Lipid peroxidation, prostacyclin and thromboxane A$_2$ in pigs depleted of vitamin E and selenium and supplemented with linseed oil

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In a 2×2 balanced factorial experiment the biochemical effects on pigs of two dietary factors were investigated. The first factor was α-tocopherol and Se supplementation and the second factor was supplementation with α-tocopherol-stripped linseed oil. In pigs fed on diets depleted of α-tocopherol and Se, increases in concentrations of markers of lipid peroxidation (4-hydroxynonenal and hexanal) were observed. However, skeletal myopathy was only observed in those pigs fed on diets depleted of α-tocopherol and Se and supplemented with oil. In those pigs, increased lipid peroxidation was observed in heart and *supraspinatus* muscle. The plasma concentration of thromboxane B$_2$ was increased in pigs fed on diets depleted of α-tocopherol and Se, suggesting an increased tendency towards platelet aggregation. However, this change was reversed in pigs depleted of α-tocopherol and Se, but supplemented with oil. This may have been a consequence of loss of arachidonic acid, the substrate for thromboxane formation, as a result of lipid peroxidation.

Vitamin E: Lipid peroxidation: 4-Hydroxynonenal: Prostacyclin: Thromboxane A$_2$

Dietetic microangiopathy is a disease of young, rapidly growing pigs, typically aged between 1 and 4 months. It is characterized by sudden death resulting from acute cardiac failure. Post-mortem lesions include myocardial necrosis and fibrinoid necrosis of myocardial arterioles and microthrombosis of myocardial capillaries (Rice & Kennedy, 1989). Necrosis of skeletal muscle, liver and myocardium have been experimentally induced by feeding oils rich in *n*-3 polyunsaturated fatty acids to pigs depleted of vitamin E (α-tocopherol) and Se (Nafstad & Tollersrud, 1970). The lesions produced experimentally were similar to, but not identical with, those of dietetic microangiopathy (Rice & Kennedy, 1989). However, investigations of biochemical alterations associated with these lesions have been largely confined to estimation of the α-tocopherol and Se status of the animals and of changes in the plasma activities of enzymes such as creatine kinase (*EC* 2.7.3.2; CK) and lactate dehydrogenase (*EC* 1.1.1.27; LDH) that are indicative of muscle damage (Ruth & Van Vleet, 1974).

It has been suggested that an imbalance between the production of two eicosanoids derived from arachidonic acid (20:4 *n*-6), namely prostacyclin and thromboxane A$_2$, may cause the microthrombi characteristic of dietetic microangiopathy (Rice & Kennedy, 1989). The eicosanoids are synthesized by the enzymes prostacyclin synthase (*EC* 5.3.99.4) and thromboxane synthase (*EC* 5.3.99.5) respectively, from a cyclic endoperoxide formed, in turn, by the action of cyclooxygenase on C$_{20:4}$.

Prostacyclin, the major product of the cyclooxygenase pathway in endothelial cells of arteries and veins, is vasodilatory and inhibits platelet aggregation (Moncada & Vane,

1979). It is unstable and is metabolized to 6-keto-prostaglandin $F_{1\alpha}$ with a half-life of about 3 min. Thromboxane A_2, the major product of the cyclooxygenase pathway in platelets, is a potent vasoconstrictor, and promotes irreversible platelet aggregation. It, too, is unstable and is metabolized to thromboxane B_2, with a half-life of about 30 s (Moncada & Vane, 1979). The balance between prostacyclin and thromboxane A_2 has been suggested to be of greater importance than individual concentrations of these compounds because of their opposing effects on the circulation and platelet aggregation (Moncada & Vane, 1979).

In the present study the effects on pigs of two factors were investigated. One factor was feeding diets depleted of α-tocopherol and Se. The other factor was feeding diets supplemented with 100 g α-tocopherol-stripped linseed oil/kg to provide a severe peroxidative challenge. The effects of these factors on lipid peroxidation, fatty acid composition and on the balance between the plasma concentration of 6-keto-prostaglandin $F_{1\alpha}$ and thromboxane B_2 were assessed in a 2×2 factorial experiment.

MATERIALS AND METHODS

Basal diet

Wheat, barley and oil-extracted soyabean meal were purchased from a local supplier. NaOH (9·25 mol/l) was applied to the wheat and barley (0·5 and 1 tonne respectively), using a commercially available applicator (Berwyn Engineering, Chippenham, Wilts.), at a level of 1 litre/20 kg grain. This treatment reduces the α-tocopherol content of the grain (McMurray *et al.* 1980). To prevent clumping, and to ensure adequate aeration of the grain, the treated grain was turned daily for at least 15 d before formulation of the complete diets. The mineral–vitamin supplement, which was purchased from Nutrition Services International (Randalstown, Northern Ireland), did not contain either α-tocopherol or Se. The α-tocopherol and Se contents of the basal diet used in the present study were 2·2 and 0·7 nmol/g respectively.

Supplementation with α-tocopherol was in the form of Rovimix E50, supplied by F. Hoffmann-La Roche AG (Basel, Switzerland). Supplementation with Se was in the form of aqueous Na_2SeO_3.

α-Tocopherol-stripped linseed oil was prepared essentially as described by Machlin (1961). Lauroyl peroxide (94 mmol) was added to commercially available linseed oil (2·5 litres). The mixture was placed in a boiling water-bath for 1 h with frequent stirring. This treatment reduced the α-tocopherol content of the oil from 180 to 3·2 nmol/g. The peroxide content of the oil was not measured.

The composition of the complete basal diet is shown in Table 1.

Animal studies

This study complied with the terms of The Animals (Scientific Procedures) Act (1986). The study was designed as a 2×2 factorial experiment, the first factor being dietary α-tocopherol and Se supplementation and the second factor being dietary supplementation with α-tocopherol-stripped linseed oil. Level 1 corresponded to a diet containing supplements of these factors and level 2 corresponded to diets without supplementation with these factors. For the testing of main effects, the null hypothesis was that there was no difference between the levels of factors.

Twenty, 6-week-old pigs were randomly allocated to four equal groups. Pigs in group A were fed on the basal diet supplemented with α-tocopherol and Se; pigs in group B were fed on the basal diet; pigs in group C were fed on the basal diet supplemented with α-tocopherol, Se and 100 g α-tocopherol-stripped linseed oil/kg; and group D was fed on the basal diet supplemented with 100 g α-tocopherol-stripped linseed oil/kg.

Table 1. *Composition of the basal diet**

Ingredient	Amount (g/kg)
NaOH-treated barley	625
NaOH-treated wheat	250
Fat-extracted soyabean meal	100
Vitamin–mineral premix	25
To provide: Na	1·00
Ca	7·40
P	1·00
Mn	0·03
Cu	0·15
Fe	0·10
Zn	0·07
Retinoyl palmitate (mg)	4·40
Cholecalciferol (mg)	0·05
Riboflavin (mg)	2·00
Cyanocobalamin (mg)	0·01
DL-α-Tocopheryl acetate (500 g/kg; mg)	250
Na_2SeO_3 (μg)	200

* DL-α-Tocopheryl acetate and Na_2SeO_3 were added to diets A and C only.

Each group was loose-housed in separate concrete-floored houses and was bedded with straw. All pigs were group-fed with approximately 1·75 kg of the appropriate diet/head per d. Fresh water was available *ad lib.*

Blood was collected by venepuncture of the cranial vena cava or jugular vein at approximately 10 d intervals. After 34 d the animals were killed by an intravenous injection of pentobarbitone sodium, necropsied and tissue samples collected and stored at $-70°$ before biochemical analysis. Samples of heart, several skeletal muscles, liver and fat were collected into 100 ml neutral-buffered formalin/l, processed in paraffin, sectioned at 5 μm and stained with haematoxylin and eosin for histopathological examination. Selected sections were examined in u.v. light for autofluorescence or stained with Ziehl-Neelsen and periodic acid–Schiff with and without pre-diastase digestion.

Analytical techniques

Plasma, tissue and dietary α-tocopherol concentrations were determined by HPLC with fluorescence detection (McMurray & Blanchflower, 1979). Tissue and dietary Se concentrations were determined by atomic absorption spectroscopy using a Varian spectra AA10 (Varian Techtron Pty Ltd, Mulgrave, Australia) complete with an automatic vapour-generation accessory (VGA-76), essentially as described by Gelman (1985).

The fatty acid content of tissues was estimated by capillary gas chromatography with flame ionization detection as described by Christie (1989). Total fatty acid content was not measured. Rather, the percentage contribution of each individual fatty acid to total fatty acids was recorded. Tissue concentrations of hexanal were measured using the same technique as described by Frankel *et al.* (1989), and Fe^{2+}-induced 4-hydroxynonenal (4-HNE) was measured by liquid chromatography–thermospray mass spectrometry as described by Blanchflower *et al.* (1993).

Whole-blood glutathione peroxidase (*EC* 1.11.1.9; GPX) activities and plasma CK and LDH activities were determined at $37°$ on an Hitachi 717 Autoanalyser (Hitachi, Tokyo,

Japan) using test kits (Ransel, CK-NAC and LD 752 respectively) supplied by Randox Laboratories, Crumlin, Northern Ireland. Plasma concentrations of thromboxane B_2 and 6-keto-prostaglandin $F_{1\alpha}$ were measured by enzyme immunoassay using commercially available kits (Amersham International PLC, Amersham, Bucks.).

Statistical analysis

A balanced factorial ANOVA was used to assess the effects of feeding the diets low in the two micronutrients and α-tocopherol-stripped linseed oil. Data are presented as means with their pooled standard errors, in accordance with the factorial design of the experiment. Results for fatty acid analysis were subjected to arcsine square root transformation, and CK and LDH to logarithmic transformation, before analysis.

RESULTS
Clinical signs and histopathology

Pigs fed on a diet low in α-tocopherol and Se and supplemented with linseed oil (group D) became dull, anorexic and recumbent from day 30 until day 34 when the study was terminated. All other pigs remained clinically normal during the study. Steatitis and microscopic lesions of skeletal and cardiac myopathy were seen in pigs from group D. Cardiac lesions were characterized by necrosis of muscle fibres and myocardial fibrosis. Occasional myocardial microthrombi were also present. Small numbers of necrotic and regenerating muscle fibres were seen in sections of a range of skeletal muscles. Necrotic fibres were swollen and hyalinized. Evidence of muscle regeneration was apparent as proliferation of muscle nuclei and formation of thin regenerated muscle fibres. Autofluorescent lipofuscin granules were present in adipose tissue. No significant histopathological changes were seen in tissues from pigs in any of the other groups (data not shown).

Plasma creatine kinase and lactate dehydrogenase activities

There were no significant inter-group differences in plasma CK and LDH activity at the commencement of the experiment (Fig. 1). On day 25 the mean plasma LDH activity in animals in group D was significantly elevated ($P < 0.0001$), by comparison with the activity on day 0. At the end of the study, on day 34, animals in group D had significantly elevated plasma CK ($P < 0.0001$) and LDH ($P < 0.0001$) activities, by comparison with the corresponding values on day 0. In contrast, there was no change in the mean plasma CK or LDH activities of animals in groups A, B and C.

Antioxidant status and indices of lipid peroxidation

Animals fed on diets depleted of α-tocopherol had mean plasma concentrations of α-tocopherol that were significantly lower than those in animals fed on diets supplemented with α-tocopherol, from day 11 until the end of the study on day 34. The mean plasma α-tocopherol concentrations at the end of the study are shown in Table 2. Tissue α-tocopherol concentrations in animals fed on diets depleted of α-tocopherol and Se were significantly lower than those in animals fed on diets supplemented with both micronutrients (Table 2). There were no significant effects of linseed oil supplementation on α-tocopherol concentrations in liver, *supraspinatus* or *longissimus dorsi* (*l. dorsi*). A significant interaction between factors resulted in an increase in the α-tocopherol concentration in heart of pigs in group C by comparison with group A. Animals fed on diets supplemented with α-tocopherol and Se (groups A and C) had significantly higher hepatic Se concentrations than
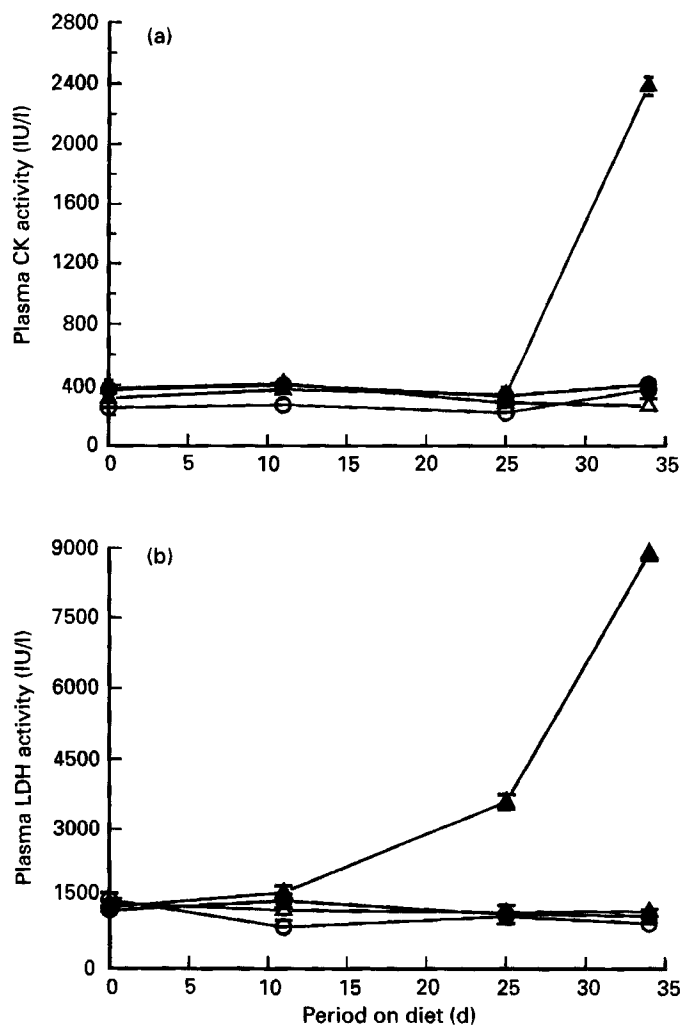
Fig. 1. (a) Creatine kinase (EC 2.7.3.2; CK) and (b) lactate dehydrogenase (EC 1.1.1.27; LDH) activities in pig plasma. Pigs were fed on a basal diet supplemented with α-tocopherol and Se (group A; ○); a basal diet (group B; △); a basal diet supplemented with α-tocopherol, Se and 100 g α-tocopherol-stripped linseed oil/kg (group C; ●); or a basal diet supplemented with 100 g α-tocopherol-stripped linseed oil/kg (group D; ▲); for 34 d. Data are geometric means with their standard errors represented as vertical bars. For details of the basal diet, see Table 1 and p. 370; and for details of analytical procedures, see pp. 371–372.

animals fed on diets depleted of both micronutrients (groups B and D). Supplementation with oil significantly decreased hepatic Se content.

Feeding diets depleted of α-tocopherol and Se increased hexanal and 4-HNE concentrations in all tissues examined (Table 2). Feeding diets supplemented with α-tocopherol-stripped linseed oil had no effect on tissue concentrations of Fe^{2+}-induced hexanal concentrations in any tissue. Only in *supraspinatus* were concentrations of 4-HNE significantly elevated by feeding diets supplemented with linseed oil. However, in both heart and *supraspinatus* a significant interaction led to increased concentrations of 4-HNE in the oil-supplemented, antioxidant-sufficient group (group C). There were no effects of oil supplementation on 4-HNE concentrations in *l. dorsi*.

Table 2. *Plasma (μmol/l) and tissue α-tocopherol, liver selenium and tissue hexanal and 4-hydroxynonenal concentrations (nmol/g) in pigs fed on the experimental diets for 34 d**

(Mean values for five pigs per treatment group)

Treatment group...	A (with E and Se, without oil)	B (without E and Se, without oil)	C (with E and Se, with oil)	D (without E and Se, with oil)	SEM	Effect of low E and Se: P <	Effect of linseed oil: P <	Interaction: P <
Plasma								
α-Tocopherol	6·92	0·44	7·26	0·06	0·44	0·0001	—	—
Heart								
α-Tocopherol	17·48	1·00	23·84	0·77	0·38	0·0001	0·005	0·002
Hexanal	0·32	9·15	0·40	9·15	1·10	0·0001	—	—
4-HNE	4·0	39·4	7·4	27·3	2·89	0·0001	—	0·02
Supraspinatus								
α-Tocopherol	10·06	0·27	11·35	0·23	0·36	0·0001	0·0001	—
Hexanal	0·73	6·43	2·70	5·73	0·57	0·0001	—	—
4-HNE	5·9	32·8	25·5	39·4	2·22	0·0001	0·0001	0·02
L. dorsi								
α-Tocopherol	15·16	0·30	12·49	0·34	0·42	0·0001	—	—
Hexanal	0·72	5·33	1·70	2·66	0·45	0·0001	—	—
4-HNE	8·5	16·7	9·6	16·7	1·49	0·0001	—	—
Liver								
α-Tocopherol	29·87	0·59	29·28	0·45	1·22	0·0001	—	—
Se	16·68	3·80	6·48	2·33	1·12	0·0001	0·0005	0·001

E, vitamin E; oil, α-tocopherol-stripped linseed oil; 4-HNE, 4-hydroxynonenal; *l. dorsi, longissimus dorsi*.
* For details of composition of diets, see Table 1 and for details of procedures, see pp. 370–372.

Table 3. *Tissue fatty acid composition (g/100 g fatty acids identified) in pigs fed on the experimental diets for 34 d**

(Mean values for five pigs per treatment group)

Treatment group... Tissue	Fatty acid	A (with E and Se, without oil)	B (without E and Se, without oil)	C (with E and Se, with oil)	D (without E and Se, with oil)	SEM	Effect of low E and Se: $P <$	Effect of oil: $P <$	Interaction: $P <$
Heart	14:0	0·77	0·40	0·36	0·57	0·11	—	—	—
	16:0	22·09	18·75	17·36	18·16	0·57	—	—	—
	16:1	2·79	0·50	0·28	0·7	0·25	—	—	—
	18:0	15·00	15·47	16·90	15·75	0·62	—	—	—
	18:1	23·81	12·89	12·77	17·34	1·34	—	—	—
	18:2	24·92	35·15	27·84	24·76	1·13	0·01	0·01	0·0001
	18:3	1·26	0·97	17·23	14·45	0·59	0·01	0·0001	—
	20:4	9·37	15·15	6·86	8·19	0·90	0·005	0·0001	—
Supraspinatus	14:0	1·06	0·97	1·02	0·98	0·06	—	—	—
	16:0	25·37	24·43	19·85	21·57	0·49	—	0·0001	0·025
	16:1	4·08	2·99	2·18	2·21	0·23	—	0·0001	0·05
	18:0	13·96	15·53	13·90	15·20	0·79	—	—	—
	18:1	32·84	34·52	27·74	25·89	1·51	—	0·0001	—
	18:2	17·14	15·71	14·45	16·90	0·94	—	—	—
	18:3	1·61	1·47	18·84	13·74	0·44	0·0001	0·0001	0·0001
	20:4	3·95	4·38	2·01	3·51	0·42	0·025	0·005	—
L. dorsi	14:0	1·68	0·91	0·79	0·84	0·36	—	—	—
	16:0	22·90	22·90	13·38	11·99	1·22	—	0·0001	—
	16:1	3·84	4·44	2·59	3·11	0·47	—	0·02	—
	18:0	12·82	11·49	8·75	9·51	0·90	—	0·005	—
	18:1	32·34	41·71	22·21	25·51	1·69	0·0025	0·0001	—
	18:2	18·05	12·70	18·54	20·33	1·62	—	—	—
	18:3	2·39	1·72	29·72	23·16	1·27	0·01	0·0001	—
	20:4	5·97	4·13	4·03	5·63	0·76	—	—	—

E, vitamin E; oil, α-tocopherol-stripped linseed oil; 4-HNE, 4-hydroxynonenal; *l. dorsi, longissimus dorsi.*
* For details of composition of diets, see Table 1 and for details of procedures, see pp. 370–372.

Table 4. Plasma concentrations (pg/ml) of 6-keto-prostaglandin $F_{1\alpha}$ (6-keto $PGF_{1\alpha}$) and thromboxane B_2 (TX B_2) in pigs fed on the experimental diets for 34 d*

(Mean values for five pigs per treatment group)

Treatment group...	A (with E and Se, without oil)	B (without E and Se, without oil)	C (with E and Se, with oil)	D (without E and Se, with oil)	SEM	Effect of low E and Se: $P <$	Effect of linseed oil: $P <$
6-keto PG $F_{1\alpha}$	38·4	52·8	48·6	51·7	10·1	—	—
TX B_2	26·1	72·9	17·2	33·2	13·1	0·05	—
6-keto PG $F_{1\alpha}$:TX B_2	1·52	0·87	2·81	1·86	0·21	0·001	0·0001

E, vitamin E; oil, α-tocopherol-stripped linseed oil.

* For details of composition of diets, see Table 1 and for details of procedures, see pp. 370–372.

Tissue fatty acids

Feeding pigs on diets deficient in α-tocopherol and Se resulted in higher proportions, rather than absolute amounts, of $C_{20:4}$ in heart and *supraspinatus* (Table 3). In *l. dorsi* the $C_{20:4}$ content was unchanged. The proportion of $C_{18:3}$ was decreased by feeding diets depleted of α-tocopherol and Se in all tissues examined. Feeding pigs on diets supplemented with α-tocopherol-stripped linseed oil significantly increased the $C_{18:3}$ content of all tissues; decreased the $C_{20:4}$ content of both heart and *supraspinatus*, but not of *l. dorsi*; and decreased the $C_{18:2}$ content of heart, but not of either *supraspinatus* or *l. dorsi*. In all tissues there were changes also in the content of some of the saturated and monounsaturated fatty acids.

Thromboxane B_2 and 6-keto-prostaglandin $F_{1\alpha}$ concentrations

Neither feeding diets depleted of α-tocopherol and Se, nor supplementation with α-tocopherol-stripped linseed oil, had any significant effect on the plasma concentration of 6-keto-prostaglandin $F_{1\alpha}$ (Table 4). Feeding diets depleted of α-tocopherol and Se did, however, increase the mean plasma concentrations of thromboxane B_2 of pigs in groups B and D. No significant effects of supplementation with linseed oil on the mean plasma concentrations of either 6-keto-prostaglandin $F_{1\alpha}$ or thromboxane B_2 were observed.

Feeding pigs on diets depleted of α-tocopherol and Se significantly reduced the 6-keto-prostaglandin $F_{1\alpha}$:thromboxane B_2 value. However, feeding diets supplemented with α-tocopherol-stripped linseed oil had the opposite effect with the ratio increasing significantly in the oil-supplemented animals.

DISCUSSION

Feeding pigs on diets depleted of α-tocopherol and Se reduced tissue concentrations of α-tocopherol to levels lower than those reported by Neilsen *et al.* (1989) or by Rice & Kennedy (1989) in cases of spontaneous dietetic microangiopathy. Similarly, the mean hepatic Se concentration in pigs in groups B and D were within the range deemed by Blood & Radostis (1990) to be indicative of Se deficiency (1·3–4·4 nmol/g).

Increases in the mean plasma CK and LDH activities of pigs in group D suggested ongoing damage to muscle, as a result of the peroxidative challenge provided by the administration of α-tocopherol-stripped linseed oil to these pigs. The principal fatty acid in linseed oil, linolenic acid (18:3 *n*-3), which constitutes more than 55% of the total fatty acids, is considerably more sensitive to peroxidative damage than is linoleic acid (18:2 *n*-6), the principal fatty acid found in maize oil (Buttriss & Diplock, 1988). We have shown than administration of α-tocopherol-stripped maize oil to pigs fed on diets depleted of both α-tocopherol and Se did not result in severe muscle damage (Nolan *et al.* 1995).

The biochemical evidence of muscle damage was confirmed at necropsy. Histopathological examination of the tissues from pigs in group D revealed necrosis of skeletal and cardiac muscle similar to those previously reported in experimental α-tocopherol and Se deficiency in pigs (Van Vleet *et al.* 1977). However, the cardiac lesions observed in the present study were not identical to those of dietetic microangiopathy (Rice & Kennedy, 1989), in that marked myocardial fibrosis was present, but myocardial haemorrhages were observed only occasionally. An unexpected result obtained in the present study was that supplementation with α-tocopherol-stripped linseed oil significantly decreased hepatic Se concentrations. However, other workers have shown a similar pattern: decreased serum and erythrocyte Se levels in response to oral supplementation of humans with fish oil (Bellisola *et al.* 1992). They also showed that fish-oil supplementation increased glutathione peroxidase activity in erythrocytes and platelets. It is possible that a similar phenomenon occurred in the present study. Unfortunately, we did not measure tissue glutathione peroxidase activity, and so cannot confirm the observations made by Bellisola *et al.* (1992).

Concentrations of 4-HNE and hexanal were increased in all tissues examined of pigs fed on a diet low in α-tocopherol and Se. Similar effects of α-tocopherol deficiency alone, or combined with Se deficiency, were reported in cattle by Walsh *et al.* (1993). We have shown previously in subclinical myopathy in pigs that levels of thiobarbituric acid-reactive substances did not reflect the degree of a peroxidative challenge (Nolan *et al.* 1993). However, we showed that Fe^{2+}-induced 4-HNE did apparently reflect an increased peroxidative challenge caused by feeding maize oil to pigs depleted of α-tocopherol and Se. Given that pigs in group D had severe muscle degeneration, one might have expected to see increased concentrations of Fe^{2+}-induced 4-HNE and hexanal in all the skeletal muscles examined. However, concentrations of hexanal remained unaffected by supplementation with α-tocopherol-stripped linseed oil. Concentrations of Fe^{2+}-induced 4-HNE were only increased by oil supplementation in *supraspinatus*. This finding suggests that it may be unimportant whether a peroxidative challenge is mild, being caused by α-tocopherol deficiency with or without linoleic acid supplementation (Nolan *et al.* 1993), or severe, being caused by α-tocopherol deficiency with linolenic acid supplementation (Table 2). There may be a maximum level of Fe^{2+}-induced 4-HNE that can be attained in any tissue, and no peroxidative challenge can increase Fe^{2+}-induced 4-HNE concentrations further. If this hypothesis is true, the threshold level of Fe^{2+}-induced 4-HNE appears to be approximately 15–40 nmol/g, depending on the tissue.

The $C_{18:3}$ content of all tissues was decreased in pigs fed on diets low in α-tocopherol and Se. This loss of $C_{18:3}$ was probably a result of lipid peroxidation since it is much more susceptible to peroxidation than $C_{18:2}$. The difference in peroxidizability arises from the additional double bond in $C_{18:3}$ (Buttriss & Diplock, 1988). In heart and *supraspinatus* the $C_{20:4}$ content increased in animals fed on diets depleted of α-tocopherol and Se. In assessing the effects of α-tocopherol and Se depletion on tissue levels of $C_{20:4}$, two opposing factors must be taken into account. This fatty acid is considerably more peroxidizable than $C_{18:3}$, thus the tissue $C_{20:4}$ content should have decreased. However, α-tocopherol deficiency has also been reported to increase the content of $C_{20:4}$ in tissues by increasing the conversion of $C_{18:2}$ to $C_{20:4}$ (Witting & Horwitt, 1967). Thus, the assumed loss of $C_{20:4}$ in heart and *supraspinatus* caused by lipid peroxidation of $C_{20:4}$ may be more than compensated for by increased production of $C_{20:4}$ from $C_{18:2}$.

Feeding pigs on diets supplemented with α-tocopherol-stripped linseed oil decreased the $C_{20:4}$ content in heart and *supraspinatus*. This decrease is probably the result of peroxidation of $C_{20:4}$, possibly ultimately to 4-HNE, a product of *n*-6 fatty acid peroxidation.

As mentioned previously, α-tocopherol deficiency has been reported to increase the content of $C_{20:4}$ in tissues by increasing the conversion of $C_{18:2}$ to $C_{20:4}$. In addition, α-tocopherol deficiency has been shown to increase phospholipase A_2 (*EC* 3.1.1.4) activity, thereby increasing the availability of free $C_{20:4}$ (Karpen *et al.* 1981; Douglas *et al.* 1986). Free $C_{20:4}$ can act as a substrate for both thromboxane synthase and prostacyclin synthase to produce prostacyclin and thromboxane A_2 respectively. Thus, one might have expected to see increased concentrations of 6-keto-prostaglandin $F_{1\alpha}$ and thromboxane B_2, the stable metabolites of prostacyclin and thromboxane A_2 respectively, in pigs fed on diets depleted of α-tocopherol and Se. However, there was no significant effect of α-tocopherol and Se on plasma 6-keto-prostaglandin $F_{1\alpha}$ concentrations (Table 4). In contrast, the plasma concentration of thromboxane B_2 increased significantly, in agreement with the findings reported by Cunnane (1988). The difference in response may be as a result of the reported inhibition of prostacyclin synthase by lipid hydroperoxides (Valentovic *et al.* 1982). This inhibition would tend to divert $C_{20:4}$ metabolism towards the pro-aggregatory thromboxane A_2 and away from the anti-aggregatory prostacyclin. The net effect of feeding diets depleted

of α-tocopherol and Se to pigs was to reduce significantly the plasma 6-keto-prostaglandin $F_{1\alpha}$:thromboxane B_2 ratio (Table 4). A reduction in this ratio is associated with an increased tendency for thrombosis to occur (Moncada & Vane, 197). A similar mechanism has been proposed to explain the occurrence of microthrombosis in dietetic micro-angiopathy (Rice & Kennedy, 1989). This hypothesis is supported by the finding of occasional myocardial microthrombi in pigs depleted of α-tocopherol and Se and fed on dietary supplements of linseed oil in the present study.

When pigs were given α-tocopherol-stripped linseed oil, the plasma 6-keto-prostaglandin $F_{1\alpha}$:thromboxane B_2 ratio was restored to a level similar to that observed in pigs in group A. Thus, the tendency to promote platelet aggregation caused by α-tocopherol depletion was, paradoxically, reversed by the severe peroxidative challenge posed by supplementation with $C_{18:3}$. This reversal may have occurred consequent to the decrease in $C_{20:4}$, the substrate for eicosanoid formation, as a result of lipid peroxidation (Table 3).

In conclusion, we have shown that clinical myopathy can be induced in pigs depleted of α-tocopherol and Se, by feeding supplementary linseed oil. Changes in tissue levels of Fe^{2+}-induced 4-HNE, $C_{18:3}$ and $C_{20:4}$ suggest that lipid peroxidation occurs in tissues of these pigs. Depletion of α-tocopherol decreases the 6-keto-prostaglandin $F_{1\alpha}$:thromboxane B_2 ratio, thus increasing the tendency of platelets to aggregate. Supplementation of pigs with a source of highly peroxidizable fatty acids reversed the changes in the 6-keto-prostaglandin $F_{1\alpha}$:thromboxane B_2 ratio, presumably as a result of increased loss of $C_{20:4}$ through lipid peroxidation.

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