumably, their diet of n-3 FAs or possibly some genetic factor as well protects cold-weather natives (Dyerberg *et al.*,1975).

Mechanism of action of n-3 and n-6 FAs: To understand the probable protective action of n-3 FAs, it is necessary to consider the "cascade" as the original materials eaten by the mammal (shorter chain unsaturated acids) are converted to longer chain n-3 FAs. From plant material the 18:3 n-3 linolenic acid is eaten by mammals (Sanders, 2000). Elongation and desaturation steps convert this 18 carbon FA to 20:5 n-3 a compound called EPA. Over two stages this is converted to DHA (22:6 n-3). These long chain unsaturated FAs are competitive inhibitors of cyclooxygenase and lipoxygenases and down regulate eicosanoid synthesis from arachidemic acid. Significantly, these long chain n-3 FAs regulate platelet function (Thorwest et al, 2000). In this case, their action is inhibitory and may explain the protective action of these materials in combating cardiovascular disease. The clumping of platelets is related to the formation of coronary clots, and it is probable that this formation is inhibited by the long chain polar bear n-3 FAs in question (Weber and Raederstorff, 2000). Although these materials may protect polar bears against cardiovascular disease, they are not protected against gallstones. Polar bears have in their bile ursodeoxycholic acid. This makes cholesterol more soluble, and it is used in the clinic to treat gallstones. However, its presence in bears does not entirely prevent gallstones. The junior author did a necropsy on a 300kg 10-year-old polar bear at Point Barrow and found the gall bladder almost entirely filled with one large gallstone.

Two controversies in this field: One school of thought favors two groups of FAs as providing protection against cardiovascular disease (Connor, 2000; Kris-Etherton et al, 2000). Mulvad (1996) and coworkers suggest that the Greenlanders who they studied having low ischaemic heart disease, are protected by high amounts of long chain monounsaturated FAs. They found high amounts of these in the fatty tissue of Greenlanders when autopsied. Support for their concept is found in our polyunsaturated FAs in both the triglycerides and cholesterol. These FAs were highest in the fed polar bears and may be protective. However, even more striking was the ratio between the fed and fasted bears in the n-3 FAs.

Another controversial matter in this field is whether to use fish-oil capsules high in n-3 FAs in the clinic (Asset *et al.*, 2000; Goodfellow *et al.*, 2000; Nestel, 2000; Smit *et al.*, 2000). Our results showing lowered cholesterol when polar bears have a high n-3 FA level in their blood compared to fasted animals eating no n-3 FAs, give some slight support to the concept of using fish-oil capsules to lower cholesterol.

Footnote\*: Abbreviations used: VLDL – very low density lipoproteins; LDL – low density lipoproteins; HDL – high density lipoproteins. Later in the text the fatty acids are abbreviated as number of carbon atoms: number of double bonds. Thus, 20:5 signifies a fatty acid containing 20 carbon atoms and 5 unsaturated bonds. 18: 2n-6 is an omega-6 fatty acid (linoleic) with the first double bond six carbon atoms in from the terminal methyl group.

## Acknowledgements

The Iowa Environmental Physiology Fund and the Canadian Department of Natural Resources, Wildlife Branch, supported these studies. Thanks are due for the willing assistance of Paul S. Cooper, D.V.M. and also to Denis Normandin of Churchill for the use of two centrifuges. This work could not have been completed without the support and encouragement of Arthur A. Spector who read the manuscript and of Max C. Brewer of the Naval Arctic Research Laboratory. We thank Pamela S. Michaud and Rosemary F. Kueny for typing and editing the manuscript.

### References

- Ando, K., K.Nagata, R. Yoshida, K. Kikugawa and M. Suzuki, 2000. Effect of n-3 polyunsaturated fatty acid supplementation on lipid peroxidation of rat organs. Lipids, 35: 401-407.
- Anderson, D.W., L.J. Filer and L.D. Stegink, 1984. Intravenous lipid emulsions in the treatment of essential fatty acid deficiency: studies in young pigs. Pediatric Res., 18: 1350-1355.
- Angerer, P. and C. von Schacky, 2000. N-3 polyunsaturated fatty acids and the cardiovascular system. Current Opinion in Lipidology, 11: 57-63.
- Asset, G., E. Bauge, R.L. Wolff, J.C. Fruchart and J. Dallongeville, 2000. Comparison of maritime pine oil and fish oil effects on plasma lipoproteins in apolipoprotein E-deficient mice. Prostaglandins, Leukotrienes, and Essential Fatty Acids, 62: 307-310.
- Aubrey, L.P., 1990. Nutrient intake of adults aged 15 to 65 years in two northern Ontario communities. Proc. Congress on Circumpolar Health, 8: 774-777.
- Bang, H.O., J. Dyerberg and A.B. Nielsen, 1971. Plasma lipid and lipoprotein pattern in Greenlandic West-coast Eskimos. Lancet., 1:1143-1145.
- Bolton-Smith, C., M.J. Gibney, P.J. Gallagher, R. Jewell and K. Hillier, 1988. Effect of polyunsaturated fatty acids on induction of atherosclerosis in rabbits. Atherosclerosis, 72: 29-35.
- Boudreau, D.A., 1993. Arctic native atherosclerosis and omega-3 fatty acids. Arct. Med. Res., 52: 73-75.
- Brenneman, D.B. and A. A. Spector, 1974. Utilization of ascites plasma very low density lipoprotein triglycerides by Ehrlich cells. J. Lipid Res., 15: 309-316.
- Carroll, K.K.and B. Serdarevich, 1967. Lipid Chromatographic Analysis. Marcel Dekker, New York.
- Caulfield, R.A., 1993. Aboriginal subsistence whaling in Greenland. Arctic, 46: 144-155.
- Choiniere, R., 1992. Mortality among the Baffin Inuit in the mid-80's. Arct. Med. Res., 51: 87-93.
- Christie, W.A., 1973. Lipid Analysis. Pergamon Press, Oxford. Connor, W.E., 2000. Importance of n-3 fatty acids in health and
- disease. Am. J. Clin. Nutr., 71: 171S-175S.

  Derocher, A.E., D. Andriashek and I. Sterling, 1993. Terrestrial foraging by polar bears during the ice-free period in Hudson
- Bay. Arctic., 46: 251-254.

  Dhew Publication 75-628. Manual of Laboratory Operations, 1974.

  Lipid and Lipoprotein analysis, 1. U.S. Govt. Printing Office,
- Washington.

  Dyerberg, J., H.O. Bang and H. Hjorne, 1975. Fatty acid composition of the plasma lipids in Greenland Eskimos. Am. J. Clin. Nutr., 28: 958-966.
- Ferguson, J.H. and G.E. Folk, 1971. Free fatty acid levels in several arctic carnivores. Comp. Biochem. Physiol., 40B: 309-312
- Folch, J., M. Lees and G.H. Sloane-Stanley, 1957. A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem., 226: 497-509.
- Folk, G.E. and R.A. Nelson, 1981. The hibernation of polar bears: a model for the study of human starvation. Proc. Intl. Symp. Cir. Health., 5: 617-619.
- Folk, G.E., Jr., 1996. Responses to seasonal changes in polar mammals. In: Fregley, M.J., Blatters, C.M. (Eds.), Handbook of Physiology. Oxford U. Press, Oxford, 541-556.
- Goodfellow, J., M.F. Bellamy, M.W. Ramsey, C.J. Jones, and M.J. Lewis, 2000. Dietary supplementation with marine omega-3 fatty acids improve systemic large artery endothelial function in subjects with hypercholesterolemia. J. Am. College of Cardiology, 35: 265-270.
- Havel, R.J., H.A. Eder and J.A. Bragdon, 1955. The distribution and chemical composition of ultracentrifugably separated lipoproteins in human sera. J. Clin. Invest., 34: 1345-1353.

- Huang, Y. and B.A. Nassar, 1990. Modulation of tissue fatty acid composition by dietary manipulation. In: Horrabin, D.F. (Ed.) Omega-6 Essential Fatty Acids. Wiley-Liss, New York.
- Kaduce, T.L., A.A. Spector and G.E. Fork, 1981. Characterization of the plasma lipids and lipoproteins of the polar bear. Comp. Biochem. Physiol., 69B: 541-545.
- Kris-Etherton P.M., D.S. Taylor, S. Yu-Poth, P. Huth, K. Moriarty, V. Fishell, R.L. Hargrove, G. Zhao and T.D. Etherton, 2000. Polyunsaturated fatty acids in the food chain in the United States. Am. J. Clin. Nutr., 71: 179S-188S.
- Kuhnlein, H.V., 1993. Dietary fat in traditional and contemporary northern indigenous food systems. Proc. Congress Cir. Health., 9: S83.
- Lees, R.S. and M. Karel, 1990. Omega-3 Fatty Acids in Health and Disease. Marcel Dekker, New York.
- Malcom, G.T., A.K. Bhattacharyya, D.A. Boudreau, H.S. Pedersen, G. Mulvad, E. Jul, J. Misfeldt and J.P. Middaugh, 1993. Omega-3 and omega-6 fatty acids in Alaska and Greenland natives. FASEB J., 7 Part 2, A799.
- Mulvad, J.E., H.S. Pedersen, J.C. Hansen, E. Dewailly and E. Jul, 1996. The Inuit diet. Fatty acids, their role in ischaemic heart disease. Arctic Med. Res., 55: 22-24.
- Nelson, R.A., G.E. Folk, E.W. Pfeiffer, J.J. Craighead and C.J. Jonkel, 1983. Behavior, biochemistry, and hibernation in black, grizzly, and polar bears. Intl. Conf. Bear Res. and Management, 5: 284-290.
- Nestel, P.J., 2000. Fish oil and cardiovascular disease: lipids and arterial function. Am. J. Clin. Nutr., 71: 228S-231S.
- Pederson, H.S., J.C. Hansen, E. Dewailly and E. Jul, 1996. The Inuit diet. Fatty acids, their role in ischaemic heart disease. Arctic Med. Res., 55: 20-24.
- Pruzanski, W., E. Stefanski, F.C. de Beer, M.C. de Beer, A. Ravandi and A. Kuksis, 2000. Comparative analysis of lipid composition of normal and acute-phase high density lipoproteins. J. Lipid Res., 41: 1035-47.

- Raheja, P.K., C. Kaur, A. Singh and I.S. Bhatia, 1973. New colorimetric method for the quantitative estimation of phospholipids without acid digestion. J. Lipid Res., 14: 695-697.
- Richard, M.J., L.D. Davis and N.L. Jacobson, 1990. The goat: a model to determine cholesterol accumulation. Comp. Biochem. Physiol., 95A: 275-280.
- Sanders, T.A., 2000. Polyunsaturated fatty acids in the food chain in Europe. Am. J. Clin. Nutr., 71: 176S-178S.
- Searcy, R.L. and L.M. Bergquist, 1960. A new color reaction for the quantitation of serum cholesterol. Clin. Chim. Acta., 6: 192-199.
- Smit, E.N., E.A. Oelen, E. Seerat, E.R. Boersma and F.A. Muskiet, 2000. Fish oil supplementation improves docosahexaenoic acid status of malnourished infants. Archives of Disease in Childhood, 82: 366-369.
- Stirling, I., N.J. Lunn, and J. lacozza, 1999. Long-term trends in polar bears in relation to climatic change. Arctic., 52: 294-306.
- Story, J.A and C.M. Weaver, 1990. Traditional fat diets. Science, 248: 1594.
- Thorwest, M., E. Balling, S.D. Kristensen, S. Aagaard, A. Hakami, S.E. Husted, J. Marqversen and V.E. Hjortdal, 2000. Dietary fish oil reduces microvascular throbosis in a porcine experimental model. Thrombosis Res., 99: 203-208.
- Uauy, R. and A. Valenzuela, 2000. Marine oils: the health benefits of n-3 fatty acids. Nutr., 16: 680-684.
- von Schacky, C., 2000. n-3 fatty acids and the prevention of coronary atherosclerosis. Am. J. Clin. Nutr., 71: 224S-227S.
- Weber, P. and D. Raederstorff, 2000. Triglyceride-lowering effect of omega-3 LC-polyunsaturated fatty acids. Nutrition Metabolism and Cardiovascular Diseases, 10: 28-37.