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The relationship between linoleic acid level in serum, adipose tissue and myocardium in humans

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A cross-sectional study of 80 consecutive cases at necropsy was undertaken to determine the relationship between linoleic acid in the serum, adipose tissue and myocardium of humans. The sample consisted of 55 males and 25 females aged 7 to 92 years who had died from cardiac and non-cardiac causes in the Southern Region of Tasmania, Australia. Fatty acids were extracted from samples of serum, adipose tissue and myocardium and separated using capillary gas liquid chromatography. Means and standard deviations were calculated for each of the main fatty acids in the three tissues studied. In serum and adipose tissue, there were significantly higher levels of linoleic acid ($p<0.001$ and $p<0.001$ in serum and adipose tissue, respectively) and total n-6 fatty acids ($p< 0.002$ and $p< 0.001$ in serum and adipose tissue, respectively) and significantly lower levels of oleic acid in females than in males ($p< 0.001$ and $p<0.05$ in serum and adipose tissue, respectively). In serum and adipose tissue, the ratio of total n-6 to total n-3 fatty acids was significantly higher in females than males ($p<0.02$ and $p<0.001$ in serum and adipose tissue, respectively). In myocardium, there were significantly higher levels of oleic acid ($p<0.05$) and linoleic acid ($p<0.001$) and significantly lower levels of arachidonic acid ($p<0.001$) and docosapentaenoic acid ($p<0.02$) in females than males. Total n-3 fatty acids in myocardium were significantly lower in females ($p<0.001$) resulting in a significantly higher ratio of total n-6 to total n-3 fatty acids in females ($p<0.001$). Highly significant Pearson correlations were found between levels of linoleic acid in adipose tissue and myocardium ($p<0.0001$), between adipose tissue and serum ($p<0.001$) and between serum and myocardium ($p<0.001$). The proportion of total polyunsaturated fatty acids (PUFA) in the myocardium was inversely related to the proportion of monounsaturated fatty acids ($p<0.001$) and inversely related to the proportion of saturated fatty acids ($p<0.001$). There was a significant positive correlation between the ratio of linoleic acid to linolenic acid in all three tissues. This study showed that there was a very strong relationship between the level of linoleic acid in adipose tissue and myocardial tissue, which suggests that dietary linoleic acid influences the level of myocardial linoleic acid. These findings support the hypothesis that dietary linoleic acid has a direct influence on myocardial membrane linoleic acid levels.

Introduction

Analysis of population trends in dietary consumption suggests an inverse relationship between dietary levels of PUFA and mortality from CHD[1]. The finding, that the fatty acid content of subcutaneous fat is a good biological indicator of fat consumption in humans[2,3], has resulted in a number of epidemiological studies using adipose tissue samples to confirm the inverse relationship between dietary levels of PUFA (in particular linoleic acid) and the risk of mortality from CHD[4,5,6]. A recent study has shown an inverse relationship between levels of linoleic acid in adipose tissue and the risk of sudden cardiac death[7]. These findings in humans are supported by results of animal studies which suggest a direct effect of dietary fatty acid levels on phospholipid fatty acid composition[8]. Such changes in the fatty acid composition of myocardial phospholipid can influence the susceptibility of the myocardium to develop arrhythmias, and may account for the association between dietary fatty acids and sudden cardiac death[9].

Given the association between dietary fat, adipose tissue, fatty acid composition and the risk of sudden cardiac death, it is important to establish whether dietary linoleic acid, as reflected in levels of linoleic acid in adipose tissue, correlates significantly with linoleic acid levels in human myocardium. The aim of this study was to examine the correlation between levels of linoleic acid in serum, adipose tissue and myocardium from humans.

Methods

Tissue samples

Where possible, samples of serum (n=62), adipose tissue (n=79) and myocardium (n=79) were taken at necropsy from 80 consecutive cases. The cases included 55 males and 25 females who died from cardiac and non cardiac causes. Ages ranged from 7 years to 92 years. Ethical approval for the collection of tissue samples was granted by the Ethics Committee of the Royal Hobart Hospital.

Serum samples were obtained from blood aspirated from cardiac chambers, usually the left ventricle. Adipose tissue was sampled from the anterior abdominal wall and

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myocardium was sampled from areas of macroscopically normal myocardium, that is, myocardium free of fibrosis and not involved in a recent myocardial infarction.

Specimens were placed into plastic containers and stored at -70° C until analysis.

Analysis of tissue fatty acids

Lipids were extracted from the thawed samples in chloroform-methanol (2:1) as described previously[10]. Aliquots of the total lipids, together with an internal standard of heptadecanoic acid, were treated with KOH in methanol followed by BF3 in methanol.[10] The resulting fatty acid methyl esters were separated by capillary gas liquid chromatography using a 50mm x 0.32mm (ID) CP Sil 88 column (Chrompack, Middelburgh, The Netherlands). The column oven was operated from 110° C to 190° C using helium as the carrier gas at a linear gas velocity of 20 cm/sec. Standard fatty acid methyl esters were routinely chromatographed to establish retention times and to determine response factors for the individual fatty acid methyl esters.

Statistical methods

Means and standard deviations were calculated for each of the main fatty acids in the three tissues studied. Comparisons of mean levels of fatty acids in the tissues studied were calculated using the Students t-test. Correlations were calculated using the Pearson correlation coefficient (r). Correlations did not vary by sex or cause of death and therefore total sample results are given.

Results

The fatty acid composition of the three tissues in males and females is shown in the Table. The main fatty acids in serum were palmitic acid (16:0), palmitoleic acid (16:1), stearic acid (18:0), oleic acid (18:1), linoleic acid (18:2n-6), arachidonic acid (20:4n-6) and docosahexaenoic acid (22:6n-3). In serum, there were significantly higher levels of linoleic acid ($p<0.001$) and total n-6 PUFA ($p<0.002$) and significantly lower levels of oleic acid in females than in males ($p<0.001$). The ratio of total n-6 to total n-3 PUFA was significantly higher in females ($p<0.02$).

The main fatty acids in adipose tissue were myristic acid (14:0), palmitic acid, palmitoleic acid, stearic acid, oleic acid and linoleic acid. In adipose tissue, there were significantly higher levels of linoleic acid ($p<0.001$) and total n-6 PUFA ($p<0.001$) and significantly lower levels of oleic acid ($p<0.05$) in females than males. The ratio of total n-6 to total n-3 PUFA in adipose tissue was significantly higher in females than in males ($p<0.001$).

The main fatty acids in myocardium were palmitic acid, stearic acid, oleic acid, linoleic acid, arachidonic acid and docosahexaenoic acid. Fatty aldehydes were also detected in this tissue and the levels are reported in the

Table. Serum, adipose tissue and myocardial fatty acid composition in males and females

		Males			Females		
		Serum n=39	Adipose n=54	Myocardium n=54	Serum n=23	Adipose n=25	Myocardium n=25
Fatty Acid		Mean±SD[a]	Mean±SD	Mean±SD	Mean±SD[a]	Mean±SD	Mean±SD
Saturated							
Myristic	14:0	1.06±0.41	3.10±0.99	1.09±0.50	1.25±0.77	2.91±0.88	1.17±0.66
Palmitic	16:09	21.78±2.48	22.19±2.17	16.33±1.77	22.12±3.50	22.25±2.50	16.74±2.45
Stearic	18:09	7.31±1.39	5.57±1.59	11.61±1.56	7.37±1.17	5.30±1.74	11.33±1.77
Monounsaturated							
Palmitoleic	16:1	2.87±1.17	5.88±1.89	1.75±0.86	2.70±1.57	5.35±2.30	1.93±1.31
Oleic	18:1[b]	25.72±3.75[f]	45.84±5.72[e]	21.22±6.42	23.03±4.15	44.38±7.43	22.54±6.78[e]
Eicosamonoenoic	20:1	0.66±0.20	1.34±0.31	0.67±0.62	0.60±0.13	1.33±0.21	0.56±0.23
Polyunsaturated							
Linoleic	18:2n-6	25.40±6.83	10.18±4.16	16.76±3.72	28.14±7.37[f]	12.26±3.67[f]	18.60±3.94[f]
Linolenic	18:3n-3	0.45±0.19	0.38±0.08	0.25±0.14	0.33±0.10	0.36±0.10	0.23±0.11
Eicosadienoic	20:2n-6	0.17±0.07	0.14±0.06	0.14±0.09	0.16±0.07	0.16±0.04	0.15±0.04
Eicosatrienoic	20:3n-6	1.27±0.56	0.15±0.07	0.81±1.48	1.24±0.42	0.20±0.10	0.58±0.18
Arachidonic	20:4n-6	6.79±1.67	0.44±0.33	15.75±4.58[f]	6.55±1.62	0.37±0.15	13.93±3.67
Eicosapentaenoic	20:5n-3	0.73±0.42	0.07±0.18	0.55±0.40	0.60±0.38	0.01±0.04	0.33±0.16
Docosatetraenoic	22:4n-6	0.21±0.08	0.09±0.07	0.33±0.16	0.17±0.08	0.13±0.11	0.30±0.09
Docosapentaenoic	22:5n-6	0.12±0.18	0.02±0.04	0.25±0.14	0.09±0.08	0.00±0.01	0.16±0.11
	22:5n-3	0.62±0.19	0.23±0.12	1.47±0.49[d]	0.51±0.19	0.27±0.17	1.08±0.40
Docosahexaenoic	22:6n-3	1.78±0.60	0.26±0.42	3.29±1.00	1.96±1.09	0.21±0.19	2.82±1.32
	n-6	33.96±7.06	11.01±4.22	34.05±6.04	36.35±8.61[e]	13.13±3.62[f]	33.79±7.18
	n-3	3.58±1.03	0.94±0.61	5.56±1.45[f]	3.41±1.45	0.85±0.40	4.46±1.68
	n-6/n-3	10.58±4.39	15.55±10.95	6.50±1.73	11.96±4.52[d]	18.61±9.07[d]	9.28±7.30[f]

a results shown as mean ±SD of g fatty acid per 100g total fatty acids. **b** 18:1n-7 and 18:1n-9. **c** $p<0.05$ **d** $p<0.02$ **e** $p<0.002$ **f** $p<0.001$ Position of superscripts **c, d, e, f** indicate gender with significantly higher level of tissue fatty acid. **g** 16 and 18-carbon aldehydes determined as dimethyl acetal derivatives (DMA) were found in myocardium at levels of 3.25±1.19 and 3.04±1.09 for 16:0 DMA for males and females respectively, and 1.48±0.82 and 1.37±0.75 for 18:0 DMA for males and females respectively.

Table. In myocardium, there were significantly higher levels of oleic acid ($p<0.05$) and linoleic acid ($p<0.001$) and significantly lower levels of arachidonic acid ($p<0.001$) and docosapentaenoic acid (22:5n-3) ($p<0.002$) in females than males. Total n-3 fatty acids were significantly lower in females ($p<0.001$) resulting in a significantly higher ratio of total n-6 to total n-3 fatty acids in females ($p<0.001$).

Figures 1 to 3 show that there were highly significant correlations between levels of linoleic acid in adipose tissue and myocardium ($p<0.0001$), between adipose tissue and serum ($p<0.001$) and between serum and myocardium ($p<0.001$).

Figure 1. Correlation between levels of linoleic acid (18:2n-6) in adipose tissue and myocardium (as a percentage of total fatty acids in adipose tissue and myocardium).

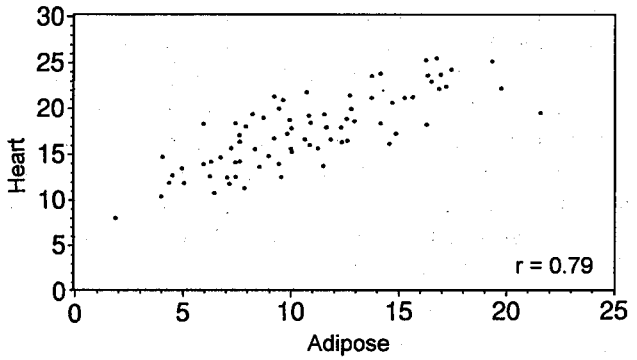


Figure 2. Correlation between levels of linoleic acid (18:2n-6) in serum and adipose tissue (as a percentage of total fatty acids in serum and adipose tissue).

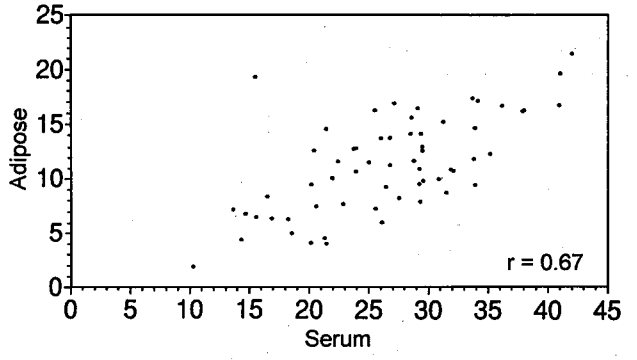
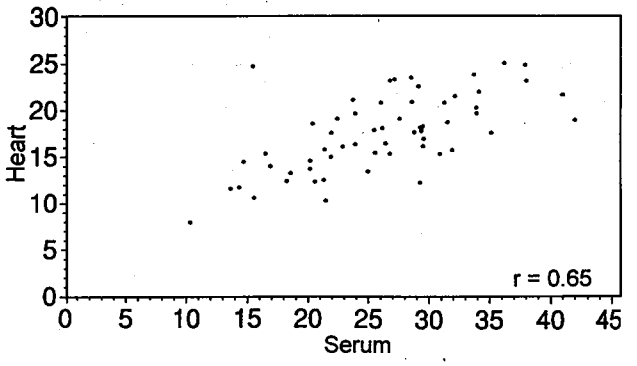


Figure 3. Correlation between levels of linoleic acid (18:2n-6) in serum and myocardium (as a percentage of total fatty acids in serum and myocardium).



Discussion

This study showed a very strong relationship between the level of linoleic acid in adipose tissue and myocardial tissue, which suggests that dietary linoleic acid influences the level of myocardial linoleic acid.

Previous studies have shown that adipose linoleic acid levels reflected dietary linoleic acid intake.[2,3] The transport of linoleic acid from the gut via chylomicrons and subsequent transport in various lipoprotein classes derived from the liver would ensure that all tissues would be likely to incorporate essential PUFA via the action of lipoprotein lipases. Thus, it was not surprising that we showed a strong relationship between the level of linoleic acid in heart and adipose tissue. These findings support the hypothesis that dietary PUFA can have a direct influence on myocardial membrane PUFA. The beneficial effects of linoleic acid might be derived by the displacement of saturated fatty acids in the myocardium as suggested by Riemersma[11]. In this study, we found that the proportion of PUFA (all n-6 and n-3 PUFA) in the myocardium was inversely related to the proportion of monounsaturated fatty acids ($r=0.962$, $p<0.001$). In particular, there was an inverse relationship between oleic acid (the main monounsaturated fatty acid) and arachidonic acid ($r=0.872$, $p<0.001$), and between oleic acid and linoleic acid ($r=0.530$, $p<0.001$). It was also found that the proportion of PUFA was inversely correlated with the proportion of saturated fatty acids ($r=0.491$, $p<0.001$). Thus, increased PUFA levels in the myocardium were mainly associated with a reduced level of monounsaturated fatty acids but also with a reduction in saturated fatty acids.

The presence of linoleic acid itself may be beneficial by acting as a precursor of specific substances in the myocardium such as 13-hydroxyoctadecadienoic acid[12], or as a result of conversion to arachidonic acid, it may stimulate production of myocardial eicosanoids[13]. There was a positive relationship between the level of linoleic acid and arachidonic acid in the myocardium ($r=0.253$, $p<0.05$).

The level of saturated fatty acids and polyunsaturated fatty acids in adipose tissue in the present study fell between those reported by Riemersma et al, for three northern European countries and Italy.[4] That is, the levels of saturated fatty acids were lower and those for linoleic acid were higher than the northern European countries.

There is an increasing interest in dietary n-3 PUFA in relation to processes involved in atherosclerosis, thrombosis and cardiac arrhythmias. It has been suggested that eicosapentaenoic acid (20:5n-3) reduces the production of a variety of eicosanoids derived from arachidonic acid, leading to a reduced production of pro-inflammatory, pro-aggregatory and pro-arrhythmogenic eicosanoids such as thromboxane and the 4-series leukotrienes[13,14]. Therefore, we investigated whether there was a relationship between the main n-6 and n-3 PUFA in the three tissues.

Since there is competition between linoleic acid and linolenic acid for metabolism to longer chain PUFA such as arachidonic acid and eicosapentaenoic acid in the liver, diets with high levels of linoleic acid relative to linolenic acid result in tissue with high levels of n-6 PUFA relative

to n-3 PUFA[15] and presumably high levels of eicosanoids derived from arachidonic acid[14].

In this study, there was a significant positive correlation between the ratio of linoleic acid to linolenic acid in all three tissues (myocardium v adipose, r=0.23, p<0.05, n=79; myocardium v serum, r=0.19, p<0.05, n=62; adipose v serum, r=0.44, p<0.001, n=62). Since others have shown that the relationship between diet and adipose tissue PUFA is correlated[2,3], these data support the concept of a positive relationship between the ratio of linoleic acid to linolenic acid in the diet and myocardial tissue. The ratio of linoleic acid to linolenic acid in adipose tissue was also significantly correlated with the ratio of total n-6 PUFA to total n-3 PUFA in myocardial tissue (r=0.42, p<0.001, n=79). Since myocardial tissue lipids contain high levels of 20 and 22-carbon PUFA derivatives of linoleic acid and linolenic acid, this correlation suggests that the ratio of dietary n-6 PUFA to n-3 PUFA influences the proportion of these two PUFA families in myocardial tissue.

Female subjects had significantly elevated levels of linoleic acid in all three tissues compared with males. This could be due to differences in dietary intake, however data from the 1990 Victorian Nutrition Survey of about 3,000 randomly selected subjects did not reveal any difference in PUFA intake (as percentage of energy) between males and females[16]. Another reason for the difference could be genetic and this is supported by several studies which have reported higher levels of linoleic acid in female subjects in plasma phospholipids[17], heart tissue phospholipids[18] and adipose tissue[19].

This study showed that there was a very strong relationship between the level of linoleic acid in adipose tissue and myocardial tissue, which suggests that dietary linoleic acid influences the level of myocardial linoleic acid. These findings support the hypothesis that dietary linoleic acid can have a direct influence on myocardial membrane linoleic acid. These findings might account for the beneficial effects of linoleic acid in reducing sudden cardiac death[7].

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The relationship between linoleic acid level in serum, adipose tissue and myocardium in humans

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亞油酸水平在人血清、脂肪組織和心肌間的關係

摘要

作者進行了 80 個連續屍體解剖，以測定亞油酸在人血清、脂肪組織和心肌之間的關係。研究對象在澳大利亞塔斯曼尼亞省（Tasmania）南區收集，包括 55 位男性和 25 位女性，年齡在 7 - 92 歲，對象是死於心臟病和非心臟病的患者。從血清、脂肪組織和心肌抽提脂肪酸，並用氣相層析法分離。計算三種組織主要脂肪酸的平均數和標準差。結果發現，血清和脂肪組織中亞油酸含量明顯增高（分別爲 $p < 0.001$ 和 $p < 0.001$），同時 N-6 脂肪酸總量也明顯增高（分別爲 $p < 0.002$ 和 $p < 0.001$）。另一方面，女性的油酸含量明顯低於男性（分別爲 $p < 0.001$ 和 $p < 0.05$）。女性血清和脂肪組織中的 N-6 脂肪酸和 N-3 脂肪酸比值明顯高於男性（分別爲 $p < 0.02$ 和 $p < 0.001$）。女性心肌中的油酸和亞油酸明顯高於男性（分別爲 $p < 0.05$ 和 $p < 0.001$），但廿碳四烯酸和廿二碳五烯酸則明顯低於男性（分別爲 $p < 0.001$ 和 $p < 0.002$）。女性心肌的 N-3 脂肪酸總量明顯下降（$p < 0.001$），結果使總 N-6 脂肪酸和 N-3 脂肪酸的比值明顯升高（$p < 0.001$）。作者還發現脂肪組織和心肌中亞油酸含量有明顯的 Pearson 相關（$p < 0.0001$），脂肪組織和血清間的相關爲 $p < 0.001$，血清和心肌之間的相關爲 $p < 0.001$。多不飽和脂肪酸（PUFA）在心肌中的總含量與單不飽和脂肪酸與飽和脂肪酸的含量呈負相關（分別爲 $p < 0.001$ 和 $p < 0.001$）。三種組織中的亞油酸與亞麻酸的比值呈明顯正常關。該研究顯示亞油酸在脂肪組織和心肌的含量有十分明顯的關係，指出了膳食亞油酸含量影響心肌亞油酸水平。該研究支持膳食亞油酸會直接影響心肌膜亞油酸含量的假說。

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Editors note:

Not all studies support the notion that the levels of tissue linoleic acid currently achieved by some individuals in industrialised, affluent societies are cardio-protective, at least insofar as coronary artery disease is concerned[20,21]. The present paper does not, of course, consider health outcome in relation to tissue fatty acid status.