

Fatty Acids and Associated Cardiovascular Risk

Caroline Le Goff¹, Jean-François Kaux², Ludovic Leroy¹, Joël Pincemail³, Jean-Paul Chapelle¹, Etienne Cavalier¹

¹Department of Clinical Chemistry, University and University Hospital of Liege, Liege, Belgium; ²Department of Clinical Sciences, University and University Hospital of Liege, Liege, Belgium; ³CREDEC, Department of Cardiovascular Surgery, University Hospital of Liege, Liege, Belgium.

Email: c.legoff@chu.ulg.ac.be

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ABSTRACT

Introduction: A fatty acid (FA) is a carboxylic acid with a long aliphatic chain, which is either saturated or unsaturated. Recently, the role of FA and particularly omega-3 and -6 has emerged as cardiovascular risk factor in the literature. The aim of our study was to establish reference values for these FA and to compare them with data obtained in a population of acute myocardial infarction (AMI) patients. **Materials and methods:** Hundred thirty five healthy subjects (59.38 ± 27.12 yo, 75 men) were selected as reference population. We also evaluated FA in thirty three patients (55 ± 9 yo, 23 men) admitted in the Emergency Department of our Institution for AMI. The fasting whole blood was drawn in vacuum container containing EDTA. Before analysis, samples were washed and transmethylated. We performed the quantification of different FA by gas chromatography associated with flame ionization detector (GCFID). **Results:** We obtained results in control healthy patients to be used as reference values. In the AMI group, levels of omega-6 were significantly higher ($p < 0.05$) for C18:2n6 and C18:3n6 than the reference population and omega-3 values were significantly lower ($p < 0.01$) compared to reference value for C22:6n3. The omega-3 index was lower and the ratio omega-6/omega-3 was higher in AMI group compared to reference values. **Conclusions:** We have established reference value for FA and have compared these values with the results obtained in AMI population. FA determination is a new tool we are able to use and to process in our laboratory which can help the clinician to screen patients with the highest cardiovascular risks because of the implication of FA in the etiopathogeny of atherosclerosis.

Keywords: Fatty Acids; Acute Myocardial Infarction; Gas Chromatography

1. Introduction

A fatty acid (FA) is a carboxylic acid with a long aliphatic chain. This chain can either be saturated or unsaturated. Most naturally occurring FA have a chain of 4 to 28 carbon atoms. FA are produced by hydrolysis of the ester linkages in a fat or biological oil (both of which are triglycerides), with the removal of glycerol. There are many families of FA: “essential FA” with omega-3, omega-6, omega-7, omega-9, “saturated” FA (SFA) and “unsaturated” (cis and trans) FA. **Table 1** shows the fatty acids with their common name, the International Union of Pure and Applied Chemists (IUPAC) name and the formula [1]. For many years, research has focused on the involvement of fatty acids and their repercussions on the cardiovascular system.

The implication of FA, particularly, omega-3 and -6 in different diseases has been widely published in literature [2-9]. SFA, abundantly found in our diet, participate in

the development of certain metabolic abnormalities such as insulin resistance or atherosclerosis [10,11]. However, all SFA have different metabolic effects. For example, palmitic acid (which consists of 50% palm oil versus a maximum of 17% for other oils such as peanut oil), unlike other SFA such as stearic acid, is a potent inhibitor of the intracellular pathway for insulin in some tissues such as skeletal muscle [10,12]. SFA are also considered as pro-inflammatory agents because they stimulate the inflammatory response in different cell types, such as adipocytes, particularly during the postprandial phase [12, 13]. Finally, this class of fatty acids probably contributes to the development of atherosclerotic plaques because it causes increased plasma concentrations of LDL-cholesterol [12]. All of this knowledge means that the current intake of SFA in western food, and in particular, palmitic acid, is a key player in the expansion of certain metabolic diseases such as type 2 diabetes, cardiovascular disease

Table 1. Fatty acids with their common name, the International Union of Pure and Applied Chemists (IUPAC) name and the formula.

Symbol	Chemical structure	Acid's common name	IUPAC name
Saturated fatty acid			
C14:0	$\text{CH}_3(\text{CH}_2)_{12}\text{COOH}$	Myristic acid	tétradécanoïque
C15:0	$\text{CH}_3(\text{CH}_2)_{13}\text{COOH}$	Pentadecanoic acid	Pentadecanoic
C16:0	$\text{CH}_3(\text{CH}_2)_{14}\text{COOH}$	Palmitic acid	hexadécanoic
C17:0	$\text{CH}_3(\text{CH}_2)_{15}\text{COOH}$	Margaric acid	heptadécanoic
C18:0	$\text{CH}_3(\text{CH}_2)_{16}\text{COOH}$	Stearic acid	octadécanoic
C20:0	$\text{CH}_3(\text{CH}_2)_{18}\text{COOH}$	Arachidic acid	eicosanoic
Unsaturated fatty acid ω -3			
C18: 3 ω -3	$\text{CH}_3(\text{CH}_2\text{CH}=\text{CH})_3(\text{CH}_2)_7\text{COOH}$	α -linolenic acid	cis,cis,cis-9,12,15-octadécatriénoic
C20: 5 ω -3	$\text{CH}_3\text{CH}_2(\text{CH}=\text{CHCH}_2)_4\text{CH}=\text{CH}(\text{CH}_2)_3\text{COOH}$	Eicosapentaenoic acid	cis,cis,cis,cis,cis-5,8,11,14,17-eicosapentaénoic
C22: 5 ω -3	$\text{CH}_3\text{CH}_2\text{CH}=\text{CH}(\text{CH}_2)_2\text{CH}=\text{CHCH}_2(\text{CH}=\text{CH}(\text{CH}_2)_2)_3\text{COOH}$	Docosapentaenoic acid	cis,cis,cis,cis,cis-4,8,12,15,19-docosapentaénoic
C22: 6 ω -3	$\text{CH}_3\text{CH}_2(\text{CH}=\text{CHCH}_2)_5\text{CH}=\text{CH}(\text{CH}_2)_2\text{COOH}$	Docosahexaenoic acid	cis,cis,cis,cis,cis,cis-4,7,10,13,16,19-docosahexaénoic
Unsaturated fatty acid ω -6			
C18: 2 ω -6	$\text{CH}_3(\text{CH}_2)_4\text{CH}=\text{CHCH}_2\text{CH}=\text{CH}(\text{CH}_2)_7\text{COOH}$	Linoleic acid	cis,cis-9,12-octadécadiénoic
C18: 3 ω -6	$\text{CH}_3(\text{CH}_2)_3(\text{CH}_2\text{CH}=\text{CH})_3(\text{CH}_2)_4\text{COOH}$	γ -linolenic acid	cis,cis,cis-6,9,12-octadécatriénoic
C20: 3 ω -6	$\text{CH}_3(\text{CH}_2)_4(\text{CH}=\text{CHCH}_2)_3(\text{CH}_2)_5\text{COOH}$	di-homo- γ -linolenic acid	cis,cis,cis-8,11,14-eicosatriénoic
C20: 4 ω -6	$\text{CH}_3(\text{CH}_2)_4(\text{CH}=\text{CHCH}_2)_3\text{CH}=\text{CH}(\text{CH}_2)_3\text{COOH}$	Arachidonic acid	cis,cis,cis,cis-5,8,11,14-eicosatetraénoic
Others unsaturated fatty acid			
C16: 1 ω -7	$\text{CH}_3(\text{CH}_2)_5\text{CH}=\text{CH}(\text{CH}_2)_7\text{COOH}$	Palmitoleic acid	cis-9-hexadécénoic
C18: 1 ω -9	$\text{CH}_3(\text{CH}_2)_7\text{CH}=\text{CH}(\text{CH}_2)_7\text{COOH}$	Oléic acid	cis-9-octadécénoic
C20: 1 ω -9	$\text{CH}_3(\text{CH}_2)_7\text{CH}=\text{CH}(\text{CH}_2)_9\text{COOH}$	Gondoic acid	cis-9-eicosénoic
Trans unsaturated fatty acid			
C18: 1 ω -9	$\text{CH}_3(\text{CH}_2)_7\text{CH}=\text{CH}(\text{CH}_2)_7\text{COOH}$	Elaidic acid	trans-9-octadécénoic
C18: 2 ω -6	$\text{CH}_3(\text{CH}_2)_4\text{CH}=\text{CHCH}=\text{CH}(\text{CH}_2)_8\text{COOH}$	Linolelaidic acid	trans,cis-10,12-octadécadiénoic

or metabolic syndrome [8,14]. Dietary long-chain, poly-unsaturated fatty acid (PUFA) also benefit the blood vessel wall by enhancing cell membrane fluidity and regulating membrane receptors [15].

Hundreds of epidemiologic studies, either of mechanisms of action, or experimental in animals have shown that dietary intake of omega-3 FA presented anti atherosclerotic potential [16-24]. The role of FA in the atherosclerosis process was already discussed by Sinclair in 1956 in the Lancet [25].

Some authors have demonstrated that the protective effect of omega-3 PUFAs in sudden cardiac death which

was most often due to ventricular arrhythmia [5,26]. However, in patients with implantable cardioverter defibrillators and cardiac failure, no benefit of supplementation with omega-3 could be established [27]. The erythrocyte fatty acid profile is modified; the omega-3 PUFA concentrations are elevated and predict the risk of ventricular arrhythmia. These results highlight an altered cardiac metabolism in patients with heart failure and may have clinical implications for early identification of subjects at high risk [28].

In general, prospective epidemiological studies have shown a relationship between intake of trans fatty acids

and increased morbidity and mortality from cardiovascular disease in Europe and North America [29,30]. The incomes of trans fatty acids compared to the intake of cis fatty acids, increase levels of LDL-cholesterol, triglycerides, lipoprotein Lp (a) and decrease levels of HDL-cholesterol and the actual particle size of LDL-cholesterol. Each point may increase the risk of coronary heart disease [31].

An important concept in the field of fatty acids is the balance omega-6/omega-3. An ideal relationship for dietary intake would be 1:1 but it is rather around the ratio of 15:1, linked to deficiency of omega-3 PUFA intakes. The increase in the proportion of omega-6 PUFA is considered as a risk factor for many diseases such as heart disease, inflammatory, autoimmune and various cancers [2]. Freije compared the fatty acid composition of erythrocyte membranes in healthy patients with those of patients with coronary heart disease in Bahrain [2]. The results mainly showed a low level of docosahexaenoic acid which increases the risk of coronary heart disease. Moreover, there was also an increased ratio of omega-6/omega-3. An omega-3 index decrease (which is the sum of the percentages of docosahexaenoic acid and eicosapentaenoic acid), was also observed in these patients [3].

The aim of our study was to establish reference values for these FA and to compare the results obtained with data observed in patients with acute myocardial infarction (AMI) seen the protector role of PUFA particularly, omega-3, and the implication of SFA in the development of atherosclerosis [32].

2. Materials and Methods

Hundred thirty five healthy subjects (59.38 ± 27.12 yo, 75 men) were selected as reference population (patient without cardiac antecedent or diabetes). We also evaluated FA in thirty three patients (55 ± 9 yo, 23 men) admitted in the Emergency Department of our Institution for AMI. All patients gave their informed consent.

Fasting whole blood samples were drawn in vacutainer tubes containing EDTA. One millilitre of whole blood was centrifuged at 3500 rpm for 5 min at 4°C. Plasma was carefully separated and the red blood cells (RBC) pellet was washed three times with equal volumes of standard saline. All the washings were carried out at 4°C. The white viscous material after each washing was carefully removed to minimize contamination by non-erythrocytic cells. The samples were finally stored at -20°C until analyzed (personal data).

Before analysis, the samples were washed and an extraction was done.

Briefly, 0.3 ml packed RBCs were placed in a 5 ml clean glass vial with 100 µl of C28 (Internal Standard). RBCs were then treated with 2 ml of methanolic-

HCl.95%/5%. The vials were sealed and incubated at 80°C for 2 h. After incubation the mixture was treated with 5 ml of hexane and centrifuged at 3500 rpm for 10 min at room temperature using a swinging rotor. The top aqueous layer comprised mainly of methylated fatty acids. Hexane mixture was then carefully collected in a separate vial. This step was repeated three times and all extractions were pooled. The methylated-fatty acids hexane mixture was then completely dried at 55°C under nitrogen. Methylated fatty acids were dissolved in 100 µl hexane and 1 µl was identified and quantified by gas chromatography with flame ionization detector (GC/FID) performed on a Shimadzu GC2010, using a capillary column SP-2380 of 30 mm × 0.25 mm × 0.20 µm dimensions (Supelco). A FID was used with an oven temperature set at 250°C. The temperature of the injector was 250°C whereas the detector was set at 260°C.

The quantification of FA is realized according to the calibration curve that we have established with standards for all peaks that we must identify.

First, we expressed the results in mg/L and after we chose to express results in percent (%) from the total of results. The sum of the FA found in a patient equals 100% and with a simple calculation we expressed them in % in relation to the sum of FA.

For the statistical analysis, we used an independent sample T-test with the statistical software Medcalc 9.1 (Mariakerk, Belgium).

3. Results

Figure 1 shows the chromatographic profile of the different FA analyzed. The **Table 2** presents the comparison of the results observed in AMI and in healthy subjects (reference values).

We obtained differences between AMI group and our healthy population.

In AMI, the levels of omega-6 were significantly higher ($p < 0.05$) for C18:3n6. The level of omega-3 was lower in comparison with reference values but not significantly. We also calculated the omega-3 index and the ratio omega-6/omega-3. The omega-3 index was significantly lower ($p < 0.0001$) in AMI compared to the reference value and the omega-6/omega-3 ratio was significantly higher ($p < 0.005$) in AMI than in reference patients. The sum of SFA was also significantly higher in AMI group ($p < 0.05$). We also noted a significantly difference ($p < 0.005$) between both types of patients for C18:1n9.

4. Discussion

The incidence of cardiovascular disease is different between the countries and increases from Southern to the Northern countries (Europe) [33,34].

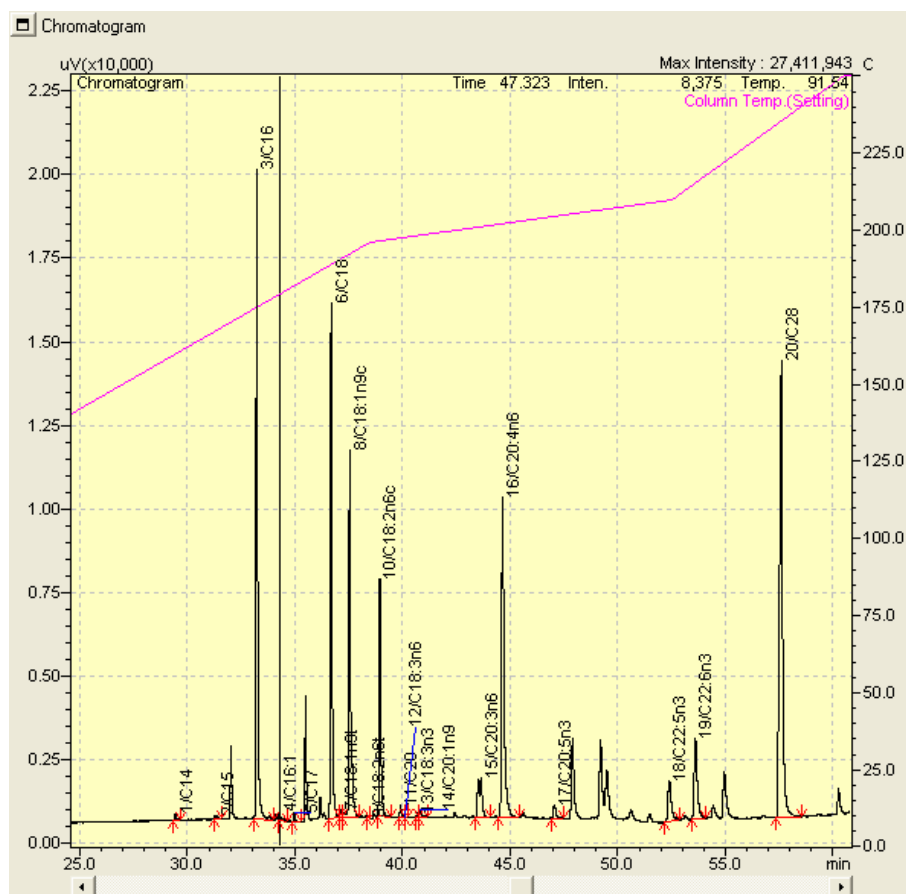


Figure 1. Example of a chromatographic profile of the different FA analyzed by gas chromatography in a healthy subject.

Cardiovascular diseases are the first cause of mortality and morbidity in the developed countries. They are the world's largest killers, claiming 17.1 million lives a year [35]. This incidence has increased with time. One explanation is probably found in changes of life style: aging, tobacco use, unhealthy diet, stress, physical inactivity and harmful use of alcohol increase the risk of heart attacks and strokes [35]. Atherosclerosis initiation and early progression is almost invariably a result of abnormal interactions between circulating oxidized lipids and blood vessel walls. Endothelial dysfunction has been linked to diverse vascular abnormalities like atherosclerosis. The first detectable stage in atherosclerosis is a slightly raised, fatty "streak" in the arterial wall. Among Western populations, these can be found in most individuals beginning as early as the teenage years [15]. In Northern of Europe, it is well known that people do not follow the Mediterranean diet, which has long been celebrated as the gold standard of healthy diet for its favourable impact on the prevention of chronic diseases, promotion of greater longevity and quality of life [36].

We have studied AMI patients because they are at risk of a second myocardial infarction due to their cardiovascular risk phenotype. Moreover, the implication of SFA

and PUFA in the etiopathogeny of atherosclerosis is well known. We found thus very interesting to be able to make the quantification of these different FA to achieve on the dietary prevention (supplementation in omega-3, decrease of intake of SFA...).

For the SFA, we observed a statistical increase between AMI and control subjects. These results are in accordance with Walrand *et al.* study [12], which explained that SFA was a key player in the expansion of cardiovascular disease. This class of fatty acids is most likely involved in the development of atherosclerosis probably due to the induced increasing of plasma concentrations of LDL-cholesterol. However, one of the limitations of our study is without any doubt the age difference between both patient groups as well as daily hygiene which we were able to define with our control group but not with the AMI group.

Our study also showed a statistically significant difference between the percentages of oleic acid (C18: 1n 9c) in the 2 groups. This increase in oleic acid has also been observed in the study of Harris *et al.* [4] who studied the lipid profile and erythrocytic plasma after myocardial infarction. It must be said that this fatty acid is often considered beneficial as it benefits from the posi-

Table 2. Comparison between AMI and reference group for FA (FA in %). p-values in red are statistically significant (p < 0.05).

	FA AMI % (n = 33)	FA reference % (n = 135)	p-value
C14	1.00 ± 0.21	0.9 ± 0.19	0.024
C15	0.34 ± 0.07	0.37 ± 0.15	<0.001
C16	25.45±1.51	31.05 ± 7.7	<0.001
C16: 1	0.56 ± 0.21	0.52 ± 0.23	0.329
C17	0.42 ± 0.09	0.41 ± 0.07	0.085
C18	20.53 ± 1.35	20.16 ± 1.56	0.342
C18: 1ω9t	0.23 ± 0.13	0.19 ± 0.09	0.003
C18: 1ω9c	15.43 ± 1.38	14.68 ± 1.03	0.005
C18: 2ω6t	0.07 ± 0.02	0.07 ± 0.04	0.513
C18: 2ω6c	9.15 ± 1.34	9.64 ± 0.89	0.043
C20	0.54 ± 0.09	0.57 ± 0.08	0.063
C18: 3ω6	0.11 ± 0.04	0.08 ± 0.02	0.034
C18: 3ω3	0.22 ± 0.04	0.24 ± 0.06	0.212
C20: 1ω9	0.27 ± 0.08	0.26 ± 0.05	0.553
C20: 3ω6	2.14 ± 0.50	2.26 ± 0.51	0.301
C20: 4ω6	15.45 ± 1.54	14.99 ± 1.15	0.112
C20: 5ω3	0.78 ± 0.26	0.72 ± 0.34	0.079
C22: 5ω3	1.94 ± 0.34	2.00 ± 1.08	0.749
C22: 6ω3	5.37 ± 1.36	6.28 ± 3.04	0.094
Sum of SFA	48.27 ± 1.38	47.57 ± 1.35	0.022
ω6/ω3	3.39 ± 0.86	2.86 ± 0.58	0.001
ω3 index	6.15 ± 1.51	7.60 ± 1.62	<0.001

tive image of olive oil, containing 70% oleic acid, and no less favourable than the Mediterranean diet. However, a portion of beneficial effects in olive oil on endothelial functions, inflammation, platelet aggregation, and perhaps even on HDL cholesterol is the lipid fraction of the olive oil [37].

In regards to trans fatty acids some statistically significant difference (p < 0.005) was observed between the 2 groups although many articles [31,38] show the harmful effects related to the consumption of trans fatty acids on the development of cardiovascular risk factors. Indeed, they are proven to increase levels of LDL-cholesterol, triglycerides, lipoprotein Lp (a), and lower levels of HDL-cholesterol as well as particle size of LDL-cholesterol. It is for all these reasons that the political authorities try to regulate the possible content of trans fat in food.

In this study, we observed differences between the two studied groups in terms of docosahexaenoic acid (C22: 6 n3), omega-6/omega-3 ratio and the omega-index, but also a significant difference for linoleic acid (C18: 2 n6c). These differences are consistent with both studies [3,39], which highlighted the same differences. However in the Freije study, the percentage of C22: 6 n3 decreased from 2.81% to 0.41% and the ω3 index from 3.14% to 0.64% in control subjects and AMI patients [2]. These results were obtained in the kingdom of Bahrain and the authors believe that these low values of C22: 6 n3 could be due to very low omega-3 fatty acids in fish inhabiting nearby waters [2]. The attempts to corroborate the higher results were obtained.

5. Conclusion

We have established reference values for FA implicated in different pathologies like cardiovascular diseases. This is a new tool we are able to use in our laboratory which can help clinicians to highlight and target patients with a higher cardiovascular risks. The clinicians could then supplement the patients with, for example, omega-3 to reduce the risk of developing cardiac diseases because of the implication of FA in the etiopathogeny of atherosclerosis.

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