Atti Accademia Nazionale dei Lincei

Classe Scienze Fisiche Matematiche Naturali

Rendiconti

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Atti della Accademia Nazionale dei Lincei. Classe di Scienze Fisiche, Matematiche e Naturali. Rendiconti, Serie 8, Vol. **61** (1976), n.6, p. 623–630.

Accademia Nazionale dei Lincei

<http://www.bdim.eu/item?id=RLINA_1976_8_61_6_623_0>

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Atti della Accademia Nazionale dei Lincei. Classe di Scienze Fisiche, Matematiche e Naturali. Rendiconti, Accademia Nazionale dei Lincei, 1976.

Chimica biologica. — *Erucic acid in serum, myocardium and subepicardial adipose tissue of human subjects.* Nota di CRISTINA ESPOSITO, LILIANA FERRI, FULVIO URSINI, MARINA VALENTE, CARLO GREGOLIN e NORIS SILIPRANDI, presentata (*) dal Corrisp. NORIS SILIPRANDI.

RIASSUNTO. — È stata condotta un'indagine sull'ingestione di olio di colza da parte di una popolazione. A questo scopo è stato determinato con tecnica gas-cromatografica il contenuto percentuale di acido erucico e di acido gondoico nel siero di sangue di 6 persone sane e di 39 pazienti con varie malattie croniche, e nel miocardio e nel tessuto adiposo subepicardico di 32 soggetti deceduti per cause diverse. L'acido erucico è stato trovato presente nel siero di tutti i controlli sani (0,62% degli acidi grassi totali) e del 79% dei pazienti (0,58% degli acidi grassi totali dei casi positivi). L'acido gondoico era presente nel siero di 5 controlli su 6 (0,16%) e nel 69% dei pazienti (0,22%). Nel miocardio l'acido erucico era presente nel 78% dei soggetti (0,76% e l'acido gondoico nell'84% dei soggetti (0,32%). Nel tessuto adiposo subepicardico l'acido erucico era presente nel 75% dei casi (1,32%) e l'acido gondoico nel 78% dei casi (0,58%). Non è stata trovata alcuna correlazione tra presenza e concentrazione dell'acido erucico e gondoico e specifiche malattie o cause di morte.

INTRODUCTION

The nutritional adequacy of rapeseed oil, its growth-retarding action and various pathological changes induced by its ingestion in organs of experimental animals have been the subject of investigation and debate for many years. The adverse effects have been attributed to erucic acid, Δ-13-cis-docosenoic, which can be present in rapeseed oil up to 50% of the fatty acid content (see review by Aaes-Jørgensen, [1]). Recently it has been shown that feeding a high calorie percent of rapeseed oil for only a few days causes a triglyceride infiltration in the hearts of rats, guinea pigs, ducks, rabbits and hamsters [2]. It has been shown by Swarttouw [3] that erucic acid is less efficiently oxidized than other fatty acids by rat heart mitochondria. Christophersen and Bremer [4] suggested that a mitochondrial metabolite of erucic acid inhibits the mitochondrial oxidation of other fatty acids.

In a previous study by our group [5] some properties of erucic acid oxidation by rat heart mitochondria were studied and it was shown that such oxidation, which is carnitine dependent, proceeds at a rate equal to 37% of that of oleic acid. Erucic acid does not inhibit oleic acid oxidation when the two acids are present in equimolar concentrations, probably because the fatty acid oxidation system has a much higher affinity for oleic than for erucic acid. As a consequence of its slower oxidation, erucic acid can accumulate in large amounts in the triglycerides of the hearts of rats fed rapeseed oil [6].

(*) Nella seduta dell'11 dicembre 1976.

In the last few years, due to the increasing consumption of rapeseed oil for human nutrition, the possibility has been discussed [7] of a long-term ingestion of even a small calorie percent rapeseed oil causing in humans alterations similar to those observed in short term experiments with higher doses in animals. In Italy, where the average consumption of rapeseed oil steadily increased in the years up to 1974, erucic acid was found present in 1972, 1973 and 1974 in the serum of apparently healthy people and in adipose tissue and myocardium of human subjects deceased for various causes [8, 9, 10, 11]. The presence of erucic acid has been taken as an index of exposure to rapeseed oil ingestion [9, 10, 11], since earlier chromatographic assays performed in Italy on serum [8, 12] as well as on adipose tissue [13] and myocardium [14, 15] did not show measurable amounts of erucic acid.

The purpose of the present study was to screen the exposure of the population of the Padua area to rapeseed oil ingestion and to investigate whether the presence and the amount of erucic acid in human tissues could be correlated with any pathological situation. Particular attention was paid also to gondoic acid (Δ-11-cis-eicosenoic) which, according to Del Carmine *et al.* [10] is present in specimens of rapeseed oil up to 10 % of fatty acid and can be formed from erucic acid by β-oxidation. It should by pointed out that the acid which is called here " gondoic " in the paper by Del Carmine *et al.* [10], by a misnomer, is called " gadoleic ". However gadoleic is Δ-9-cis-eicosenoic acid, which cannot be the product of β-oxidation of erucic acid. From another paper by the same Authors [11] it is apparent that the name gadoleic acid refers to Δ-11-cis-eicosenoic, which is commonly known as gondoic acid.

Materials and methods

Blood sample were obtained at random from 39 patients of the Institutes of Medical Clinics and Medical Pathology of the University of Padua. The age of patients was 32 to 78. Both sexes were included. The patients were suffering from atherosclerosis, hyperlipemia, diabetes, gastrointestinal or chronic liver diseases. Blood for control samples was obtained from 6 apparently healthy volunteers (age 19 to 51). After clotting, serum was stored at —20° C until extracted and analyzed. Samples of approximately 2 g of myocardium from the left ventricle and 500 mg of subepicardial adipose tissue were removed at ramdom from 32 cadavers of both sexes during autopsy (24–30 h after death) in the Institutes of Forensic Medicine and Pathology of the University of Padua. Deaths were due to head trauma, myocardial infarction, hepatic, pulmonary and neurological diseases, and tumors. Age was 19 to 75. Samples were quickly fronzen and stored at — 20° C until processed.

All samples for this research were obtained during the period October 1974-March 1975.

Lipids were extracted from serum with methanol and chloroform, according to the method of Sperry and Brand [16]. Lipid extracts from 1 ml

of serum were saponified in the presence of 5 ml of 3 % KOH in methanol, at 70 °C for 30 minutes, by refluxing under a nitrogen stream. Following the addition of water and the evaporation of methanol, the insaponifiable matter was extracted with petrol ether (b.p. 40°–60° C). The residue was acidified and the fatty acids were extracted two times with three volumes of petrol ether and brought to dryness under a nitrogen stream.

Tissues were minced and specimens of 200 to 500 mg were saponified with excess 20 % KOH in 85 % methanol at 70 °C for two hours, under nitrogen. The mixtures were acidified and the fatty acid extracted three times with three volumes of petrol ether and brought to dryness under a nitrogen stream.

Fatty acids extracted from serum or tissue samples were methylated with 2 ml of 15 % boron trifluoride in methanol, for 4 minutes at 100 °C, and following the addition of 1 ml of water, methyl esters were extracted with petrol ether and concentrated. The fatty acid methyl esters were analyzed with a Perkin-Elmer F 30 gaschromatograph equipped with a flame ionization detector and with a 6 ft long stainless-steel column, 1/8 inch. o.d., packed with 10 % dietylenglycosuccinate (DEGS) on Chromosorb W (80–100 mesh). Nitrogen was used as the carrier gas. Column temperature was programmed from 170° to 200 °C and methyl esters of fatty acids were identified by comparing their retention times with those of authentic standards (Analabs, Inc.). Results are expressed in percent by weight in the total mixture.

RESULTS

Erucic and gondoic acid in serum lipids.

The percentages of erucic and gondoic acid present in the fatty acid profile of the serum of diseased subjects and normal controls are reported in Table I. Erucic acid was present in the serum of 31 or 39 patients and in the serum of all controls. Gondoic acid was present in the serum of 27 or 39 patients and in 5 out of 6 control sera. No significant correlation is apparent between the nature of the diseases studied here and the presence and the percent of erucic and gondoic acid. For this reason it is felt justified to unify the different percent values of erucic and gondoic acid in the sera of diseased subjects in average percentages and to compare them with the corresponding percentages for normal subjects. Only the cases in which a detectable amount of erucic or gondoic acid was found were included in the calculations. It can be seen that no important difference exists between the average values of diseased and normal subjects. In both cases oleic, linoleic, palmitic and arachidonic acid were, as expected, the most abundant fatty acids in serum. On the other hand, erucic and gondoic acid were present in percentages similar to those of trace fatty acids. The average percentage of erucic acid in the diseased cases, when present, was 0.58, with a range of 0.24–1.63. The average percentage for the controls was 0.62, with a range of 0.36–0.89. The average

TABLE I

Erucic and gondoic acid in serum fatty acids of patients and healthy subjects.

Patient	Age	Sex	Clinical diagnosis	% Erucic acid [(a)]	%Gondoic acid [(a)]
B.E.	69	M	Cyrrosis of the liver . . .	0,55	0,29
B.M.	59	F	Cyrrosis of the liver . . .	—	—
F.M.	63	M	Cyrrosis of the liver . . .	0,67	0,25
F.A.	55	F	Cyrrosis of the liver . . .	0,25	0,25
L.A.	52	M	Cyrrosis of the liver . . .	0,62	—
M.C.	78	M	Cyrrosis of the liver . . .	0,42	—
M.I.	50	F	Cyrrosis of the liver . . .	0,44	0,16
T.R.	54	M	Cyrrosis of the liver . . .	0,51	0,19
V.D.	60	M	Cyrrosis of the liver . . .	0,34	0,14
B.E.	49	F	Regional enteritis	0,91	0,39
B.S.	60	M	Regional enteritis	0,24	0,24
B.R.	60	F	Regional enteritis	0,56	0,37
Z.I.	48	M	Ulcerative colitis	0,45	—
C.F.	31	M	Ulcerative colitis	0,34	—
B.A.	57	M	Ulcerative colitis	0,57	0,15
C.L.	41	M	Malabsorption syndrome .	0,93	—
M.B.	59	F	Malabsorption syndrome .	0,56	0,10
M.E.	74	F	Malabsorption syndrome .	—	0,13
F.B.	34	M	Peptic ulcer	0,71	0,14
F.R.	57	F	Peptic ulcer	0,44	0,19
Z.V.	70	M	Peptic ulcer	0,90	0,15
D.D.	51	M	Chronic active hepatitis .	0,94	—
D.O.	33	M	Chronic active hepatitis .	0,48	0,36
F.C.	64	M	Chronic active hepatitis .	0,43	0,32
M.S.	33	M	Chronic active hepatitis .	—	—
M.A.	25	M	Chronic active hepatitis .	0,38	0,20
R.O.	62	F	Alcoholic hepatitis	0,64	0,15
B.R.	42	M	Alcoholic hepatitis	0,59	0,12
C.G.	32	M	Alcoholic hepatitis	0,67	0,16
C.M.	37	F	Alcoholic hepatitis	1,63	0,40
V.C.	32	M	Alcoholic hepatitis	—	—
P.G.	38	M	Ischemic heart disease . .	0,28	—
S.A.	69	F	Ischemic heart disease . .	—	0,13
F.L.	51	M	Ischemic heart disease . .	0,36	0,19
S.G.	73	F	Ischemic heart disease . .	—	0,38
Z.C.	41	F	Ischemic heart disease . .	—	0,12
P.R.	47	F	Diabetes	—	—
Z.B.	58	M	Diabetes	0,61	0,20
Z.L.	43	F	Diabetes	0,66	—
Cases in which the acid is present				79%	69%
Mean [(b)]				0,58	0,22
Range				0,24–1,63	0,10–0,40
Controls					
Cases in which the acid is present				100%	83%
Mean [(b)]				0,62	0,16
Range				0,36–0,89	0,14–0,20

(*a*) The percentages are expressed by weight.

(*b*) The means are calculated relative to the cases in which the fatty acid was found in detectable amounts.

TABLE II

Erucic and gondoic acid in myocardium and subepicardial adipose tissue.

Patient	Age	Sex	Death Cause	Myocardium fatty acids (a)		Subepicardic fatty acids (a)	
				% Erucic	% Gondoic	% Erucic	% Gondoic
M.C.	20	M	Head trauma	0,18	0,34	1,02	0,53
S.M.	70	M	Head trauma	—	—	—	1,55
C.G.	33	M	Head trauma	1,20	0,60	1,48	0,53
R.S.	78	F	Head trauma	0,48	0,15	1,09	1,41
B.A.	46	M	Head trauma	1,07	0,18	1,37	—
N.R.	36	M	Head trauma	0,33	0,15	—	1,25
I.B.	19	M	Head trauma	—	0,55	0,82	0,49
C.A.	31	M	Head trauma	0,86	0,51	0,78	0,61
B.P.	62	F	Head trauma	—	—	—	—
S.M.	22	M	Head trauma	—	—	0,59	0,59
Z.P.	38	M	Head trauma	0,30	0,37	—	0,51
C.A.	43	F	Anaphylactic shock	0,95	0,31	1,40	0,63
L.A.	64	M	Cerebrovascular embolism	—	0,50	0,72	0,50
B.R.	47	M	Subarachnoid hemorrage.	1,25	0,26	0,18	0,37
F.P.	49	M	Toxic encephalitis	1,52	0,34	1,49	0,37
F.L.	66	M	Myocardial infarction	2,07	0,29	3,55	0,34
B.G.	61	M	Myocardial infarction	—	0,16	—	—
T.A.	66	F	Cyrrosis of the liver	0,41	0,26	0,99	0,52
B.R.	62	F	Cyrrosis of the liver	0,53	0,32	0,59	0,53
G.B.	50	F	Cyrrosis of the liver	1,36	0,53	1,47	0,52
B.F.	69	M	Cancer of the lung	0,29	—	—	—
F.E.	44	M	Hodgkin's disease	0,38	0,12	—	—
C.P.	59	F	Breast cancer	1,57	0,35	3,73	0,38
M.A.	66	F	Breast cancer	0,89	0,44	2,00	0,33
C.A.	43	F	Mesenteric vascular occlusion	0,92	0,12	2,50	—
P.M.	75	F	Cancer of the pancreas	1,00	0,32	1,34	0,45
G.O.	39	M	Acute pancreatitis	0,20	—	—	—
P.R.	51	M	Pulmonary edema	0,92	0,43	1,09	0,60
B.A.	65	F	Pulmonary embolism	—	0,15	1,01	0,32
U.A.	72	M	Ischemic heart disease	0,26	0,26	0,34	0,37
V.R.	55	M	Ischemic heart disease	0,22	0,16	0,67	0,41
F.A.	64	M	Pleural empyema	0,76	0,45	1,55	0,44
Case in which the acid is present				78%	84%	75%	78%
Mean (b)				0,79	0,32	1,32	0,58
Range				0,18–2,07	0,12–0,60	0,18–3,73	0,32–1,55

(*a*) The percentages are expressed by weight.

(*b*) The means are calculated relative to the cases in which the fatty acid was found in detectable amounts.

percentage of gondoic acid in the diseased cases was 0.22, with a range of 0.10–0.40. The average percentage for the controls was 0.16, with a range of 0.14–0.20.

Erucic and gondoic acid in myocardium.

Also in myocardium, which represents a tissue where the turnover of fatty acids is presumably slower than in serum, erucic and gondoic acid were not constantly present: erucic acid was found in 25 and gondoic acid in 27 or 32 of the cases studied. The percentages, in the cases in which the fatty acids were found, were 0.79 (range 0.18–2.07) and 0.32 (range 0.12–0.60) for erucic and gondoic acid, respectively (Table II). No correlation can be appreciated between the presence and the amount of erucic and gondoic acid and any death cause, (e.g. trauma in a healthy subject or chronic degenerative and neoplastic diseases). As in serum, no correlation was found with age.

Erucic and gondoic acid in subepicardial adipose tissue.

Subepicardial adipose tissue was chosen for analysis because its metabolic rate is slower than that of subcutaneous fat and it can therefore presumably accumulate exogenous fatty acids for a longer time. The fatty acid composition of this tissue differs from that of serum and myocardium because polienoic acids are present in it in lower amounts. On the contrary, erucic acid, when present, was found in this tissue in a higher percentage than in the other two tissues. It was present in 24 out of 32 cases, with a percentage, in the cases in which it was detectable, of 1.32 (range 0.18–3.73). It is interesting to note that the two cases presenting the highest concentration of erucic acid in myocardium present the highest percentage of erucic acid in subepicardial fat. In four cases erucic acid was absent both in myocardium and in subepicardial fat. Also gondoic acid was present in a percentage higher in this than in the two other tissues: 0.58 (range 0.32–1.55) in 25 out of 32 cases. However, also from these data no correlation emerges between the percentage of these two fatty acids and any metabolic alteration or disease.

Discussion

From the present results it is apparent that the population in the Padua area, from which the specimens for this work were obtained at random, was less exposed—probably because of different eating habits—to rapeseed oil ingestion than the groups of subjects studied by Maranesi *et al.*, presumably in the Bologna area [8] and by Del Carmine *et al.* in the Rome area [9]. In this investigation erucic acid was present in serum in 82 % of the cases studied as 0.58 % of total fatty acids in the serum of diseased subjects and 0.62 % of healthy subjects, versus 1.58 % in the report by Maranesi *et al.* [8] on healthy subjects and 1.1 % in the report by Del Carmine *et al.* [9] on healthy railway workers. No term of comparison is available for gondoic acid in serum.

In myocardium erucic acid was present, in 78 % of the cases reported here, as 0.79 % of the total fatty acids, versus 0.7 % found by Del Carmine *et al.* [10]. There was 0.32 % gondoic acid in the present research and 0.6 % in the paper by Del Carmine *et al.* [10]. For adipose tissue the comparison must be made considering that the present results refer to subepicardial fat and those by Del Carmine *et al.* [10] to subcutaneous fat. In the present cases there was 1.32 % erucic acid and 0.58 % gondoic acid, while the comparable figures by Del Carmine *et al.* are 2.6 % and 2.2 % respectively [10]. Erucic and gondoic acid are present, in these results as in those of other Authors, in the majority but not in all of the cases. Analyzing all the pathological situations studied in the present study and the percent amounts of the two acids determined, it is not possible to establish any correlation between exposure to rapeseed oil ingestion and development of specific toxicologic consequences. Maranesi *et al.* [8], by comparing the serum fatty acid composition of a set of subjects studied in 1968 with that of another set studied in 1972 suggest that the average serum fatty acid composition evolved in this period to an atherosclerotic pattern characterized by increase in erucic, palmitoleic and eicosatrienoic acids and decrease in arachidonic acid. They point out that it is improbable that erucic acid is the only cause of this evolution. The present results seem to exclude that cardiovascular diseases are linked to a higher presence of erucic acid than other diseases. Actually the human organism can metabolize erucic acid to a considerable extent. Tremoliéres *et al.* [17] showed that rapeseed oil ingested by adult persons in an amount of 0.5 g/kg causes an increase in serum lipids, triglycerides, non-esterified fatty acids, acetoacetate and β-hydroxybutyrate and a decrease in the respiratory quotient, which suggests a regular utilization of this amount of rapeseed oil, comparable to that of a similar amount of arachis oil. Very little is known on the long term effects of exposure to rapeseed oil. But in this sense the experiments of Beare-Rogers [18] are important. He showed that rapeseed oil at a level of 10 % of ingested calories has a zero effect in the long–term fat deposition in organs of young animals. It is highly probable that the population on which this investigation was performed was not exposed to this or higher levels of rapeseed oil.

Acknowledgment. We wish to thank Mrs. Giuliana Giungarelli for secretarial work.

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