Quantification of the major ω-3, ω-6 PUFAs using the Purdie assay and their ratios in different cholesterol types and the effects of gender and cholesterol on PUFA levels

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This study used the Purdie assay, a new assay, to quantify the levels of omega-3 (ω-3) and omega-6 (ω-6) polyunsaturated fatty acids (PUFAs) in mol/L and the ratio of ω-6:ω-3 in Total cholesterol (TC), high-density lipoprotein cholesterol, (HDL-C) and low-density lipoprotein cholesterol (LDL-C) (referred to throughout as "non-HDL-C" in this study) fractions in 35 samples of human serum and also assessed the influence of gender and cholesterol types on levels of the analyzed PUFAs.

Three principal components explained 89% of the total variance based on the variables measured. The ratio of ω-6:ω-3 PUFAs was significantly influenced by the type of cholesterol ($F = 10.84$, $df = 2, 99$, $P = < 0.001$) but not gender or interaction between gender and type of cholesterol, while the total PUFAs and the levels of ω-3 and ω-6 PUFAs were significantly influenced by gender, but age did not have a significant effect on the levels for total PUFAs.

The findings of this study imply that, for males, more focus should be on the ratios of ω-6:ω-3 PUFAs in the non-HDL-C fraction and that the use of the ω-6:ω-3 ratio of PUFAs in serum is a better predictor of coronary heart disease than estimating LDL-C.

Keywords: Coronary heart disease (CHD); High-density lipoprotein cholesterol (HDL-C); Human serum; Low-density lipoprotein cholesterol (LDL-C); Non-high-density lipoprotein cholesterol (non-HDL-C); Omega-3 (ω-3); Omega-6 (ω-6); Polyunsaturated fatty acid (PUFA); Purdie assay; Total cholesterol (TC)

Abbreviations: AA, Arachidonic acid; ALA, Alpha-linolenic acid; ANOVA, Two-way Analysis of Variance; CHD, Coronary heart disease; CLA, Conjugated linoleic acid; CVD, Cardiovascular heart disease; DHA, Docosahexaenoic acid; EPA, Eicosapentaenoic acid; FF, Friedewald formula; HDL, High-density lipoprotein; HMC, Hillcrest Medical Center; LCFA, Long chain fatty acid; LDL-C, Low-density lipoprotein cholesterol; LNA, Linoleic acid; LSP, Laboratory Standardization Panel; mg/dL, Milligram/deciliter; NCEP, National Cholesterol Education Program; PC, Principal Component; PCA, Principal Component Analysis; PUFA, Polyunsaturated fatty acid; SAS, Science Analysis System; TC, Total cholesterol; TG, Triglyceride

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1. Introduction

Cardiovascular diseases are the world's largest killers, claiming 17.1 million lives a year [1]. In the USA, heart diseases are the leading cause of death and are a major cause of disability [1]. The most common heart disease in the USA is coronary heart disease (CHD). In 2010, an estimated 785,000 Americans had a new coronary attack, and about 470,000 had a recurrent attack [1].

Many researchers have cited that the chance of developing CHD can be reduced by taking steps to prevent and to control factors that put people at greater risk [1–4]. This calls for a more elaborate method for diagnosing, predicting and reporting CHD risk factors (e.g., TC and the PUFAs in TC, HDL-C and LDL-C). The current methods used in the measurement

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of TC include colorimetric, enzymatic, chromatographic, and near infrared reflectance spectrometry detection methods [3–6], while those used to measure serum lipids/TGs include ultracentrifugation, selective precipitation, electrophoresis, and immunochemical methods. These methods fail to report the quantitative values of the individual major PUFAs in TC, HDL-C, and LDL-C. Further, serum LDL-C concentrations in clinical practice are most often obtained as a calculated value derived from the Friedewald formula (FF) [7]. This formula is known to be inaccurate in estimating the levels of LDL-C, especially when serum-TG concentrations are >4.52 mmol/L and may also be inaccurate among specific patient groups at these lower TG concentrations [8–10].

It is therefore essential to have a method that can effectively quantify serum-PUFA concentrations whether greater, equal to or less than 400 mg/dL. However, it is important to have a paradigm shift that might change the focus away from cholesterol lipofraction as the major risk factor to the effects of the components of the TGs. The ''real'' numbers for the TG levels are reported in mg/dL and percentages [5]. It is evident that TGs biochemically break down into long-chain saturated fatty acids (LCFAs) and polyunsaturated fatty acids (PUFAs). Of these, the latter are considered as potential biomarkers for deriving quantitative health-risk prediction models [5,6] and are divided into two groups (i.e. ω-3 and ω-6) [6]. These are divided into the ω-3 (ALA, EPA and DHA) and ω-6 (LNA, CLA and AA) – sub-types that are considered ''good'' and ''bad'', respectively, for maintaining human health.

In the search for a new, early diagnostic biomarker, it is important to assess the presence of elevated levels of ω-6 fatty acids, perhaps even before considering serum-cholesterol levels or both. The concern is that, although cholesterol and total TGs, are both routinely reported in terms of mg/dL, in clinical laboratories, only cholesterol can be converted into the proper mmol units.

Cholesterol and PUFA molecules share the same allylic –(CH═CH–CH_2) functional group that is susceptible to oxidative stress; that group is absent in both saturated LCFAs and apolipoproteins [5]. If that functional group, present in the B-ring of the cholesterol molecule, is the origin of its role as a CHD risk factor, most probably the PUFAs share a role as a CHD risk factor greater than cholesterol.

According to the NCEP, decreasing the levels of LDL-C is the primary strategy for reducing the risk of CVD. However, despite the intervention aimed at LDL-C, 60–70% of coronary events still occur [11]. This strongly suggests that factors other than LDL-C might be more important in determining the risk of CVDs. Thus, we contend that inclusion of other factors (e.g., the levels of ω-3 and ω-6 PUFAs in TC, HDL-C and non-HDL-C fractions and the ω-6:ω-3 in the patient's diagnoses) might give a more elaborate meaning.

This study uses the Purdie assay simultaneously to quantify the ω-3 and ω-6 PUFAs in TC, HDL-C, non-HDL-C fractions in mol/L and the ratio of ω-6:ω-3 PUFAs in TC, HDL-C, and non-HDL-C fractions. The method meets precision and accuracy requirements of LSP [12]. The serum samples for this study were provided by HMC Tulsa, OK, USA.

2. Samples, subjects, chemicals and method used

2.1. Samples and Subjects

The study analyzed human-serum samples obtained from 35 subjects aged between 23 and 99 years (54.8 ± 15.8 years).

2.2. Chemicals used

98% acetyl chloride (Acros) perchloric acid (70% ACS reagent grade, GFS); DL and LDL/VDL Cholesterol Assay Kit (ab65390) from abcam; 2X LDL/VDL precipitation buffer.

2.3. Methods

2.3.1. Analysis of ω-3 and ω-6 in TC, HDL-C and LDL-C in human serum. Blood serum was obtained from volunteering patients from HMC Tulsa after fasting overnight. The anonymous samples from HMC were from volunteers who had already requested a lipid profile and had given consent. No attempt was made to solicit samples nor was any extensive medical information derived from the samples except for the TC, HDL-C, and TG levels, which were determined by the HMC clinicians. Prior to obtaining blood from an antecubital vein, patients assumed a sitting position for 5 min, since posture change can alter the serum-cholesterol concentrations. The blood samples were collected into tubes without anticoagulant and centrifuged to obtain serum after separation from the clot within 2 h. The sera were stored at a temperature of −80°C and transported to the Purdie Laboratory.

There, all the blood samples were analyzed for the levels of the ω-3 and ω-6 PUFAs in the TC using the Purdie assay and the HDL and LDL-C fractions were separated from each serum sample on the same day using HDL and LDL/VDL Cholesterol Assay Kit HDL and LDL/VLDL Cholesterol Assay Kit (ab65390) obtained from abcam and stored at −80°C. During these analyses, a 1-mL aliquot acetyl chloride was added into a 13 × 100 borosilicate disposable test tube containing exactly 10 μL of serum sample. To the acetyl chloride-sample mixture, a 40 μL aliquot of perchloric acid (70% ACS reagent grade, GFS) was carefully added. The mixture was shaken for 20 s, and the test tube was covered with a Teflon cap, placed into a centrifuge and spun for 3 min at 3400 RPM.

After centrifugation, the reagent solution was transferred to a 10-mm path-length optical glass