Abnormal myocardial lipid composition in an infant with type II glutaric aciduria

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Abstract We have analyzed the myocardial lipids of an infant with glutaric aciduria type II (GAII) who died from sudden cardiac failure and of five infants who died suddenly from indeterminate causes (sudden infant death syndrome, SIDS). Histology of the SIDS hearts was normal, but there was marked fatty deposition in the GAII heart. Fatty acid composition of myocardial lipids was determined by thin-layer chromatography–gas–liquid chromatography. Total lipid was elevated 20-fold in the GAII heart. Of total fatty acids, 75% was derived from phospholipids in SIDS heart and 89% from neutral lipids in GAII heart. Increased levels of free oleic acid and a 6-fold elevation in the (n-6)/(n-3) fatty acid ratio in phospholipid were noted in GAII heart compared to SIDS hearts. —**Galloway, J. H., I. J. Cartwright, and M. J. Bennett.** Abnormal myocardial lipid composition in an infant with type II glutaric aciduria. *J. Lipid Res.* 1987. **28:** 279–284.

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Glutaric aciduria Type II (GAII) is a disorder biochemically characterized by decreased capacity to oxidize various fatty acyl-CoA esters. The biochemical lesion lies in the common electron transport chain which serves the fatty acyl-CoA dehydrogenase (1).

We have described the investigation and treatment of a neonatal case of GAII (2). The patient was treated with a controlled lipid diet and regular DL-carnitine therapy and progressed well up to 4 months of age. During this time, the expected elevation in plasma triglycerides was successfully halted, but plasma free fatty acids continued to rise to abnormally high levels. The patient died suddenly from cardiac arrest at 18 weeks of age.

Postmortem examination showed normal histology of skeletal muscle and spinal cord, but there were accumulations of fat in various organs including kidney, liver, and smooth muscle. The heart, which was almost pure white in appearance, contained especially large amounts of fat. It was suggested that the cardiac failure was probably attributable to this accumulation of fat in the heart (2).

Myocardial lipids appear to play an important role in the control of heart function and changes in the fatty acid composition of these lipids may influence the development of myocardial necrosis (3).

The maintenance of heart function depends upon the availability of cellular ATP (3). The translocation of ATP-ADP across the mitochondrial membrane may be a physiological control step in the energy metabolism of the heart. This transport of ATP is stimulated by Ca^{2+} and K^{+} but inhibited by fatty acids, particularly certain fatty acyl-CoA derivatives such as oleyl CoA (4, 5).

The relative amounts of the polyunsaturated fatty acids of the (n-6) and (n-3) series of myocardial phospholipids may also be important in the maintenance of normal cardiac function (3). The (n-6) fatty acid, arachidonic acid (C20:4 (n-6)) serves as a substrate for synthesis of prostaglandins and numerous other substances (6). These products of C20:4 (n-6) have various regulatory functions. Some may be involved in the regulation of coronary tone (7). Other products control the release of noradrenaline from nerve terminals in the heart (8).

The (n-3) fatty acids, particularly docosahexaenoic acid (22:6 (n-3)) may play a role in the maintenance of normal heart rate (9).

The purpose of this study was to perform a detailed quantitative fatty acid analysis of the various lipid fractions of the GAII heart muscle and to compare the results to those of a study of age-matched infants who had also died suddenly.

In view of the important role of lipids in heart function, it seemed possible that changes in the lipid composition of GAII heart muscle might help to explain the cardiac failure in our patient.

Abbreviations: GAII, glutaric aciduria type II; SIDS, sudden infant death syndrome; TLC, thin-layer chromatography; GLC, gas–liquid chromatography.

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MATERIALS AND METHODS

Subjects

A postmortem sample of heart muscle was obtained from the patient with GAII as previously described (2). Samples of heart muscle were obtained from five age- and sex-matched controls. The cause of death of these infants was sudden infant death syndrome (SIDS) and postmortem examination showed no sign of fatty heart or liver.

Tissue preparation

Heart muscle was stored at −20°C until required for lipid extraction which was performed within 6 weeks of death. Tissue (0.5 g) was added to 2 ml of Tris buffer (pH 7.4) (15 mmol Tris-HCl; 134 mmol NaCl/1; 5 mmol KCl) at 4°C, and homogenized using a Teflon homogenizer, and maintained on melting ice. One ml of chilled homogenate was taken for lipid extraction and another aliquot was used for protein estimation.

Lipid extraction

Preparation of lipid extracts of heart muscle was performed as previously described for platelets (10), but with the following modifications. One ml of heart homogenate was mixed for 15 min with 7.5 ml of methanol, followed by 7.5 ml of chloroform for a further 15 min. This extraction procedure was repeated twice.

Two-dimensional thin-layer chromatography (2D-TLC)

The dry, crude lipid extracts were redissolved in 600 μl of chloroform–methanol 1:1 (v/v) and applied to the TLC plates. The 2D-TLC method allowed complete separation of the major myocardial phospholipid subclasses, as previously described (10). The following components were readily resolved: phosphatidylcholine (PC), phosphatidylethanolamine (PE), lysophosphatidylethanolamine (LPE), phosphatidylserine (PS), phosphatidylinositol (PI), sphingomyelin (SM), and diphosphatidylglycerol (cardiolipin, DPG).

One-dimensional thin-layer chromatography (1D-TLC)

The dry, crude lipid extracts were redissolved in 600 μl of chloroform–methanol 1:1 (v/v) and applied to the same types of TLC plates as for the phospholipid separation. Plates were run for approximately 45 min in the solvent system hexane–diethyl ether–glacial acetic acid 180:20:2 (v/v/v) and developed as previously described for phospholipids (10). Four distinct neutral lipid bands were resolved. These were in order of decreasing mobility; cholesteryl ester (CE), triglyceride (TG), free fatty acid (FFA), and cholesterol.

Methylation of lipid fatty acids

The extracted lipids were treated with 1.5 ml of boron trifluoride–methanol complex (Sigma Chemical, St. Louis, MO) for 1 hr, under nitrogen, at 65°C. Total lipid and sphingomyelin extracts were further incubated at 90°C for 1 and 2 hr, respectively, since the amide linkages of sphingomyelin are more resistant to derivatization than the ester linkages of lipid and neutral lipid fatty acids.

TABLE 1. Percentage fatty acid composition of total myocardial lipids in SIDS infants (n = 5) and a GAII infant

Fatty Acid	SIDS	GAII
	%	
C16:0	15.7 (1.64)	27.6
C16:0 DMA	3.12 (0.44)	
C16:1 (n-9)	0.95 (0.40)	8.98
C18:0	12.3 (1.03)	8.31
C18:0 DNA	2.30 (0.35)	
C18:1 (n-9)	21.4 (2.80)	41.8
C18:1 DMA	1.32 (0.24)	
C18:2 (n-6)	20.8 (1.91)	11.8
C20:3 (n-6)	1.22 (0.20)	
C20:4 (n-6)	15.9 (1.09)	1.48
C22:4 (n-6)	0.70 (0.25)	
C22:5 (n-6)	0.45 (0.18)	
C22:5 (n-3)	0.63 (0.14)	
C22:6 (n-3)	1.58 (0.38)	Tr

Results expressed as mean ± SD; DMA, dimethyl acetal; Tr, trace.

Gas-liquid chromatography (GLC) of fatty acids

GLC was essentially that described by Galloway et al. (10) with modifications. Temperature programming was applied; an initial 9 min at 196°C, rising by 6°C/min, to 235°C; the final temperature was held for 20 min. The detector temperature was 200°C.

Before injection, all samples were diluted in a solution containing an internal standard, heneicosanoic acid (C21:0) methyl ester in ethyl acetate (0.05 μmol/l). Heneicosanoic acid was chosen as it is not normally found in tissue lipids. Quantitation of fatty acids was made against this internal standard.

TABLE 2. Percentages of total fatty acids derived from phospholipids and neutral lipids in myocardial tissue of SIDS infants (n = 5) and a GAII infant

	SIDS	GAII
	% of total fatty acids	
Phospholipids		
Diphosphatidylglycerol	4.11 (0.75)	0.40
Phosphatidylcholine	36.7 (6.13)	6.42
Phosphatidylethanolamine	22.4 (4.77)	1.39
Lysophosphatidylethanolamine	0.39 (0.48)	0.33
Phosphatidylserine	2.97 (0.42)	0.47
Phosphatidylinositol	4.46 (1.05)	1.19
Sphingomyelin	2.17 (0.35)	0.35
Neutral lipids		
Free fatty acid	16.7 (2.60)	1.01
Triglyceride	8.23 (3.92)	88.08
Cholesteryl ester	1.75 (0.40)	0.31

Results expressed as mean ± SD.

TABLE 3. Concentrations of (n-6), (n-3), and total fatty acids (FA) of myocardial phospholipids (PL), neutral lipids (NL), and total lipids in SIDS infants (n = 5) compared to a GAII infant

	PL		NL		Total Lipids (PL + NL)	
	SIDS	GAII	SIDS	GAII	SIDS	GAII
	μmol/g protein					
C18:2 (n-6)	34.8 (7.47)	153.9	14.1 (3.27)	588.3	47.0 (9.88)	742.2
C20:4 (n-6)	37.7 (11.8)	61.5	10.1 (1.57)	3.84	46.0 (8.61)	65.3
C22:6 (n-3)	3.66 (1.14)	1.78	0.78 (0.36)	1.99	4.31 (1.09)	3.77
Total fatty acids	206.5 (39.3)	678.0	66.5 (26.9)	5931	272.9 (30.2)	6609
(n-6)/(n-3)*	20.5 (3.70)	122.9				
C20:4 (n-6)/(n-3)	10.5 (1.48)	34.6				

Results expressed as mean ± SD.

*$(n-6)/(n-3) = \frac{C18:2 + C20:4}{C22:6}$.

Protein determination

Myocardial tissue protein was estimated by the method of Lowry et al. (11).

RESULTS

Analysis of the fatty acid composition of total myocardial lipids showed marked differences between GAII and SIDS (**Table 1**). The relative percentages of linoleic acid (C18:2 (n-6)) and arachidonic acid (C20:4 (n-6)) were depressed by one-half and tenfold, respectively, in GAII compared to SIDS. The other polyunsaturated fatty acids were undetectable in GAII. Generally, the saturated and monounsaturated fatty acids were markedly increased in GAII, except for a reduction in C18:0. The dimethyl acetals of the plasmalogens were completely absent in GAII total lipid.

The percentages of total fatty acids derived from phospholipids and neutral lipids of myocardial tissue are shown in **Table 2.** In SIDS, 75% of total fatty acids was derived from phospholipids. However, in GAII almost 90% of total fatty acids was derived from neutral lipids. Data on the quantitative analysis of SIDS and GAII heart tissue are presented in **Table 3** and **Table 4.**

The gross difference in total lipid fatty acid concentration between SIDS and GAII is depicted in Table 3. Total fatty acids were elevated over 20-fold in GAII compared to SIDS. There was a 3-fold increase in phospholipid fatty acids but, despite this, there was only a slight increase in levels of C20:4 (n-6) in GAII, and levels of long-chain polyunsaturated fatty acids were decreased. Levels of

TABLE 4. Myocardial neutral lipid fatty acid concentrations in SIDS infants (n = 5) compared to a GAII infant

	Free Fatty Acid		Triglyceride		Cholesteryl Ester	
	SIDS	GAII	SIDS	GAII	SIDS	GAII
	μmol/g protein					
C16:0	6.95 (1.36)	11.4	5.86 (2.38)	1786	0.63 (0.25)	6.00
C16:0 DMA	nd	nd	2.31 (1.24)	nd	nd	nd
C16:1 (n-9)	0.66 (0.43)	7.10	0.47 (0.35)	558.5	0.08 (0.03)	1.57
C18:0	5.06 (1.61)	4.14	2.85 (0.95)	403.7	0.63 (0.31)	2.33
C18:1 (n-9)	15.1 (3.63)	32.7	9.74 (4.16)	2547	0.61 (0.22)	5.33
C18:2 (n-6)	8.56 (2.38)	6.76	4.65 (2.62)	578.4	0.86 (0.28)	3.11
C20:4 (n-6)	8.73 (1.63)	2.65	0.93 (0.50)	nd	0.42 (0.12)	1.21
C22:4 (n-6)	0.17 (0.08)	nd	Tr	nd	Tr	nd
C22:5 (n-3)	0.28 (0.05)	1.50	0.64 (0.35)	nd	Tr	0.64
C22:6 (n-3)	0.39 (0.37)	1.45	0.22 (0.06)	nd	0.18 (0.11)	0.54
Total fatty acids	47.7 (10.3)	67.6	31.9 (8.10)	5874	4.18 (2.09)	21.1

Results expressed as mean ± SD; nd, none detected, Tr, trace.

TABLE 5. Fatty acid composition of myocardial phospholipids of SIDS infants (n = 5) compared to a GAII infant

	Phosphatidylcholine		Phosphatidylethanolamine		Phosphatidylserine	
	SIDS	GAII	SIDS	GAII	SIDS	GAII
	mol/100 mol					
C16:0	28.6 (1.95)	37.6	3.50 (0.77)	5.83	2.94 (0.23)	4.59
DMA	5.28 (1.56)		6.44 (1.12)	4.41		
C16:1 (n-9)	0.51 (0.37)	2.26	Tr	0.68		0.55
C18:0	5.51 (0.82)	8.26	16.7 (1.20)	25.1	40.9 (0.86)	43.6
DMA	1.07 (0.35)		9.48 (1.87)	5.21		
C18:1 (n-9)	27.1 (4.50)	25.7	7.32 (1.79)	12.2	16.7 (0.90)	21.6
DMA	1.25 (0.32)		4.11 (0.20)	2.18		
C18:2 (n-6)	18.9 (2.39)	21.4	7.71 (1.33)	13.3	5.47 (1.26)	8.62
C20:2 (n-6)	0.53 (0.35)		0.48 (0.21)		1.66 (0.40)	0.69
C20:3 (n-6)	1.28 (0.39)		1.14 (0.19)	1.53	4.16 (0.46)	4.43
C20:4 (n-6)	8.46 (1.55)	4.80	35.4 (2.51)	25.3	9.39 (1.24)	3.89
C20:5 (n-3)	Tr		0.47 (0.08)		0.31 (0.19)	
C22:4 (n-6)	0.26 (0.17)		1.49 (0.31)	1.13	2.70 (0.74)	4.13
C22:5 (n-6)	Tr		0.89 (0.24)	0.91	3.28 (0.94)	2.71
C22:5 (n-3)	0.54 (0.30)		1.40 (0.47)	1.09	2.56 (0.63)	2.75
C22:6 (n-3)	0.56 (0.22)		3.14 (0.44)	1.09	9.31 (0.77)	2.43

Results expressed as mean ± SD; Tr, trace.

neutral lipids were elevated in GAII (Table 4). Triglyceride levels were massively elevated (more than 100-fold) and cholesteryl esters were elevated 5-fold. In the free fatty acids, of particular interest was the 2-fold increase (4 SD above mean of SIDS hearts) in oleic acid (C18:1 (n-9)).

Table 5 and **Table 6** show the fatty acid compositions of the phospholipids of SIDS infants and the GAII infant. The decrease in the level of polyunsaturated fatty acids in the GAII infant is readily apparent. The percentages of C20:4 (n-6) in each phospholipid were decreased in GAII. Thus, despite the marked increase in phospholipid (Table 3), the quantity of C20:4 (n-6) in GAII was only slightly increased.

Finally, Table 3 shows that linoleic acid (C18:2 (n-6)), a precursor of arachidonic acid (C20:4 (n-6)), was elevated 4-fold in phospholipids and 40-fold in neutral lipids in GAII. Despite this, arachidonic acid (C20:4 (n-6)) levels in GAII were only slightly increased in phospholipids and decreased in neutral lipids. Additionally the (n-6)/(n-3) ratio in heart muscle phospholipids was elevated 6-fold in GAII compared to SIDS.

DISCUSSION

This study reports a comprehensive analysis of the fatty acids of heart muscle lipid classes in a GAII infant and SIDS infants. The analysis shows both gross and complex differences in the lipid composition of the GAII heart compared to the SIDS hearts. The fatty acid composition of myocardial phospholipids in SIDS (Table 5) is comparable to previously published data (12). However, our study reports the most detailed qualitative and quantitative analysis of cardiac lipids to date. We suggest that changes in the fatty acid composition of some of the lipid species in GAII might help to explain the sudden cardiac failure.

In humans, plasma free fatty acids are the major source of fuel for heart muscle contraction. Fatty acids are oxidized in the mitochondria to produce ATP. ATP is then transported across the mitochondrial membrane to the contractile proteins actin and myosin. This ATP transport may be inhibited by certain fatty acyl CoAs (3). Free oleic acid levels were increased twofold in GAII heart (Table 4) and, since oleyl CoA inhibits transport of ATP from mitochondria to actin/myosin, this may have led to an inhibition of heart muscle contraction (4, 5).

Changes in the fatty acid composition of myocardial phospholipids of GAII may also have been partly responsible for cardiac failure. The (n-6) and (n-3) polyunsaturated fatty acids of myocardial phospholipids appear to play an important role in the normal function of the heart. Cyclooxygenase products of arachidonic acid are involved in the regulation of coronary tone (7), while docosahexaenoic acid (C22:6 (n-3)) is involved in the maintenance of normal heart beat (9).

The levels of the (n-6) and (n-3) fatty acids of myocardial neutral lipids and phospholipids are shown in Table 3. Levels of C20:4 (n-6) in phospholipids were remarkably similar in GAII and SIDS, despite a gross difference in the 20:4 (n-6) percentage in total myocardial lipid (Table 1).

TABLE 6. Fatty acid composition of myocardial phospholipids of SIDS infants (n = 5) compared to a GAII infant

	Phosphatidylinositol		Diphosphatidylglycerol		Sphingomyelin	
Fatty Acid	SIDS	GAII	SIDS	GAII	SIDS	GAII
	moles/100 moles					
C16:0	2.81 (0.73)	4.81	1.76 (0.66)		16.2 (2.26)	19.0
DMA	Tr					
C16:1 (n-9)		2.24	Tr	1.99	Tr	0.50
C18:0	42.3 (2.73)	45.1	2.02 (0.74)	4.37	19.1 (1.90)	17.1
DMA	Tr					
C18:1 (n-9)	9.85 (2.48)	13.9	8.19 (1.57)	9.61	3.68 (0.77)	3.83
C18:2 (n-6)	8.58 (1.11)	11.3	78.3 (9.33)	84.0		
C20:0					6.33 (1.10)	9.57
C20:2 (n-6)	0.26 (0.17)		0.86 (0.23)			
C22:0					18.8 (4.60)	18.9
C20:3 (n-6)	2.28 (0.51)	2.93	0.81 (0.21)			
C23:0					5.38 (0.58)	7.52
C20:4 (n-6)	29.9 (4.45)	19.7	4.22 (0.72)			
C22:1 (n-9)					3.74 (0.55)	3.58
C20:5 (n-3)	0.23 (0.14)		Tr			
C24:0					10.8 (2.33)	8.12
C22:4 (n-6)	Tr		Tr			
C24:1 (n-9)					10.3 (1.44)	9.63
C22:5 (n-6)	Tr					
C24:2 (n-6)					1.09 (0.28)	1.12
C22:5 (n-3)	0.66 (0.31)		1.11 (0.31)			
C26:0					0.89 (0.46)	2.00
C22:6 (n-3)	0.53 (0.15)		0.79 (0.27)			
C26:1 (n-9)					2.40 (0.47)	

Results expressed as mean ± SD; Tr, trace.

The data in this study suggest an optimum level of arachidonic acid in myocardial phospholipids for the maintenance of heart function. It is possible that in the GAII heart there had been an attempt to maintain normal levels of C20:4 (n-6) despite the increased availability of lipid. The marked increase in linoleic acid in GAII heart (Table 3), the precursor of C20:4 (n-6), suggests that such a mechanism may be in operation. Therefore, the myocardial phospholipid fatty acid composition of GAII heart may have been regulated to maintain an optimum level of C20:4 (n-6).

The massive increase in triglyceride levels in GAII heart (Table 4) would contribute to the gross lipid abnormalities observed. The regular DL-carnitine therapy may have contributed to the cardiac lipidosis in GAII heart, since carnitine is known to enhance lipid uptake by tissues.

These results suggest that the use of DL-carnitine as a regular supportive measure should be considered with some caution.

The ratio of (n-6)/(n-3) fatty acids in phospholipids of normal heart has been reported to decrease with age. This was due to a decrease in linoleic acid and an increase in C22:6 (n-4), while the levels of C20:4 (n-6) were unchanged (9). However, in men who died from sudden cardiac failure without signs of atherosclerosis, the C20:4 (n-6)/(n-3) ratio has been found to be abnormally high (3).

The C20:4 (n-6)/(n-3) ratio was three times greater in GAII compared to SIDS heart phospholipids (Table 3). Levels of C22:6 (n-3) were depressed in phospholipids of GAII heart (Table 3). In view of the importance of C22:6 (n-3) in the maintenance of normal heart rate, the low levels of this fatty acid in GAII heart may have partly led to the sudden cardiac failure.

In summary, the elevation of oleic acid in the neutral lipids of GAII heart may have inhibited the translocation of ATP from mitochondria to the contractile proteins, thereby inhibiting heart muscle contraction. In phospholipids of GAII heart there was an increased ratio of C20:4 (n-6)/(n-3) which is important in the regulation of coronary tone and heart rate. High ratios of C20:4 (n-6)/(n-3) have been observed in men who died from sudden cardiac failure. We suggest that other infants with fatty acid oxidation defects and lipid deposition in the heart may be at high risk of sudden cardiac arrest.

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