CLINICAL STUDY

The effect of gamma-linolenic acid on plasma and membrane lipids and renal prostaglandin synthesis in older subjects

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Abstract

Senescence is associated with a decreased activity of enzyme delta-6 desaturase, which converts linoleic acid to gamma-linolenic acid. This enzymatic defect may alter the composition of plasma and membrane lipids, and influences the biosynthesis of renal prostaglandins. Exogenous supplementation of GLA during 3 months increases the plasma level of dihomo-gamma-linolenic acid ($p<0.002$), and to a smaller degree, the level in erythrocyte membrane lipids. This treatment was associated with a beneficial reduction of cardiovascular risk factors (arterial hypertension, total cholesterol, apolipoprotein B, HDL-cholesterol, apolipoprotein A-I) and the renal function has become stable reached. Epogam treatment also increased the biosynthesis of renal prostaglandins, especially that of prostaglandin E_2, which has a vasodilatory effect on vessel walls and reduces the elevated blood pressure.

Conclusion: Dietary supplementation of essential fatty acids such as gamma-linolenic acid to old subjects has beneficial effect on their health condition. ***(Tab. 6, Fig. 5, Ref. 37.)***

Key words: gamma-linolenic acid, delta-6-desaturase, prostaglandins, old subject.

Senescence in humans is characterized by the appearance of several cardiovascular complications such as atherosclerosis, ischemic heart disease, arterial hypertension, stroke and decrease in renal function. Aging is also associated with various metabolic defects such as decreased activity of delta-6-desaturase (7, 9—11, 21, 24, 25), which converts cis-linoleic acid (C 18:2n-6) to gamma-linolenic (GLA, C 18:3n-6) and alpha-linolenic acids (ALA, 18:3n-3) to stearidonic acid (18:4n-3) (2) (Fig. 1). However, aging affects more the affinity of the enzyme with respect to the (n-6) series substrates than to (n-3) series substrates (26). Therefore a deficit in delta-6-desaturase may contribute to disequilibrium in plasma and membrane lipids as well as in prostaglandin biosynthesis. It has been suggested that the resulting deficiency may be one of the key factors involved in aging (1, 21, 24).

The aim of this study

was to test whether a prolonged supply of exogenous gamma-linolenic acid to older subjects: a) may overcome the presumed enzymatic defect and may improve the plasma and membrane lipid profiles, b) may ameliorate their cardiovascular, renal and metabolic states and renal synthesis of prostaglandins (PG) and thus improve the general health state in older subjects (5, 8, 15—18, 20, 29—31).

Subjects and methods

Subjects

Ten mobile elderly subjects were included into the study, 1 man and 9 women of mean age of 83±8 (SD) years (range 69—90) who were hospitalized initially at the Geriatric Department. All

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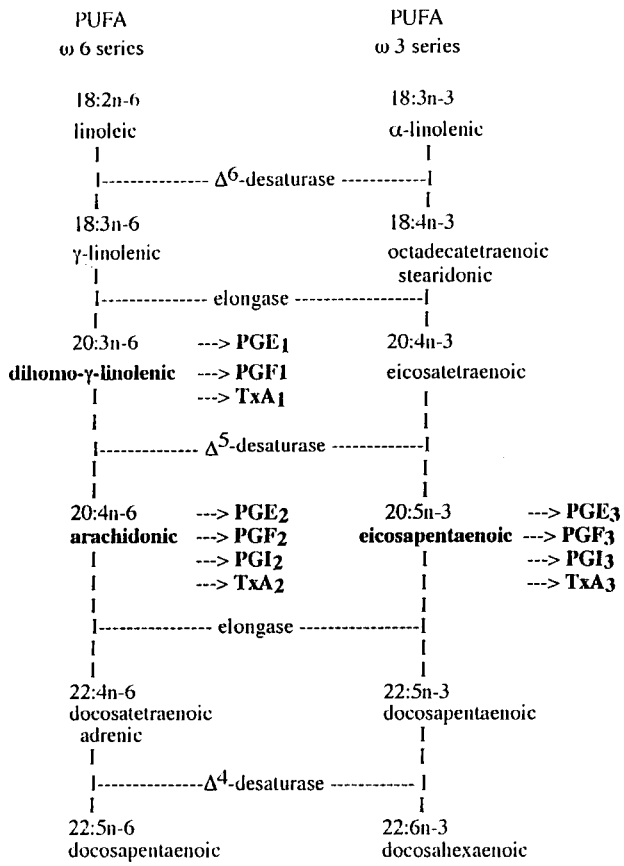

Fig. 1. Enzymatic transformation of essential fatty acids and the generation of prostaglandins 1,2,3 series by the cyclooxygenase pathway from corresponding polyunsaturated fatty acids.

subjects were without specific metabolic or degenerative diseases and were in satisfactory mental states: They all gave written consent to the study. The study protocol was approved by the Ethic Committee of Hôtel-Dieu Hospital, Paris. After the initial hospitalization, they were discharged from hospitaland followed up by ambulatory treatment with a daily suplement of 320 mg of gamma-linolenic acid given as Epogam capsules (Scotia Pharmaceuticals Ltd) 4x2/day; each capsule containing 500 mg of evening primrose oil supply 40 mg of GLA and 10 mg of vitamin E. The composition of primrose oil (27) has been shown in Table 1.

Clinical investigation

This was a three-month open study with an inclusion period on day 0 (D0) and three-month treatment with clinical and biochemical monitorings on D30 and the study was ultimated on D90. The clinical examination assessed body weight, arterial blood pressure (BP) twice, and heart rate in the supine and upright (sitting) position, and general physical and psychological states.

Tab. 1. Composition of evening primrose oil expressed in % of total fatty acids.

Palmitic acid	C 16:0	6.6 %
Palmitoleic acid	C 16:1n-7	0.1 %
Stearic acid	C 18:0	1.7 %
Oleic acid	C 18:1n-9	10.9 %
Linoleic acid	C 18:2-6	71.5 %
gamma-linolenic acid	C 18:3n-6	8.6 %
alpha-linolenic acid	C 18:3n-3	0.2 %
arachidic acid	C 20:0	0.3 %
gadolenic acid	C 20:1n-9	0.1 %

Biochemical examinations

Biochemical evaluation included: 1) the measurement of plasma electrolytes, Na, K, and alkali reserve from 5 ml of heparinized blood; 2) creatinine, total cholesterol, HDL cholesterol, apolipoprotein (apo)A 1 and apolipoprotein (apo)B from 5 ml of blood sampled in a dry tube, 3) sampling of 10 ml of blood in EDTA for the measurement of plasma and membrane (erythrocyte) fatty acids; 4) renal investigation including morning (4 hours) urinary sample for the measurement of urinary Na, K, creatinine and urinary PG: 6-keto-$PGF_{1\ alpha}$ (metabolite of prostacyclin PGI_2), PGE_2, $PGF_{2\ alpha}$ and Thromboxane (Tx) B_2 (metabolite of TxA_2). The samples for the measurement of plasma and membrane lipids and urinary samples for the measurement of PG were frozen at -30 °C, and sent to corresponding laboratories to be measured.

Methods

Arterial blood pressure was measured by sphygmomanometer. Plasma and urinary electrolytes and plasma lipids were measured by usual automated techniques at the Biochemistry Department of the St. Perine Hospital. Plasma and erythrocyte membrane fatty acids (C14—C22) were measured by automated gas chromatography at Efamol Research Institute, Kentwille, Nova Scotia, Canada (37). Urinary PG was measured by radioimmunoassay after previous extraction and chromatographic separation by HPLC (19, 20) at the Eicosanoid Laboratory of Broussais Hospital. The statistical analysis was performed by non parametric Friedman test.

Results

A. Clinical results

The arterial systomic, but especially the diastolic blood pressure, were moderately but not significantly decreased over the 3-month period, as shown in Table 2 and Figure 2. Heart rate was unchanged during the treatment in both, supine and upright positions, the mean being about 70—72 beats/min. The body weight was stable in all subjects and there were no apparent other clinical modifications. The treatment with Epogam capsules was well tolerated throughout the study: two patients reported an acceleration of intestinal transit but there were no other apparent side effects.

Tab. 2. The effect of Efamol treatment on arterial blood pressure (x±SD).

	D0	D30	D90	p
Supine BP mmHg				
systolic	144±12	140±15	142±16	NS
diastolic	87±13	84±11	83±14	NS
Sitting BP mmHg				
systolic	144±12	139±15	142±16	NS
diastolic	86±12	83±11	81±13	NS

Tab. 3. The effect of Efamol treatment on plasma lipids.

		D0	D30	D90	p
Cholesterol	mmol/l	6.07±1.30	5.66±0.77	5.81±1.06	NS
HDL	mmol/l	1.81±0.50	1.90±0.50	1.90±0.51	0.15
Triglycerides	mmol/l	1.21±0.46	1.24±0.89	1.37±0.95	NS
$ApoA_1$	g/l	1.35±0.30	1.38±0.35	1.51±0.30	NS
ApoB	g/l	1.05±0.28	1.02±0.26	1.12±0.37	NS

B. Biochemical results

B.1. Plasma Na, K, alkali reserve were unchanged. Values for plasma creatinine were as follows: D0 73±17, D30 75±17, D90 77±19 μmol/l. The effect of Epogam treatment on plasma lipids is shown in Table 3. There was a decrease in total cholesterol with a simultaneous increase in HDL-cholesterol and apoA1, however, none of these changes were statistically significant. No changes in triacylglycerol and apoB concentrations in serum were confirmed. *Renal functions:* Diuresis, natriuresis, kaliuresis and creatinine clearance were not significantly changed during the time of Epogam treatment.

B.2. Urinary prostaglandins

The results are shown in Figs 3 and 4. The basal 6-keto-PGF_1 alpha excretion was low and not influenced by treatment.

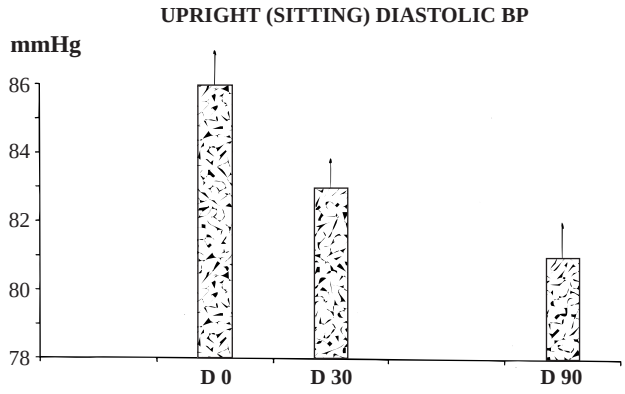

Fig. 2. Effect of Efamol on sitting diastolic blood pressure (BP) after 30 and 90 days of treatment. The results are expressed by mean value±SD.

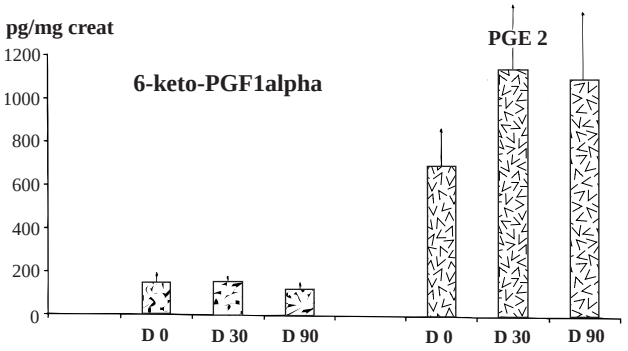

Fig. 3. Effect of Efamol treatment on urinary excretion of prostaglandins 6-keto-$PGF_{1\ alpha}$ and PGE_2.

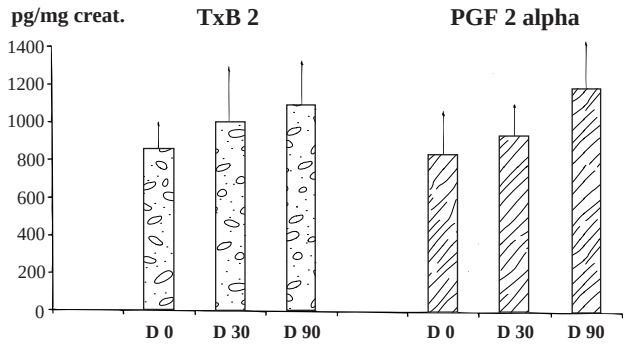

Fig. 4. Effect of Efamol treatment on urinary excretion of thromboxane B2 and $PGF_{2\ alpha}$.

On the other hand, all other prostaglandins measured, and especially PGE_2 increased non significantly after 30 and 90 days of treatment (+86 %), however without statistic significance.

B.3. Plasma essential fatty acids

The effect of Epogam treatment on plasma fatty acids is shown in Table 4 and Figure 5. The treatment with exogenous supply of gamma-linolenic acid increased significantly the plasma levels of dihomo-gamma-linolenic acid ($p<0.02$), non-significantly the adrenic acid ($p<0.12$) and decreased significantly the leic acid ($p<0.02$) and non significantly the docosahexaenoid acid ($p<0.06$). The other fatty acids were not sinificantly modified, including the linoleic acid.

B.4. Membrane fatty acids

The analysis of erythrocyte membrane fatty acids before and after the treatment with Epogam shows Table 5. There was a moderate non significant increase in membrane dihomo-gamma-liniolenic acid (+16 %) and linoleic acid (+11 %) and non significant decrease in both docosapentaenoic acids. Otherwise, there were no significant modifications in membrane lipids.

Tab. 4. Plasma essential fatty acids before (D0) and after 30 (D30) and 90 (D90) days of treatment with evening primrose oil (Epogam). Results expressed in % of total fatty acids.

Fatty acid		D0	D30	D90	p
C 14:0	myristic	0.49±0.14	0.48±0.15	0.39±0.11	0.20
C 16:0	palmitic	26.65±1.29	27.08±0.75	27.32±1.00	0.30
C 16:1n-7	palmitoleic	1.64±0.46	1.83±0.43	1.66±0.40	0.67
C 18:0	stearic	8.31±1.68	8.69±1.22	8.85±0.43	0.50
C 18:1n-9	oleic	10.95±1.76	10.24±1.18	10.59±0.04	<0.02
C 18:2n-6	linoleic	23.60±4.0	23.80±3.7	23.30±2.8	0.67
C 18:3n-3	α-linolenic	0.243±0.057	0.238±0.059	0.244±0.073	0.98
C 20:2n-6	eicosadienoic	0.426±0.146	0.392±0.152	0.433±0.179	0.31
C 20:3n-6	dihomo-τ-linolenic	3.60±0.72	4.26±0.89	4.12±1.02	<0.002
C 20:4n-6	arachidonic	13.39±1.90	13.61±2.06	13.29±2.70	0.90
C 20:5n-3	eicosapentaenoic	1.52±0.51	1.31±0.69	1.33±0.50	0.30
C 22:4n-6	adrenic	0.371±0.148	0.397±0.113	0.411±0.099	0.12
C 22:5n-6	docosapentaenoic	0.46±0.30	0.43±0.15	0.44±0.11	0.50
C 22:5n-3	docosapentaenoic	1.03±0.15	1.01±0.24	1.02±0.22	0.27
C 22:6n-3	docosahexaenoic	5.83±0.92	5.44±0.76	5.35±1.40	0.06

Discussion

The age-dependent reduction of delta-6-desaturase (7, 9—11, 21, 24, 25) may produce metabolic defects in the synthesis of n-6 essential fatty acids in old subjects (21), especially that of gamma-linolenic acid as well as higher unsaturated fatty acids such as dihomo-gamma-linolenic acid and arachidnic acid. The objective of our study was to overcome this possible enzymatic defect by the exogenous supply of gamma-linolenic acid in form of evening primrose oil (Epogam). The mean plasma concentration of gamma-linolenic acid before the treatment was low in comparison with otherstudies (Tab. 6).

The 3-month treatment with Epogam in 10 old subjects increased significantly the plasma levels of dihomo-gamma-linolenic acid in all of them, especially after the first month (Tab. 4, Fig. 5). It should be noted that the initial levels of dihomo-gamma-linolenic and arachidonic acids before treatment were higher than in published American studies (Harrison study, World study) (Tab. 6), but similar to the data of Lassere et al (35). These differences may depend on defferent dietary habits in different populations. The significant increase in dihomo-gamma-linolenic acid is produced by the exogenous supply of gamma-linolenic acid and its elongation, but may also be due to the increased activity of delta-6-desaturase itself, because gamma-linolenic acid supplementation in old animals stimulates the enzyme (26, 28). Therefore the administration of GLA in form of evening primrose oil can reverse the decline in delta-6-desaturase activity (6, 26). This helps to increase not only the essential fatty acids of (n-6) series but also those of the (n-3) series, the effect of which would reverse the fall in membrane unsaturation index and membrane fluidity associated with aging (6) and improve the synthesis of PG (3, 4). However in our study the increase in dihomo-gamma-linolenic acid was associated with a moderate decrease in (n-3) acids such as eicosapentaenoic and docosahexaenoic acids (13) (Tab. 4). A similar observation was reported in humans by Darcet et al (8, 12). This may be due to the saturation of the delta-5-desaturase activity by an increased afflux of dihomo-gamma-linolenic acid. The significant decrease of oleic acid may be a consequence of the inhibition of delta-9-desaturation. The explanation of adrenic acid increase is more hypothetical: it could be due to the decrease in eicosapentaenoic acid which may open on part of the elongase activity to the n-6 chain allowing the transformation of arachidonic acid into adrenic acid. Arachidonic acid concentration was apparently not modified by the treatment. However it has been shown that the 5-desaturase step which converts dihomo-gamma-linolenic acid to arachidonic acid is rate limiting and particularly slow in humans (24), the fact of which may explain the small variation of plasma arachidonic acid concentration.

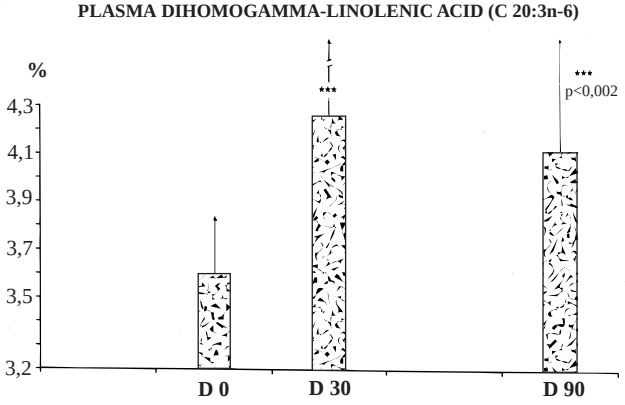

Fig. 5. Effect of Efamol treatment on plasma levels of dihomo-gamma-linolenic acid after 30 and 90 days of treatment. Results are expressed in % of total fatty acids.

Membrane fatty acids

An increase in dihomo-gamma-linolenic acid in erythrocyte membrane phospholipids was also achieved but it was non sig-

Tab. 5. Erythrocyte membrane essential fatty acids before (D0) and after 30 (D30) and 90 (D90) days of treatment with evening primrose oil (Epogam). Results expressed in % of total fatty acids.

Fatty acid		D0	D30	D90	p
C 14:0	myristic	0.51±0.24	0.51±0.07	0.55±0.19	0.89
C 16:0	palmitic	27.50±7.8	27.80±3.6	29.00±8.00	0.72
C 16:1n-7	palmitoleic	1.13±0.55	1.16±0.37	1.06±0.14	0.31
C 18:0	stearic	15.90±2.0	16.10±2.6	16.40±3.2	0.72
C 18:1n-9	oleic	17.20±3.3	16.80±2.2	17.40±3.4	0.64
C 18:2n-6	linoleic	7.80±2.8	9.30±2.4	8.70±2.8	0.46
C 18:3n-3	α-linolenic	0.20±0.03	0.25±0.04	0.23±0.08	0.51
C 20:2n-6	eicosadienoic	0.32±0.08	0.32±0.04	0.36±0.09	0.85
C 20:3n-6	dihomo-τ-linolenic	1.27±0.81	1.42±0.20	1.48±0.91	0.50
C 20:4n-6	arachidonic	12.30±5.4	12.80±3.9	11.90±6.0	0.72
C 20:5n-3	eicosapentaenoic	0.79±0.19	0.60±0.28	0.78±0.23	0.51
C 22:4n-6	adrenic	2.97±0.98	2.92±0.95	2.57±0.91	0.46
C 22:5n-6	docosapentaenoic	1.54±0.52	1.47±0.44	1.18±0.31	0.12
C 22:5n-3	docosapentaenoic	2.15±0.44	1.51±0.58	2.22±0.42	0.14
C 22:6n-3	docosahexaenoic	4.53±2.71	4.27±1.55	3.94±2.67	0.89

Tab. 6. Comparison of plasma essential fatty acids in Epogam study and in two American studies before any treatment. Results expressed in % of total fatty acids.

Fatty acid		Epogam study n=10	Harrison study n=32	World study n=434
C 16:0	palmitic	26.65±1.29	26.72±1.71	26.84±2.34
C 18:0	stearic	8.31±1.68	9.12±2.02	10.04±2.88
C 18:1n-9	oleic	10.95±1.76	12.07±1.31	13.69±3.16
C 18:2n-6	linoleic	23.60±4.00	26.82±3.73	25.68±4.05
C 18:3n-3	α-linolenic	0.243±0.05	0.010±0.03	0.045±0.13
C 18:3n-6	τ-linolenic	0.152±0.04*	0.246±0.011	0.242±0.28
C 20:3n-6	dihomo-τ-linolenic	3.60±0.72	2.94±0.68	2.56±0.59
C 20:4n-6	arachidonic	13.39±1.90	11.10±1.52	10.84±2.23
C 20:5n-3	eicosapentaenoic	1.52±0.51	1.41±0.76	1.39±2.34
C 22:4n-6	adrenic	0.37±0.14	0.32±0.13	0.28±0.23
C 22:5n-6	docosapentaenoic	0.46±0.30	0.30±0.25	0.17±0.21
C 22:5n-3	docosapentaenoic	1.03±0.15	1.06±0.26	0.97±0.32
C 22:6n-3	docosahexaenoic	5.83±0.92	4.33±0.97	4.45±1.69

nificant (16 % increase) (Tab. 5). Red blood cells cannot synthesize phospholipids "de novo": in contrast to other cells. They can only exchange their membrane phospholipids with phospholipids of plasma lipoproteins. The result show that this exchange is probably slow and modest. The increase of membrane dihomo-gamma-linolenic acid was associated with a decrease in both docosapentaenoic acids.

Plasma lipids

The modification of plasma lipids induced by the treatment are not significant, probably because the number of patients is small and the Friedman test is a relatively severe test. In spite of this we observed interesting biological improvements: Total plasma cholesterol decreased after 30 and 90 days of treatment from 6.07 to 5.66 and 5.81 mmol/l respectively (Tab. 3). A similar moderate decrease in total cholesterol after treatment with gamma-linolenic acid was described by Darcet et al (12). In our study, we observed a moderate non significant increase in HDL cholesterol and apo A I plasma concentration after Epogam treatment. The concentration of apo A-I increased simultaneously with the increase in dihomo-gamma-linolenic acid concentration in plasma. Similar changes in lipid parameters were published by other authors as well (17, 23).

This decrease in plasma cholesterol may be due to the hypocholesterolemic action of gamma-linolenic acid and linoleic acid as well (22), as the latter represents 71 % of acids present in evening primerose oil (Tab. 1). It was also shown that linoleic acid may decrease the total cholesterol, LDL-cholesterol and apoB, and may increase in apoA1 (r=0.54, p=0.10). Therefore the decrease in total cholesterol and an increase in apoA 1

in our study may be due to the synergic action of both linoleic and gamma-linolenic acids. This plasma cholesterol lowering effect of linoleic and gamma-linolenic acids is of clinical importance, especially in old subjects because high cholesterol level inhibits delta-6 desaturase (23) and profounds GLA deficiency in older subjects with all the concequences on their metabolism.

Urinary prostaglandins

Our results show an increase in all prostaglandins measured at the end of the study except for 6-keto-$PGF_{1\ alpha}$ (Figs 3, 4). This means that renal cyclooxigenase is not yet affected in our subjects by aging. This conclusion is also corroborated by relatively high basal values of these prostanoids before treatment at D0, which are not depressed (20). On the contraty, the mean basal urinary excretion of 6-keto-$PGF_{1\ alpha}$ at D0 was low, as we observed in old subjects of similar age (20), and it was not influenced by Epogam treatment. These results may suggest a reduced activity of prostacyclin synthase. Our data are in good agreement with experimental data showing that it is prostacyclin synthase which is the first enzyme depressed by aging (31, 34), and not cyclooxygenase, because PGE_2 synthesis may be increased at the same time (36). This was the case also in our study. The selective inhibition of PGI_2 biosynthesis is neither ameliorated by an excess of exogenous arachidonate (33) nor by PGH_2 endoperoxide as substrates (3), which confirms the primary inhibition of prostacyclin synthase. These data may explain the poor response of renal prostacyclin synthase to gamma-linolenic acid supply in our study.

On the contrary, the evening primrose oil preparation stimulated the biosynthesis of TxA_2, $PGF_{2\ alpha}$ and PGE_2 by 28 %, 42 % and 86 % respectively (Figs 3, 4). An important increase in vasodilator and natriuretic PGE_2 may contribute to the decrese in arterial blood pressure and to the maintenance of renal functions. We did not measure the urinary PGE_1 excretion, nor its concentration in plasma, but it is known that the exogenous supply of gamma-linolenic acid increases PGE_1 synthesis (12, 24, 32). Therefore an increased generation of this PG in our study is likely as well. As PGE_1 is not only a vasodilator and natriuretic but also a potent antiaggregant agent, its generation may be beneficial to old subjects.

Clinical effects

Epogam treatment moderately reduces the arterial, but especially the diastolic blood pressure (Fig. 2, Tab. 2). Normally, arterial blood pressure increases with age. The period of treatment is relatively short (3 months) but still this hypotensive effect may be beneficial. Our results are less significant in comparison with the study of Dillon (14), who observed a significant decrease in diastolic blood pressure after 3 months of treatment with dietary supplementation of gamma-linolenic acid. However his population was not so old as ours. Heart rate was not changed by the treatment. The body weight and renal functions remained stable throughout the study. The treatment was tolerated well with good compliance to the protocol. Only two subjects had moderately accelerated intestinal transit and one subject had more frequent micturitions.

In summary, the treatment was associated with a beneficial reduction of cardiovascular risk factors as represented by the decrease in arterial blood pressure and plasma cholesterol and the increase in HDL cholesterol and apoA I. The exogenous supply of gamma-linolenic acid increased the levels of higher unsaturated essential fatty acids and substrates for the biosynthesis of PG. The increased renal PGE_2 biosynthesis represents a supplementary renal and cardiovascular benefits.

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