The Occurrence of Cardiac Lipidosis and Necrotic Lesions in the Hearts of Rats Following Long-Term Feeding of Different Lipid Supplemented Diets

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Summary The effects of “long-term” feeding of different lipid supplemented diets (12% added fat, w/w) on the incidence of lipidosis or the severity of necrosis was examined in the cardiac muscle of male Hooded Wistar rats, after at least 12 months on the diets. The effects of supplementation with either n-6 polyunsaturated fatty acids (PUFA’s) added as sunflower seed oil (the SSO diet), or one enriched with n-3 PUFA’s added as a low cholesterol, low vitamin (A & D) fish oil preparation obtained from eviscerated Southern Bluefin Tuna (the TFO diet) were compared to those found in the hearts of rats fed either a relatively low fat commercially available stock diet (REF) which contained 4% (w/w) of mixed fats of animal, vegetable and marine origin, or after this stock diet had been supplemented to the same extent by the addition of 12% (i.e. 12:88 g) sheep kidney (perirenal) fat, the SF diet.

Extensive cardiac lipidosis was seen after feeding either the TFO or the SF diets, but was not observed in the hearts of experimental rats from either the SSO or REF fed groups. Conversely, in these mature animals, grade 1 necrotic lesions were uniformly found in the cardiac muscles of all rats examined, but neither their incidence nor severity could be attributed to any dietary effect. These necrotic lesions are therefore more probably a reflection of the age of the animals at the time of sacrifice, rather than to any of the dietary supplements employed.

Some evidence of “Yellow Fat” disease was found by the presence of lipofuscin pigmentation in the storage fat of rats receiving n-3 PUFA’s (the TFO diet) but was not observed in any other dietary group nor in the livers or kidneys of any animals. This extent of storage fat pigmentation was not associated with any retardation of growth in this dietary group.

Key Words cardiac lipidosis, necrotic lesions, rat-hearts, lipid-supplemented diets
The accumulation of lipid droplets in the heart muscle cells of laboratory animals fed a variety of lipid supplements has been noted by many observers (1–4). In some instances this has been thought to arise from specific but naturally occurring fatty acid components of the dietary supplement which are not easily oxidized by the mitochondria of the cardiac muscle (2, 5), while in other studies the effect has been attributed to the presence of partially hydrogenated components derived from the commercial processing of natural oils (4, 6, 7). This latter suggestion has been used by some government agencies to limit the permitted levels of certain fatty acids in oils and fats for human consumption (8), and has prompted a large scale life-span study of the phenomena in laboratory rats (7).

While it is clear from the work of Barer (9) and Duthie and Barlow (7) that a causal relationship between cardiac lipidosis and the subsequent development of necrosis and fibrosis of the myocardium is far from established, it is important to consider this possibility in any study of the physiological sequelae of dietary lipid supplementation, particularly if the period of dietary study spans a considerable proportion of the animals normal life-span (10).

In our laboratory we have recently carried out an examination of the effects of long-term dietary lipid supplementation on both the chemical composition of rat cardiac muscle membranes (11–13) and changes in cardiac muscle function (14–16) which occurred after feeding either sunflower seed oil, saturated animal fat or a specially prepared low cholesterol tuna fish oil preparation for periods of at least 12 months. At the time of sacrifice of the animals, portions of the left ventricular wall (LVW) and interventricular septum (IVS) were collected for histological examination. The result of a semi-quantitative assessment of the occurrence and extent of both lipidosis and histopathological lesions in the hearts of these rats following ingestion of these experimental diets is presented in this report.

MATERIALS AND METHODS

Animals and diets. Eighty-day old male Hooded Wistar rats weighing 259 ± 2 g (mean ± SEM) were randomly assigned to the various experimental groups, the first of which (n = 6) was fed a standard commercial laboratory diet (obtained from Milling Industries, Australia Limited) which contained 4% (w/w) total fat of mixed animal, vegetable and marine origin, and sufficient linoleic acid to avoid any essential fatty acid deficiencies (11–13). This group served as an age-related reference group (REF) for this and other related studies of cardiac function published elsewhere (14–16).

Three other groups of age and sex matched rats received the standard diet which had been supplemented (12 : 88 g) with either sunflower seed oil (the SSO diet, n = 5) obtained from Nuttelex Pty Ltd., Victoria, sheep kidney (perirenal) fat (the SF diet, n = 6) obtained from a local slaughter house (Metro Meats, Noarlunga Division, South Australia) or a low-cholesterol (and low vitamin A & D) fish oil preparation (the TFO diet, n = 9) obtained from the flesh of eviscerated Southern...
Bluefin tuna processed by SAFCOL Seafoods Pty Ltd., Victoria.

The preparation of the various refabricated diets, the fatty acid composition of the different lipid supplements (SSO, SF and TFO respectively) and the fatty acid composition, cholesterol content and combustible energy of the diets have all been described in detail in several previous publications from this laboratory (11–16), as has the feeding regime, the average energy intake and mean group body weights of the rats both during the experiment and at the time of sacrifice (12, 13).

In general, supplementing the diet with 12% (w/w) sunflower seed oil (SSO) increased the level of n-6 PUFA’s as the major component of this vegetable oil is linoleic acid (C18:2, n-6); whereas supplementing the diet with 12% (w/w) tuna-fish oil (TFO) greatly increased the level of long-chain n-3 PUFA’s as both eicosapentaenoic acid (C20:5, n-3) and docosahexaenoic acid (C22:6, n-3) are major components of the Southern Bluefin tuna-oil preparation used in these experiments (13). That is, while the P/S ratios of the n-6 and n-3 supplemented diets were 3.7 and 1.1 respectively, the n-6/n-3 ratios were 45 and 0.4. Cetoleic acid (C22:1) occurred to a maximum level of 0.7% in the tuna-oil employed in these experiments and was diluted below this level in the refabricated diets (13).

Supplementing the diet with 12% sheep fat (SF) rich in saturated fatty acids produced a refabricated diet identical to that used before (12, 13) and resulted in an experimental diet with a P/S ratio of 0.2 and an n-6/n-3 ratio of 3.8.

All animals were fed the various experimental diets for at least 12 months (maximum 13.5 months). At the completion of the “long-term” feeding period the rats were fasted overnight before sacrifice under light ether anaesthesia, their chests opened and the hearts removed and dissected for chemical (11–13), physiological (14–16) and histological examination.

For the study of cardiac lipidosis, the left ventricular wall (LVW) and a small portion of the interventricular septum (IVS) were removed and fixed in 10% phosphate buffered formalin for at least 24 h before sectioning. The LVW was then cut from base to apex into three longitudinal strips approximately 2 mm wide. The central portion of the LVW and a longitudinal strip of the IVS were examined for the accumulation of lipid droplets by the procedure described below. Between 1,500–2,000 cells/longitudinal section were examined.

The remaining strips of cardiac muscle were processed for examination by other histological staining methods (H & E; long Ziehl-Neelsen etc.) referred to later. Samples of liver, kidney, and storage fat (perirenal adipose tissue) were also collected for histological examination for the possible occurrence of ‘Yellow Fat’ disease (17, 18) which has been reported to occur in both pigs and rats fed a high n-3 PUFA supplemented diet (18).

Histology. Preliminary experiments had confirmed that the osmium tetroxide method of Hall et al. (19) was suitable for the detection of lipid droplets in myocardial cells as well as in liver. However unlike the cryostat sections used by previous investigators the method of Hall et al. provides sections of uniform thickness, and the staining procedure avoids any tendency for lipids to diffuse out of
cells (9). The fixed sections of LVW and IVS were rinsed briefly in distilled water and stained by immersion in Marchi reagent (20), i.e. 1% osmium tetroxide and 5% potassium dichromate (1:1, v/v) thus causing specific blackening of hydrophobic unsaturated lipids, cholesterol esters and free fatty acids. After 6 h, the sections were washed in distilled water, dehydrated through graded alcohols and cleared in acetone before infiltration and embedding in Araldite CY 212 according to a processing schedule similar to that described by Hall et al. (19). Two μm sections were cut with a Jung (model K) microtome and mounted in distrene-dibutylphthalate xylene (DPX) synthetic mounting medium for examination by light microscopy. The remaining strips were also processed through graded alcohols, cleared in acetone and embedded in Araldite CY 212. Sections were then cut from each block, the resin removed using a saturated solution of sodium ethoxide and stained by the following procedures: haematoxylin and eosin (H & E), Hall’s modification of Masson’s trichrome stain for collagen (21) and a long Ziehl-Neelsen procedure for lipofuscin pigmentation (22).

Assessment of lipidosis. As previously noted by Barer (9), the estimation of the volume fraction of lipid in the cardiac muscle sections using stereological procedures could not be applied by either manual or automated procedures as the majority of the lipid droplets that were observed have diameters less than the section thickness and considerable overlapping of small droplets was apparent as was some variation in the staining intensity of the droplets. A semi-quantitative method similar to that of Opstvedt et al. (3) was therefore adopted in which the extent of intracellular myocardial lipidosis was graded according to the scale shown in Table 1. Because of the uneven distribution of myocardial cells with lipid droplets it was necessary to assess all areas in a section in a standard manner without reference to the dietary code of the experiment. The percentage of myocardial cells with intracellular lipid droplets was calculated for the heart tissue of each animal, based upon the assessment of individual sections. For each animal in a dietary group, this percentage was then assigned the grade described in Table 1, and a final lipidosis index calculated for the group by dividing the sum of the lipidosis grading for the

| Estimated % of cells with lipid droplets | Assigned grade |
|----------------------------------------|----------------|
| Nil                                    | 0              |
| <5% of myocardial cells                | 0.5            |
| 5–25%                                  | 1.5            |
| 26–50%                                 | 2.5            |
| 51–75%                                 | 3.5            |
| 76–100%                                | 4.5            |

*From Opstvedt et al. (see Ref. 3).
individuals by the number of animals in each group. Because of the semi-quantitative (graded) nature of the values obtained by this method of scaling, statistical analysis of this data was assessed by a ranking T-test described by White (23) which is particularly suitable where the total sample size is small and it is not necessary to assume normal distributions with equal variance.

Assessment of the incidence and severity of cardiac lesions. Two longitudinal strips of LVW from each heart were embedded in Araldite as described above. Three sections were cut at regular intervals (about 0.25 mm apart) from each block (i.e. six sections per heart) and stained with H & E for examination under high power light microscopy. Each high power field (HPF) covered an area of 0.181 mm².

Both collagen containing lesions, which were the predominant type in the hearts of these older animals and which were regarded as a scar resulting from muscle cell necrosis, and those in which more than 10 leukocytes or histiocytes were grouped together with or without evidence of muscle cell necrosis, were graded semi-quantitatively according to the empirical criteria employed by Svaar (10). In this system, lesions smaller than 1 HPF are graded as 1, lesions covering 1–3 HPF are graded as 2 and lesions greater than 3 HPF are graded as 3.

Each strip of LVW was then graded for overall severity based upon the number and size of the lesions in the six sections examined. The severity index (SI) for each group of dietary manipulated rats was then determined from the sum of the combined grades of the lesions divided by the total number of sections.

Yellow Fat disease. Lipofuscin pigmentation of storage fat (and other organs such as liver and kidney) has been employed as a means of identifying the presence of ‘Yellow Fat’ disease thought to be associated with vitamin E deficiency in both pigs and rats (17, 18). This histological procedure was used in this study, particularly as n-3 PUFA’s were provided in the TFO diet (13) and have been implicated by Danse and Verschuren (18) in the induction of this condition in rats.

RESULTS

Cardiac lipidosis

The average results from each dietary group are given in Fig. 1, where the lipidosis index for both the rats fed the standard diet (REF) and those receiving the n-6 polyunsaturated fatty acid supplement as sunflower seed oil (SSO) is shown to be about 1.0. In general this means that in none of the sections examined from these dietary groups did the extent of lipidosis exceed 25% of all cells examined, and that in many sections less than 5% of the cells contained lipid droplets.

On the other hand, the extent of cells with marked lipidosis (>50%) was very high following lipid supplementation with either n-3 PUFA’s as tuna fish oil (TFO)—which also contains about 30% of the common saturated fatty acids, myristic, palmitic and stearic—or the saturated animal fat supplement (SF) which contains about 64% of these saturated fatty acids (11–14).
Fig. 1. Extent of cardiac lipidosis in the hearts of rats receiving various lipid supplemented (12%, w/w) diets for more than 12 months. * is significantly different \((p<0.05)\) from either REF or SSO group.

Table 2. The incidence, distribution and severity of lesions in the left ventricular wall of rats receiving different lipid supplements for at least 12 months.

| Dietary group | Animals per group | Incidence of lesions (%) | Distribution of lesions by zone (%) |
|---------------|-------------------|-------------------------|-----------------------------------|
|               |                   | Grade 1    | Grade 2   | Grade 3   | Subendocardial | Central myocardial | Subepicardial | Severity index |
| REF           | 6                 | 95         | 3         | 2         | 57           | 23              | 20           | 0.9           |
| TFO           | 9                 | 100        | —         | —         | 32           | 18              | 50           | 1.0           |
| SSO           | 5                 | 97         | 3         | —         | 27           | 20              | 53           | 1.1           |
| SF            | 6                 | 100        | —         | —         | 34           | 18              | 48           | 1.0           |

*From Svaar (See Ref. 10).

That is, feeding either TFO or SF produces a lipidosis index between 2.5–3.0 which is significantly greater \((p<0.05)\) than that seen in either the REF or the SSO group (23). These results are also illustrated in the photographs of typical sections shown in Fig. 2.

Cardiac lesions

After H & E staining, the LVW from all animals irrespective of their dietary group, exhibited extensive grade 1 lesions having small foci of inflammation and small patchy areas of scarring (Fig. 3). The incidence of grade 2 or 3 lesions was less than 5\% in all sections examined and was not readily related to the nature of the
Fig. 2. Accumulation of lipid droplets in the cardiac muscle from rats fed different dietary lipid supplements for at least 12 months. Panel A, REF diet; panel B, TFO supplemented diet; panel C, SSO supplemented diet; panel D, SF supplemented diet as described under METHODS section and as in Fig. 1. All sections stained by Hall's osmium tetroxide procedure as described in METHODS section and Ref. 19. All photomicrographs × 580.
Fig. 3. Typical grade 1 lesions in the left ventricular wall of rats after at least 12 months feeding of any of the experimental diets. Panel A, inflammatory lesion stained by H & E; Panel B, collagen containing lesion stained by Masson's modified trichrome procedure, as in Ref. 21. All photomicrographs × 230.

Fig. 4. Evidence of Yellow Fat disease in perirenal adipose tissue of rats fed n-3 fatty acid enriched TFO diet. Panel A: Interstitial acid-fast pigment accumulation stained by long Ziehl-Neelsen procedure. Photomicrograph × 480. Panel B: Inflammatory infiltration around degenerate fat cell (H & E). Photomicrograph × 240.
dietary supplement. However, the lesions in the LVW of all fat supplemented animals was predominantly (i.e. 50% or more) in the sub-epicardial zone whereas those in the LVW of the low fat-fed reference group were predominantly in the sub-endocardial zone.

There was no dietary induced difference in the severity indices of any dietary group as all groups gave values of S.I. between 0.9 and 1.1. Mean group values for all these parameters are given in Table 2.

No other histological evidence of any dietary induced effect was found in the sections of rat heart muscle that were examined.

Yellow Fat disease

The perirenal adipose fat deposits of rats fed long-term n-3 PUFA’s as the TFO supplement exhibited marked occurrence of lipofuscin pigmentation as well as some evidence of infiltration by predominantly mononuclear cells. This was not observed in any other dietary group including the mature rats in the low fat REF group (Fig. 4). Nor was it observed in any other tissues examined in this study.

DISCUSSION

In recent years the advantages and disadvantages from a diet rich in either the n-6 PUFA’s of vegetable oils or the n-3 PUFA’s of marine oils have been widely discussed, particularly in relationship to coronary heart disease in both man and experimental animals (1-18, 24-36). That many fish oils contain relatively high levels of both C22:1 fatty acids (and cholesterol) has been noted (20, 21) as has the possible connection between long-chain monounsaturated fatty acids and the occurrence of cardiac lipidosis (5, 25-29).

However, most fish oils or fish oil products rich in C22:1 fatty acids (like that of Norwegian Herring oil) are usually derived from fish species which are caught in Nordic or Arctic waters (30), while many species of fish caught in tropical or subtropical waters contain much less of this “unusual” fatty acid. In a recent survey of fish caught in ‘Australian’ waters, Gibson reported that 8 out of 9 species examined contained less than 1% C22:1 and that with the highest value (which was found in a local species of Red Cod) was only 1.2% of the total fatty acids (31).

The specially prepared tuna oil which was used in this study (see also Refs. 13, 16, 35 and 36) was obtained from eviscerated Southern Bluefin tuna also contained less than 1% of its total fatty acids as C22:1, and by dilution, the refabricated TFO diet had a maximum level of less than 1% of this potentially cardiotoxic monounsaturated fatty acid (13, 16, 27, 36).

However the TFO diet is comparatively rich in EPA (6.3%) and DHA (16.6%), but also contains about 30% of its total fatty acids as saturated fatty acids (mainly palmitic and stearic). In this respect, the TFO diet is very similar to the fatty acid composition (proportional %) of the commercially available stock diet (REF) used in our studies (12-16) which also contains (albeit at a lower level) proportionally
more than 30% of its total fatty acids as saturated animal fatty acids.

From the observations presented in Figs. 1 and 2, it is clear that a high incidence of cardiac lipidosis occurred following dietary supplementation with either TFO or SF diets, but was not found in the hearts of rats receiving either the "low fat" REF diet or the "high fat" SSO supplemented diet for more than 12 months. Therefore, because the sunflower seed oil supplemented diet was also low in saturated fats and did not contain any C22 : 1 fatty acids, it seems more likely that the cardiac lipidosis found in the TFO and SF groups, arises from the levels of saturated fats in the diet rather than from the presence of "unusual" long-chain monounsaturates. Indeed it is most unlikely that the presence of trace amounts of C22 fatty acids in the SF diet is the explanation for the incidence of cardiac lipidosis observed in the group fed a supplement of saturated animal fat!

This view is strengthened when it is recalled that C22 : 1 did not accumulate in any major phospholipid fraction of the heart; that is in any major component of the structural lipids of the heart of rats fed the TFO diet for more than 12 months(13, 24, 35).

The relationship between dietary induced cardiac lipidosis and either the incidence or severity of so-called "long-term lesions" in the hearts of experimental animals subjected to a wide variety of lipid supplemented diets has been studied by numerous other investigators (1-7). In general it has been difficult to produce convincing experimental evidence for a causal relationship between cardiac lipidosis and necrosis.

The findings from this study also do not support such a view as the incidence of grade 1 lesions was essentially identical in all the dietary groups including the hearts from those rats which had received the "low-fat" reference diet. This suggests that the lesions observed were more likely to be associated with the age of the animals than any specific dietary effect. Certainly it is not possible to ascribe any potentially deleterious effect to the long-term ingestion of any of the dietary lipid supplements.

However it should also be pointed out that in parallel studies with long term feeding of both n-6 and n-3 PUFA's to rats, we have obtained strong evidence that dietary polyunsaturated fatty acid supplements are of great benefit to the mechanical performance of the heart (14, 16), result in a significant decrease in the susceptibility of the heart to experimentally induced arrhythmias and infarction (15), and a general reduction in several of the risk factors associated with thrombogenesis (36).

Whether the change in distribution of the lesions from predominantly the subendocardial zone in the "low-fat" fed reference group, to predominantly the subepicardial zone of the hearts of all lipid supplemented groups, has any biological significance cannot be determined from this study. Nor does it appear to be related to any of the dietary induced functional changes in myocardial performance we have reported elsewhere (13, 14). However, this observation should be noted for future comparison if it were found to also occur in the hearts of other species of dietary manipulated experimental animals.

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The accumulation of lipofuscin pigment and infiltration by mononuclear cells of the perirenal storage fat deposits of the rat following the long term ingestion of the TFO supplemented diet may be taken as evidence of degenerative changes in this tissue which are usually associated with "Yellow Fat disease" in pigs (17). This condition is thought to arise from a dietary deficiency of vitamin E and has also been reported (18) to occur in rats when large amounts of n-3 fatty acids (such as in the TFO supplemented diet employed in this experiment) are ingested. However, the constant gain in body weight of these animals throughout the whole experimental period (13, 16, 24) and the absence of similar lipofuscin pigmentation in either the liver or kidney sections examined strongly suggests that the amount of vitamin E supplied in the basic (REF) diet was sufficient to prevent serious vitamin E deficiency in these animals.

Nevertheless, it would be prudent to provide additional dietary vitamin E in studies involving the long-term feeding of n-3 PUFA's to rats, and this had been done in several subsequent studies in our laboratory without obvious beneficial or detrimental effect upon the parameters studied (13, 16, 35).

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REFERENCES

1) Abdellatif, A. M. M., and Vles, R. O. (1970): Pathological effects of dietary rapeseed oil in rats. *Nutr. Metab.*, 12, 285–295.

2) Schiefer, B., Lowe, F. M., Laxdal, V., Prasad, K., Forsyth, G., Ackman, R. G., and Offert, E. D. (1978): Morphologic effects of dietary plant and animal lipids rich in docosenoic acids on heart and skeletal muscle of cynomologous monkeys. *Am. J. Pathol.*, 90, 551–558.

3) Opstvedt, J., Svaar, H. J., Hansen, P., Pettersen, J., Langmark, F. T., Barlow, S. M., and Duthie, I. F. (1979): Comparison of lipid status in the hearts of piglets and rats on short term feeding of marine oils and rapeseed oils. *Lipids*, 14, 356–371.

4) Kramer, J. K. G., and Sauer, F. D. (1983): Results obtained with feeding low erucic acid rapeseed oils and other vegetable oils to rats and other species, in *High and Low Erucic Acid Rapeseed Oils*, ed. by Kramer, J. K. G., and Sauer, F. D., Academic Press, New York, pp. 414–471.

5) Beare-Rogers, J. L. (1977): Docosenoic acids in dietary fats. *Prog. Chem. Fats Lipids*, 15, 29–56.

6) Vergroesen, A. J., and Gottenbos, J. J. (1975): Colon and introduction, in *The Role of Fats in Human Nutrition*, ed. by Vergroesen, A. J., Academic Press, New York, pp. 2–36.

7) Duthie, I. F., and Barlow, S. M. (1982): A rat life-span study comparing partially hydrogenated fish oils, partially hydrogenated soyabean and rape-seed oil included in the diet at high levels: Outline, description and interim communication, in *Nutritional Evaluation of Long-Chain Fatty Acids in Fish Oil*, ed. by Barlow, S. M., and Stansby,
8) Craig, B. M., Mallard, T. M., Wright, R. E., Irvine, G. N., and Reynolds, J. R. (1973): Influence of genetics, environment and admixtures of low erucic acid rapeseed in Canada. *J. Am. Oil Chem. Soc.*, **50**, 359–399.

9) Barer, R. (1982): Lipidosis in the rodent heart and other organs, in Nutritional Evaluation of Long-Chain Fatty Acids in Fish Oil, ed. by Barlow, S. M., and Stansby, M. E., Academic Press, London and New York, pp. 185–214.

10) Svaar, H. (1982): The long-term heart lesions phenomenon in animals and humans, in Nutritional Evaluation of Long-Chain Fatty Acids in Fish Oil, ed. by Barlow, S. M., and Stansby, M. E., Academic Press, London and New York, pp. 163–184.

11) Charnock, J. S., Abeywardena, M. Y., McMurchie, E. J., and Russell, G. R. (1984): The composition of cardiac phospholipids in rats fed different lipid supplements. *Lipids*, **19**, 206–213.

12) Charnock, J. S., McLennan, P. L., Abeywardena, M. Y., and Russell, G. R. (1985): Altered levels of n-6/n-3 fatty acids in rat heart and storage fat following variable dietary intake of linoleic acid. *Ann. Nutr. Metab.*, **29**, 279–288.

13) Charnock, J. S., Abeywardena, M. Y., and McLennan, P. L. (1986): Comparative changes in the fatty-acid composition of rat cardiac phospholipids after long-term feeding of sunflower seed oil or tuna fish oil supplemented diets. *Ann. Nutr. Metab.*, **30**, 393–406.

14) Charnock, J. S., McLennan, P. L., Abeywardena, M. Y., and Dryden, W. F. (1985): Diet and cardiac arrhythmia: Effects of lipids on age-related changes in myocardial function in the rat. *Ann. Nutr. Metab.*, **29**, 306–318.

15) McLennan, P. L., Abeywardena, M. Y., and Charnock, J. S. (1985): Influence of dietary lipids on arrhythmias and infarction after coronary ligation in rats. *Can. J. Physiol. Pharmacol.*, **63**, 1411–1417.

16) McLennan, P. L., Abeywardena, M. Y., and Charnock, J. S. (1987): A comparison of the long-term effects of n-3 and n-6 polyunsaturated fatty acid dietary supplements and the action of indomethacin upon the mechanical performance and susceptibility of the rat heart to dysrhythmia. *Prost. Leuk. Med.*, in press.

17) Nafstad, I., and Tollersrud, S. (1970): The vitamin E deficiency syndrome in pigs. I. Pathological changes. *Acta Vet. Scand.*, **11**, 452–480.

18) Danse, L. H., and Verschuren, P. M. (1978): Fish oil-induced yellow fat disease in rats: Histological changes. *Vet. Pathol.*, **15**, 114–124.

19) Hall, P., Gormley, B. M., Jarvis, L. R., and Smith, R. D. (1980): A staining method for the detection and measurement of fat droplets in hepatic tissue. *Pathology*, **12**, 605–608.

20) Adams, C. W. M. (1969): Lipid histochemistry. *Adv. Lipid Res.*, **7**, 1–62.

21) Hall, J. B., and Mathews, G. A. (1979): Simple routine trichrome staining for epoxy resin embedded histological sections. *Aust. J. Med. Technol.*, **10**, 61–64.

22) Stevens, A. (1982): Pigments and minerals: A long Ziehl-Neelsen staining method for lipofuscin, in Theory and Practice of Histological Techniques, ed. by Bancroft, J. D., and Stevens, A., Churchill Livingstone, Edinburgh and New York, pp. 242–266.

23) White, C. (1952): The use of ranks in a test of significance for comparing various treatments. *Biometrics*, **8**, 33–41.

24) Charnock, J. S. (1985): Dietary fats and cardiac function. *Proc. Nutr. Soc. Aust.*, **10**, 25–33.

25) Sinclair, H. M. (1980): Advantages and disadvantages of an Eskimo diet, in Drugs Affecting Lipid Metabolism, ed. by Fumagalli, R., Kritchevsky, D., and Paoletti, R., Elsevier/North Holland, Amsterdam, pp. 363–370.

*J. Nutr. Sci. Vitaminol.*
26) Barlow, S. M., and Stansby, M. E. (1982): Conference participants discussion and summing up, in Nutritional Evaluation of Long Chain Fatty Acids in Fish Oil, ed. by Barlow, S. M., and Stansby, M. E., Academic Press, London and New York, pp. 283–314.

27) Editorial (anon.) (1983): Eskimo diet and disease. Lancet i, 1139–1141.

28) Sauer, F. D., and Kramer, J. K. G. (1980): The metabolism of long-chain monoenoic fatty acids in heart muscle and their cardiopathogenic implications. Adv. Nutr. Res., 3, 207–230.

29) Christophersen, B. O., Norseth, J., Thomassen, M. S., Christiansen, E. N., Norum, K. R., Osmundsen, H., and Bremer, J. (1982): Metabolism and metabolic effects of C22:1 fatty acids with special reference to cardiac lipidosis, in Nutritional Evaluation of Long-Chain Fatty Acids in Fish Oil, ed. by Barlow, S. M., and Stansby, M. E., Academic Press, London and New York, pp. 89–139.

30) Ackman, R. G. (1974): Myocardial alterations resulting from feeding partially hydrogenated marine oils and peanut oils to rats. Lipids, 9, 1032–1035.

31) Gibson, R. A. (1983): Australian fish: An excellent source of both arachidonic acid and ω3 polynsaturated fatty acids. Lipids, 18, 743–752.

32) Glomset, J. A. (1985): Fish, fatty acids and human health. New Engl. J. Med., 312, 1253–1254.

33) Kromhout, D., Bosschieter, E. B., and Coulander, Cor de L. (1985): The inverse relation between fish consumption and 20-year mortality from coronary heart disease. New Engl. J. Med., 312, 1205–1209.

34) Dyerberg, J. (1986): Linolenate-derive polyunsaturated fatty acids and prevention of atherosclerosis. Nutr. Rev., 44, 125–134.

35) Abeywardena, M. Y., McLennan, P. L., and Charnock, J. S. (1987): Long-term saturated fat feeding induced changes in rat myocardial phospholipid fatty acids are reversed by cross-over to polyunsaturated diets: Differences between n-3 and n-6 lipid supplements. Nutr. Res., in press.

36) McIntosh, G. H., McLennan, P. L., Lawson, C. A., Bulman, F. A., and Charnock, J. S. (1985): The influence of dietary fats on plasma lipids, blood pressure and coagulation indices in the rat. Atherosclerosis, 55, 125–134.