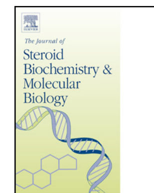
Journal of Steroid Biochemistry & Molecular Biology 169 (2017) 111–122

Contents lists available at ScienceDirect

Journal of Steroid Biochemistry & Molecular Biology

journal homepage: www.elsevier.com/locate/jsbmb



The value of surrogate markers to monitor cholesterol absorption, synthesis and bioconversion to bile acids under lipid lowering therapies

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ARTICLE INFO

Article history:
Received 30 November 2015
Received in revised form 21 March 2016
Accepted 22 March 2016
Available online 6 April 2016

Keywords:
Fecal balance
Cholesterol precursors
Bile acid precursors
Oxysterols
Non-cholesterol sterols

ABSTRACT

Introduction: Regulation of cholesterol (Chol) homeostasis is controlled by three main fluxes, i.e. intestinal absorption, *de novo* synthesis (ChS) and catabolism, predominantly as bile acid synthesis (BAS). High serum total Chol and LDL-Chol concentrations in particular are considered risk factors and markers for the development of atherosclerosis. Pharmaceutical treatments to lower serum Chol have focused on reducing absorption or ChS and increasing BAS. Monitoring of these three parameters is complex involving isotope techniques, cholesterol balance experiments and advanced mass spectrometry based analysis methods. Surrogate markers were explored that require only one single fasting blood sample collection. These markers were validated in specific, mostly physiological conditions and during statin treatment to inhibit ChS. They were also applied under cholesterol absorption restriction, but were not validated in this condition. We retrospectively evaluated the use of serum campesterol (Camp), sitosterol (Sit) and cholestanol (Cholol) as markers for cholesterol absorption, lathosterol (Lath) as marker for ChS and 7α-hydroxycholesterol (7α-OH-Ch) and 27-hydroxycholesterol (27-OH-Ch) as markers for BAS under conditions of Chol absorption restriction. Additionally, their values were corrected for Chol concentration (R_sterol or oxysterols).

Methods: Thirty-seven healthy male omnivore subjects were studied under treatments with placebo (PLAC), ezetimibe (EZE) to inhibit cholesterol absorption, simvastatin (SIMVA) to reduce cholesterol synthesis and a combination of both (EZE + SIMVA). Results were compared to those obtained in 18 pure vegetarian subjects (vegans) whose dietary Chol intake is extremely low. Relative or fractional Chol absorption (FrChA) was measured with the continuous feeding stable isotope procedure, ChS and BAS with the cholesterol balance method. The daily Chol intake (DICh) was inventoried and the daily Chol absorption (DACh) calculated.

Results: Monitoring cholesterol absorption, R_Camp, R_Sit and R_Cholol responded sensitively to changes in FrChA. R_Camp correlated well with FrChA in all omnivore treatment groups and in the vegan group. R_Camp confirmed reduced FrChA under EZE treatment and reduced DACh in the vegan subjects. R_Sit and R_Cholol did not accurately reflect FrChA or DACh in all situations. Monitoring endogenous cholesterol synthesis, R_Lath correlated with ChS in the vegan group, but in none of the omnivore treatment groups. R_Lath confirmed increased ChS under EZE treatment and was reduced under SIMVA treatment, while ChS was not. An increased ChS under EZE + SIMVA treatment could not be confirmed with R_Lath. R_Lath responded very insensitively to a change in ChS. Monitoring BAS, R_7α-OH-Ch but not R_27-OH-Ch correlated with BAS during PLAC, EZE and SIMVA treatments. In line with BAS, R_7α-OH-Ch did not differ in any of the omnivore treatment groups. R_7α-OH-Ch responded insensitively to a change in BAS.

Conclusions: Under Chol absorption restriction, serum R_Camp is a sensitive and valid marker to monitor FrChA in a population with a normal DICh. Also, major changes in DACh can be detected in vegans. Serum R_Lath does not reflect ChS measured with the cholesterol balance method during EZE treatment. This result initiates the question whether the measured ChS reflects pure *de novo* synthesis. Serum R_7α-OH-Ch appears to be a valid but insensitive marker for BAS.

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Abbreviations: 7α-OH-Ch, 7α-hydroxycholesterol; 27-OH-Ch, 27-hydroxycholesterol; BAS, bile acid synthesis; Camp, campesterol; Chol, cholesterol; Cholol, cholestanol; ChS, daily cholesterol synthesis rate; DACh, daily dietary cholesterol absorption rate; DICh, daily dietary cholesterol intake; EZE, ezetimibe; FBAE, daily fecal bile acid excretion; FChE, daily fecal cholesterol excretion; FrChA, fractional cholesterol absorption; Lath, lathosterol; SIMVA, simvastatin; Sit, sitosterol.

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http://dx.doi.org/10.1016/j.jsbmb.2016.03.030
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1. Introduction

Cholesterol (Chol) metabolism consists of a complex set of interactive input and output fluxes with the aim to provide the large membrane bound Chol pool with sufficient Chol molecules. For this purpose, two input fluxes are active. One is the absorption of dietary Chol, the other is *de novo* Chol synthesis (ChS). Furthermore, Chol is also catabolized to produce steroid hormones and bile acids. Bile acid formation is quantitatively most important. Bile acids serve to keep biliary Chol dissolved in the bile and to support transit of dietary Chol and fat through the small intestine [1]. Bile acids are also involved in the regulation of their own synthesis and that of glucose and lipid metabolism [2,3]. High serum Chol concentration, and in particular that of LDL-Chol, is considered a risk factor for atherosclerosis. The accumulation of serum LDL-Chol can be explained by a malfunctioning control of the LDL receptor-mediated uptake of Chol into peripheral tissues or an oversupply due to an excessive *de novo* synthesis or a high dietary intake. In the ultimately controlled situation, Chol absorption, Chol *de novo synthesis* and bile acid formation are interactive. Increased Chol absorption is thus compensated by reduced ChS and increased bile acid synthesis (BAS) [4]. The fractional absorption of Chol (FrChA) from the intestine has been measured using radioactive and stable isotope techniques applying continuous dual isotope feeding and feces collection [5–7] or single dual isotope administration and blood sampling [8,9]. In individual healthy subjects, values between 10% and 80% have been reported. While in textbook terminology, the mean FrChA is reported as 50%, mean values obtained in groups of healthy subjects vary between 24% and 70% [10]. Stable isotope enrichments of Chol are low and measured with complex gas chromatography/mass spectrometry techniques. Isotope techniques were developed to study the incorporation of ^{13}C-acetate or $^{2}H_2O$ into plasma cholesterol in order to express ChS activity [11–14]. A high fraction of newly synthesized Chol represents a high synthesis rate. However, no information is obtained on the daily ChS. Therefore, the classic way of ChS measurement is the cholesterol balance method [5]. Based on total body Chol metabolism, Chol and bile acids excreted with the feces are compensated by ChS and daily dietary Chol intake (DICh). Measurement of daily fecal Chol (FChE) and bile acid excretion (FBAE) allows the calculation of ChS as [(FChE + FBAE) – DICh]. The measurement of Chol catabolism involves the measurement of BAS. This can be performed with the measurement of FBAE based on the steady state principle that BAS compensates for FBAE. Alternatively, stable isotope labeled bile acids are administered and the decay of isotope enrichment of bile acids is measured in blood over a period of four days [15–17]. A direct comparison of both methods has been published [18] indicating that lower values are obtained with the Chol balance method.

Measurement of Chol absorption, synthesis and catabolism would be very helpful to determine the underlying cause of hypercholesterolemia in individual patients at risk for atherosclerosis. The information could help to determine the best therapeutic approach to reduce Chol absorption, to reduce Chol synthesis, or to increase BAS. However, the complexity of the different experimental procedures prevents application as routine clinical diagnostic tests. Therefore, alternative procedures have been developed based on the measurement of surrogate markers in fasting serum that reflect FrChA, ChS and BAS. Plant sterols have been shown to use the same endothelial transport system for absorption as cholesterol [19]. Reduction of FrChA results in a similar reduction in the fractional plant sterol absorption. For over 25 years, the fasting serum levels of campesterol (Camp) and sitosterol (Sit) corrected for the Chol levels (R_Camp and R_Sit) have been used to monitor the daily Chol absorption rate [20–24]. Also, serum cholestanol (Cholol), the 5α-saturated derivative of Chol, acts as a surrogate marker for FrChA [25]. Lathosterol (Lath) is the second last step of the Kandutsch-Russell part of the steroidal cholesterol synthesis pathway. Its serum concentration corrected for Chol concentration (R_Lath) has been identified as a valid surrogate marker to express the activity of ChS [26]. As surrogate markers for BAS, serum 7α-hydroxycholesterol or its follow-up product 7α-hydroxy-4-cholesten-3-one (C4) were established [27–29] and shown to correlate to BAS measurement performed with stable isotopes [30]. Bile acid synthesis is known to contain a neutral and major pathway based on cholesterol 7α-hydroxylation as the first and rate-limiting step as well as an acidic and minor pathway based on cholesterol 27-hydroxylation as the initial step. The validity of serum 7α-hydroxy-cholesterol (7α-OH-Ch) as surrogate marker for BAS was documented in a number of studies [28,29]. Serum 27-hydroxy-cholesterol (27-OH-Ch) has not been investigated as a marker to date.

Serum surrogate markers for Chol metabolism have been validated by comparison with established methods determining real functionality (absorption, synthesis, catabolism) or reflecting the expected change in function in patients during therapy. Possibly interfering dietary factors were studied and found to not disturb the validity of the markers [31].

Recently, Jakulj et al. published a comparison of serum plant sterol concentrations and FrChA determined by the blood based stable isotope approach [9,32] and concluded that the serum plant sterol concentration does not reflect FrChA [33].

Interestingly, while surrogate marker technology has been applied in patients undergoing ezetimibe (EZE) treatment [34–38], application under EZE treatment has never been validated. The effect of EZE treatment alone or in combination with SIMVA treatment on FrChA and ChS has been studied applying the original function tests [39,40]. In particular, the combined ezetemibe/statin treatment is a major challenge for surrogate markers when both Chol synthesis and absorption are downregulated. An intriguing aspect is the observation that EZE leads to increased ChS and increased total input calculated as ChS + DACh, however, eventually resulting in decreased serum total Chol and LDL-Chol concentrations [40]. Therefore, we decided to retrospectively investigate the validity of all of the different surrogate markers for FrChA, ChSand BAS in different combinations of EZE-induced reduced absorption and SIMVA-induced reduced synthesis. As a control situation, we also investigated FrChA and ChS and the corresponding markers in pure vegetarians (vegans), whose Chol intake is extremely low and whose FrChA was shown not to be impaired [41].

2. Subjects and methods

The data of 37 healthy omnivores and 18 vegans were retrospectively analyzed. The details of subject selection and the designs of the studies have been published previously and are summarized in Table 1 [40,41]. Briefly, the omnivore study was a computer-randomized, double-blind, placebo-controlled, 4-period, balanced, crossover study comparing the effects of 10 mg ezetimibe in combination with 20 mg simvastatin (EZE + SIMVA), 10 mg ezetimibe (EZE) alone, 20 mg simvastatin (SIMVA) alone and placebo (PLAC) in the same subjects [40]. Each treatment period lasted seven weeks. In the vegetarian study, vegans were studied under PLAC and EZE treatment [41]. For this validation study, the PLAC data from the vegan subjects were compared to the PLAC data from the omnivore subjects. In the original omnivore study, only cholesterol absorption and cholesterol synthesis as well as BAS functions were measured. In the original vegetarian study, function

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