Prostaglandins and their Precursors in Tissues from Rats Fed on *trans,trans*-Linoleate

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Feeding *trans,trans*-9,12-linoleate to rats as 50 and 100% of the dietary fat decreased the concentrations of *n*-6 fatty acids, i.e. 18:2, 20:3 and 20:4, in heart, kidney, lung, adipose tissue and platelets of rats. The concentrations of prostaglandin products prostaglandins E₁, E₂ and F_{2α} in serum were significantly decreased in rats receiving high concentrations of dietary *trans,trans*-linoleate.

Dietary fat is strongly implicated in atherosclerosis and coronary heart disease, and the public has been advised to consume more unsaturated vegetable fats (Levy *et al.*, 1979). Though a clear cause and effect has not been shown, the elucidation of the potent effects of prostaglandins and related metabolites provides strong evidence of an ameliorating role of dietary unsaturated fatty acids in coronary heart disease (Vergroesen, 1975*a,b*, 1977). Dietary fatty acids affect several tissues whose physiological functions are regulated by prostaglandins (Vergroesen, 1975*a,b*). For example, thrombosis caused by platelet aggregation is a frequent lethal complication of coronary heart disease and may be involved in atherogenesis (Hornstra, 1975). Saturated dietary fat is associated with increased platelet aggregation, whereas unsaturated fatty acids, e.g. linoleic acid (18:2, *n*-6), reduce aggregation (Iacono, 1974; Vergroesen, 1975*b*; Hornstra, 1975; Fleishman *et al.*, 1975). This effect is apparently modulated by the relative production of prostaglandins which control platelet aggregation (Anonymous, 1976; Silver *et al.*, 1977). Thus prostaglandin endoperoxides, prostaglandins G₂ and H₂, thromboxane and prostaglandin E₂, synthesized from arachidonic acid (20:4, *n*-6) by platelets after stimulation, promote platelet aggregation. These are counteracted by prostacyclin produced from prostaglandin H₂ by enzymes located in vascular endothelial cells (Anonymous, 1976; Silver *et al.*, 1977; Marcus, 1978; Gorman, 1979). In addition prostaglandin E₁, synthesized from eicosatrienoic acid (20:3, *n*-6), may retard platelet aggregation (Hornstra, 1971).

Dietary fatty acids, by affecting the synthesis and availability of prostaglandin precursors, i.e. 20:3, *n*-6 and 20:4, *n*-6, may influence platelet functions and thrombosis (Hornstra, 1975). Dietary fatty acids affect prostaglandin production (Hwang *et al.*, 1975). The *n*-6 dietary unsaturated fatty acids,

e.g. 18:2, *n*-6, apparently enhance prostaglandin production, whereas *n*-9 unsaturated fatty acids interfere with their synthesis (Hulan & Kramer, 1977; Van Evert *et al.*, 1978). Essential fatty acid deficiency decreases prostaglandin synthesis in rat tissues (Kaa, 1976; Dunham *et al.*, 1978). Little is known of the effects of *trans*-fatty acids on prostaglandin metabolism. Both Kummerow (1974) and Mann (1977) alluded to the potentially undesirable effects of dietary *trans*-fatty acids, and Privett *et al.* (1977) reviewed their effects in experimental animals with particular emphasis on the disruption of normal metabolism of unsaturated fatty acids.

Because of the occurrence of *trans*-fatty acids in various amounts in processed vegetable shortenings and margarines (Kummerow, 1974; Mann, 1977; Carpenter & Slover, 1973), we have studied the effects of high concentrations of dietary *trans*-fatty acids on the concentration of prostaglandin precursors in rat tissues and the relative production of prostaglandins by platelets to ascertain if dietary *trans,trans*-18:2 fatty acids affect their synthesis.

Materials and Methods

Weanling male Sprague-Dawley rats (approx. 50g) were kept individually in stainless-steel cages in a controlled-atmosphere animal room. The rats were randomly divided into four groups, and each group (10 rats each) was fed on identical balanced rations (Hwang *et al.*, 1975), except that the fat was different. Thus group 1 received only *cis,cis*-linoleate (18:2, *n*-6,9); group 2 received a 50:50 mixture of *cis*-18:2 and *trans*-18:2 fatty acids; group 3 received *trans,trans*-linoleate (*trans*-18:2, *n*-6,9); group 4 received hydrogenated coconut fat, at 5% of the diet (11% of total calories). Diets were mixed daily to minimize autoxidation, and water was available *ad libitum*. The fatty acids (99% pure) were custom-

produced by Nu-Chek-Prep (Elysian, MN, U.S.A.). After 12 weeks, the rats were anaesthetized, and blood was withdrawn from the abdominal aorta. Serum and platelets were prepared from portions of the blood samples pooled from each group (Hwang *et al.*, 1975). The remainder was incubated at 37°C to maximize prostaglandin production by the platelets during clotting (Hwang & Kinsella, 1978; Silver *et al.*, 1972).

The concentration of prostaglandins was determined by the radioimmunoassay technique (Hwang *et al.*, 1975). Although endoperoxides, thromboxane and prostacyclin are the most active physiologically (Silver *et al.*, 1977; Gorman, 1979), we measured prostaglandin end products, i.e. prostaglandins E₁, E₂ and F_{2α}, because we had appropriate antisera, and, furthermore, on the basis of the rationale of other workers (Dunham *et al.*, 1978; Clausen & Srivastava, 1972; Pace-Asciak & Rangaraj, 1978), we assumed that the concentrations of these products reflected the relative capacities of the platelets to synthesize these and their precursors. The validity of the assay system was tested and the data were analysed as described previously (Hwang *et al.*, 1975).

Organs were removed from rats immediately *post mortem*, weighed and frozen in liquid N₂, and their compositions subsequently determined. Lipids were extracted by the method of Folch *et al.* (1957). Phospholipids and protein were quantified by the procedures of Raheja *et al.* (1973) and Lowry *et al.*

(1951) respectively. Phospholipids were separated by t.l.c. (Kinsella & McCarthy, 1968), and their fatty acid compositions determined by g.l.c. (Kinsella *et al.*, 1977). The fatty acids of the phospholipids from heart, kidney and lung were examined because these, via phospholipase A₂ action, are major sources of prostaglandin precursors, i.e. 20:3, *n*-6 and 20:4, *n*-6, and prostaglandins are involved in controlling the functions of these organs (Hornstra, 1975; Vergroesen, 1977; McGiff, 1979). Other unsaturated fatty acids, which may be released from phospholipids and possibly inhibit prostaglandin synthesis (Nugteren, 1970; Flower & Vane, 1974; Hulan & Kramer, 1977), were also quantified.

Results and Discussion

Increasing concentrations of dietary *trans,trans*-18:2 fatty acid markedly decreased growth (Hwang & Kinsella, 1978), and organ weights (Table 1). The rats receiving 100% of their dietary fat as *trans,trans*-18:2 fatty acid showed aggravated classical symptoms of essential fatty acid deficiency (Holman, 1978). The *trans*-18:2 fatty acids generally decreased the total lipids, phospholipids and proteins in the organs analysed. Marked changes in the fatty acid composition of the phospholipids were observed (Table 1). The *n*-9 series of fatty acids increased with intake of *trans*-18:2 fatty acid, particularly oleic acid (18:1), and, as is characteristic of essential fatty acid deficiency, the concentration of 20:3, *n*-9 was

Table 1. Average composition and fatty acids of organs from rats receiving various amounts of *trans,trans*-linoleate for 12 weeks

Dietary <i>trans,trans</i> -linoleate concentration (%) ...	Heart			Kidney			Lung			Adipose		
	0	50	100	0	50	100	0	50	100	0	50	100
Body wt. (g)	373	334	228	373	334	228	373	334	228	373	334	228
Organ wt. (g)	1.06	0.92	0.72	2.52	2.50	2.03	1.95	1.63	1.49	1.18	1.16	1.04
Lipid	39.8	38.6	33.9	42.6	40.9	32.2	94.6	116.2	32.4	863	827	830
Phospholipid } (mg/g)	23.9	22.6	18.5	21.6	19.5	18.6	43.0	52.9	15.3	50.4	47.1	22.8
Protein	214.3	212.5	180	216.0	172.7	155.7	205.5	211.1	199.0	—	—	—
Fatty acids	Fatty acid composition of phospholipids (% by wt.)											
16:0	10.9	13.5	7.0	18.2	17.1	15.3	60.1	56.8	45.8	26.1	19.0	25.0
18:0	23.4	28.5	23.6	23.3	23.6	18.1	7.0	7.0	3.4	7.8	13.1	8.1
16:1, <i>n</i> -7	—	—	7.0	1.6	1.6	6.1	6.0	5.2	13.9	11.5	9.2	13.1
18:1 } <i>n</i> -9	9.4	12.5	39.3	14.3	16.5	39.1	10.6	14.8	17.1	29.4	25.1	28.6
20:3 }	—	—	5.9	—	—	5.4	—	—	1.7	—	—	3.7
18:2- <i>trans</i>	—	2.8	6.1	—	1.1	3.1	—	1.0	3.3	—	1.6	2.5
18:2 }	22.6	28.2	8.3	11.6	14.1	4.3	3.4	6.0	1.7	12.2	10.8	4.7
20:3 }	—	—	—	0.7	—	—	—	—	—	1.7	4.5	—
20:4 } <i>n</i> -6	18.0	11.1	2.4	27.0	24.3	7.5	1.7	1.1	1.3	3.6	3.1	2.5
22:4 }	2.6	0.9	—	1.0	0.6	—	1.1	0.8	—	—	1.1	2.2
22:5 }	12.2	1.6	—	1.3	—	—	—	—	—	2.2	—	—
20:5 }	—	—	—	0.2	0.1	0.3	—	—	0.3	2.2	2.3	2.0
22:5 } <i>n</i> -3	—	—	—	—	—	—	0.4	0.4	0.2	—	—	0.6
22:6 }	—	—	—	—	0.4	0.1	1.2	1.0	0.3	—	—	—

increased. These fatty acids, which can inhibit prostaglandin synthetase (Nugteren, 1970; Flower & Vane, 1974; Hulan & Kramer, 1977; Dunham *et al.*, 1978), increased at the expense of the *n*-6 series, i.e. essential fatty acids (18:2, *n*-6, 20:4, *n*-6), particularly in the organs from rats receiving *trans*-18:2 fatty acids as sole source of dietary fat. It is noteworthy that, in the heart, kidney and lungs from rats on the diet containing an equi-mixture of *trans*- and *cis*-18:2 fatty acids, the concentration of 18:2, *n*-6 fatty acid was increased compared with the rats fed on dietary *cis*-18:2 fatty acid alone. Arachidonic acid (20:4, *n*-6), the precursor of the principal endoperoxides and prostaglandins, was markedly decreased by dietary *trans*-18:2 fatty acid in phospholipids in heart and kidney, but it was only slightly affected in lung and adipose tissue where its normal concentration is low. Eicosatrienoic acid (20:3, *n*-6), the precursor of prostaglandin *E*₁, was not detectable in the phospholipids of heart or lung, but was present in kidney and adipose tissue. The *trans*-18:2 fatty acid apparently decreased synthesis of prostaglandin *E*₁ in kidney tissue. Both 22:4, *n*-6 and 22:5, *n*-6 fatty acids, which occurred most abundantly in heart tissue, were depleted in hearts from rats fed on *trans*-18:2 fatty acids. These fatty acids by retroconversion (Sprecher, 1977) can be converted to 20:4, *n*-6 fatty acid, and thus they may represent a reserve form of prostaglandin precursors. The *n*-3 series, which was absent from cardiac tissue, was present in small but varying amounts in the other tissues.

These data corroborate those from earlier studies (Privett *et al.*, 1977), which showed that mixtures of *trans*-fatty acids decreased essential fatty acid production and also inhibited some enzymes. In contrast with observations of essential-fatty acid-deficient animals, in which the fatty acid composition of different organs may be depleted in varying degrees (Weston & Johnson, 1978), our data revealed that dietary *trans,trans*-linoleate caused rather similar changes in the fatty acid composition of the organs studied.

The 20:3, *n*-6 and 20:4, *n*-6 fatty acid contents of the total lipids of heart, liver, serum and platelets were also markedly decreased by dietary *trans*-18:2 fatty acid, and 100% dietary *trans*-18:2 fatty acid was more severe in its effects than the hydrogenated coconut fat diet (Table 2). Significantly, prostaglandins *E*₁, *E*₂ and *F*_{2α} were even more markedly decreased than their respective precursors in platelets or serum. The effect of 50% *trans*-18:2 fatty acid in significantly decreasing all these prostaglandins, even when adequate amounts of precursors were apparently available, is noteworthy. This may indicate that the *trans,trans*-18:2 fatty acid or metabolic derivatives thereof inhibited some of the enzymes involved in prostaglandin synthesis. In

Table 2. Relative tissue concentrations of eicosatrienoic and eicosatetraenoic acids (percentage by weight) and serum prostaglandins *E*₁, *E*₂ and *F*_{2α} (ng/ml) in rats receiving *cis*, *cis*-linoleic acid, *trans,trans*-linoleic acid or hydrogenated coconut oil as dietary fat for 12 weeks
The results for prostaglandin concentrations are means ± s.e.m. for four determinations. Abbreviations: t, trace, <0.05; u, undetected; 18:2, linoleate.

Dietary fat	Fatty acid and prostaglandin concentrations												
	Eicosatrienoic acid (20:3, <i>n</i> -6)						Eicosatetraenoic acid (20:4, <i>n</i> -6)						
	Prostaglandin			Prostaglandin			Prostaglandin			Prostaglandin			
	Kidney	Heart	Liver	Serum	Platelets	E ₁	Kidney	Heart	Liver	Serum	Platelets	E ₂	F _{2z}
100% <i>cis</i> -18:2	0.01	t	0.50	0.15	0.35	5.70 ± 0.70	9.30	23.50	26.7	30.0	17.6	24.90 ± 4.30	27.40 ± 3.20
50% <i>cis</i> -18:2+50% <i>trans</i> -18:2	t	u	0.30	0.10	0.10	3.50 ± 0.60	6.50	20.50	21.3	25.2	14.1	11.60 ± 2.60	14.10 ± 2.90
100% <i>trans</i> -18:2	u	u	t	u	t	0.20 ± 0.02	2.30	5.20	3.6	0.8	1.4	0.10 ± 0.03	0.30 ± 0.60
100% Hydrogenated coconut oil	u	u	t	u	u	1.10 ± 0.02	u	u	5.0	u	2.0	2.20 ± 0.85	3.20 ± 0.90

essential fatty acid deficiency, Dunham *et al.* (1978) suggested that the increased concentrations of 20:3, *n*-9 fatty acid may interfere with the enzymes of prostaglandin synthesis.

These data indicate that the decrease in prostaglandin production by platelets from rats on high dietary contents of *trans*-18:2 fatty acid is much more marked than the decrease in the corresponding fatty acid precursors of these prostaglandins. Further studies to monitor the effects of much lower concentrations of dietary *trans*-18:2 fatty acids on these parameters are required.

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