Measurement of cholesterol homeostasis health essay

Health & Medicine

In the following sections, we will first briefly review the methods that are used to measurecholesterol homeostasis in humans. We will then review the available data pertaining tocholesterol homeostasis in metabolic syndrome, abdominal obesity and insulin resistance aswell as its association with CHD risk. 2. 1. Measurement of cholesterol homeostasisThe various processes regulating cholesterol homeostasis can be measured directly withisotopes or indirectly with surrogate markers. The gold standard measurement ofcholesterol homeostasis has traditionally used cholesterol labelled with radioisotope butmore reliable techniques are now based on the use of stable isotopes and massspectrometry [26]. Using radioisotopes, one can assess cholesterol absorption or synthesisby various methods including balance methods, single dose isotopic feeding, dual isotopeplasma ratio, continuous isotope feeding and intestinal perfusion. Every method has its ownstrengths and limitations, and it is beyond the scope of this review to discuss thesetechniques in detail (see review by Matthan and Lichtenstein [27] and Stellaard and Kuipers[26]). It must be stressed that the use of radiolabeled isotope is no longer allowed in somecountries due to ethical considerations on exposing participants to radiation. Also, radioisotopes cannot be used in older populations and children and are very costly. Stable isotope methods label cholesterol as a tracer with a stable isotope given toparticipants orally, intravenously or a combination of those two. Incorporation of the traceris measured in either blood or faecal samples by gas chromatography and massspectrometry (GC-MS) to derive various measures of cholesterol homeostasis. Methodsbased on stable isotopes use the dual isotope plasma ratio or continuous isotope feedingapproaches [26, 27]. These methods

involve labour-intense processes to purify thecholesterol and its tracer prior to analysis. Their use may also be limited by availability ofspecific tracers for research. Collection of faecal samples may be a limitation in some cases. On the other hand, continuous infusion methods can assess cholesterol absorption overtime and this is a significant strength. The stable isotope techniques are also extremelyprecise, sensitive and safe [27, 28]. Intestinal cholesterol absorption and endogenous synthesis can also be estimated usingsurrogate markers in plasma such as non-cholesterol sterols, stanols and phytosterols. Recognized surrogate markers of endogenous cholesterol synthesis are plasma cholestenol, desmosterol, lathosterol and squalene concentrations [29, 30]. These molecules aresequential precursors in the cholesterol biogenesis pathway and have been validated asrelatively strong correlates of isotopically measured cholesterol synthesis [29, 31]. Plasmalathosterol concentrations have been shown to correlate particularly strongly with directmeasures of cholesterol endogenous synthesis [32]. Intestinal cholesterol absorption canalso be assessed using surrogate markers, i. e. plasma cholesterol metabolites (cholestanol)and plant-sterols (campesterol, beta-sitosterol) [27, 29, 30]. Campesterol and beta-sitosterolare plant-derived sterols, or phytosterols, that are present in small quantity in westernizeddiets. Phytosterols have a higher affinity for micelles in the intestine than cholesterol andare absorbed through similar pathways in the gut. Their rate of absorption, however, isabout a thousand times lower than that of cholesterol [33]. Unlike cholesterol, phytosterolsare not synthesized in the human body, and therefore their plasma concentrations can beused to reflect the capacity of the body to absorb cholesterol [32].

Researchers have developed and adapted methods to measure cholesterol homeostasissurrogate markers in blood plasma by capillary gas-liquid chromatography (GLC) [29, 30, 32, 33]. A major strength of this method is that it is technically both fast and relatively simple. This is the reason why it currently represents the only method available to assess cholesterolhomeostasis in large-scale clinical trials and epidemiological studies. Indirect methods basedon surrogate markers are limited by the fact that they do not provide actual rates ofcholesterol synthesis and absorption. Surrogate markers only reflect the balance betweencholesterol synthesis and absorption in the body. Furthermore, the validity of using plasmaphytosterol concentrations as surrogates of cholesterol absorption becomes questionablewhen there are significant variations in the intake of plant sterols by participants over timeor when participants have genetic disorders like phytosterolemia [27]. 2. 2. Association between cholesterol homeostasis markers and CHD riskThe study of the association between surrogate markers of cholesterol homeostasis andCHD risk is emerging and has become of great interest as preliminary data suggest that suchmarkers may predict CHD risk independent of other traditional risk factors including plasmaLDL-cholesterol concentrations. Matthan et al. [30] have shown using retrospective datafrom the Framingham Offspring Study Cycle-6 (N = 155 cases and 414 controls respectively)that plasma concentrations of cholesterol synthesis surrogates (desmosterol andlathosterol) and of cholesterol absorption surrogates (campesterol, beta-sitosterol andcholestanol) were significantly correlated with the risk of CHD. Specifically, a 1-SD increase incholesterol synthesis surrogates was

associated with an approximately 40% lower odds ofhaving CHD. In contrast, a 1-SD increase in each of the cholesterol absorption surrogateswas associated with a 147%, 87% and 57% increase in the risk of CHD respectively. Surrogatemarkers were expressed per mole of plasma cholesterol and therefore their associationswith CHD risk were to some extent independent of variations in plasma cholesterolconcentration. Cases and controls were matched for age, body mass index (BMI) and systolicblood pressure and no significant difference in waist circumference was observed betweencases and controls. Furthermore, associations were significant even after adjustment formedication use, diabetes and diastolic blood pressure. Results from a smaller case-controlstudy in non-diabetic subjects (N= 66 cases and 111 controls respectively) indicated that anincreased plasma lathosterol-to-cholesterol ratio and a reduced campesterol-to-cholesterolratio both predicted lower odds ratios for CHD [34]. Taken together, these results suggestthat enhanced endogenous cholesterol synthesis and reduced intestinal cholesterolabsorption may be associated with a lower risk of CHD, independent of several traditionalrisk factors, including plasma cholesterol concentrations. These are not consistent findings, however. Escurriol et al. [33] have shown in a nested casecontrolstudy (N= 299 cases and 584 controls) that having moderately elevated plasma betasitosterollevels reflecting increased cholesterol absorption was associated with a reducedrisk of having CHD, subjects in the highest beta-sitosterol-to-cholesterol tertile showing a41% lower odds ratio for CHD than those in the lowest tertile. Associations were weaker forplasma campesterol. These results are consistent with the findings from the LongitudinalAging Study Amsterdam

(LASA) by Fassbender et al. [35], who showed in a cohort of 1242elderly Dutch subjects (> 65 years) that increased plasma beta-sitosterol concentrations wereassociated with a significant 22% reduction in the risk of CHD. Variations in the plasmaconcentration of other plant sterol and surrogate markers of endogenous cholesterolsynthesis showed no significant association with CHD risk [35]. A 22-year prospective studyof 232 men (mean age 60 years) at high risk of CHD suggested that lower cholesterolsynthesis and higher absorption profiles were associated with lower total and CHD mortality[36]. Finally, the Coronary Risk factors for Atherosclerosis in women study (CORA) is aretrospective study of 186 cases and 231 controls with comparable plasma LDL-cholesterolconcentrations that showed no association between plasma phytosterol concentrations andthe risk of CHD [37]. Inconsistent associations in these studies between cholesterol homeostasis surrogates andCHD risk can be attributed to a number of factors. First and foremost, the variety in studydesigns (cross-sectional vs. prospective, nested case control vs. population-based) and instudy outcomes (total cardiovascular vs. coronary heart disease) can explain many of the10 inconsistencies between studies. The age and gender of patients in these studies may alsohave confounded results. Another key point to emphasize pertains to the fact that various groups of subjects arecompared to each other in epidemiological studies in the absence of any treatment. It is wellaccepted that statin treatment inhibits endogenous cholesterol synthesis, which in turn isassociated with increased intestinal cholesterol absorption, and that overall this is alsoassociated with a lower risk of CHD. That certainly does not imply that increasing

cholesterolabsorption per se may be beneficial from a cardiovascular health perspective. Data suggestthat assessing surrogates of endogenous cholesterol synthesis at baseline may identifypatients in whom statin may not reduce the risk of recurrent coronary events. Specifically, Finnish researchers investigated how plasma cholestanol concentrations at baselinemodulated the effectiveness of statin treatment in a subsample of 868 patients withcoronary heart disease from the Scandinavian Simvastatin Survival Study (4S). The meanreduction in the risk of coronary events in 4S was 34% [38]. Sub-analyses showed that thisreduction in the risk of recurrent coronary events was significant among patients in thelowest quartile of plasma cholestanol concentrations at baseline (-37% reduction in risk) butnot among those in the highest quartile (+16%). These findings suggested that CHD patientsCHD with a high absorption and low synthesis of cholesterol may not benefit from statintreatment alone [39]. The Prospective Study of Pravastatin in the Elderly at Risk (PROSPER)[40], which investigated the association between plasma markers of cholesterol synthesis(desmosterol, lathosterol) and cholesterol absorption (campesterol, beta-sitosterol) atbaseline and after treatment with pravastatin in elderly male and female patients at risk ofCHD, came to a different conclusion. Pravastatin reduced concentrations of the cholesterolsynthesis markers desmosterol (-12% in cases vs. -11% in controls) and lathosterol (-50% incases vs. -56% in controls) and increased the concentrations of the cholesterol absorptionmarkers campesterol (48% in cases vs. 51% in controls) and beta-sitosterol (25% in cases vs. 26% in controls). Because these changes were similar between cases and controls, it wassuggested

that inter-individual variations in cholesterol homeostasis in response totreatment do not explain differences in CHD outcomes between patients treated withpravastatin [40]. Therefore, the extent to which the magnitude of the change in thecholesterol synthesis and absorption profile of any given individual with treatment predictsdifferent CHD outcomes needs to be further investigated. It is virtually impossible based on the available data from these epidemiological studies toconclude which cholesterol homeostasis profile (high vs. low endogenous cholesterolsynthesis, high vs. low cholesterol absorption) is more favourable from a cardiovascularstandpoint. Other evidence from association studies may be helpful in trying to address thatquestion. The following sections will review the emerging yet limited literature on the interrelationshipbetween cholesterol homeostasis, metabolic syndrome, obesity and insulinresistance. We will also discuss potential clinical implications from a cardiovascular healthpoint of having high vs. low cholesterol synthesis and absorption profiles in these states. 2. 3. Cholesterol homeostasis in metabolic syndromeGylling et al. [29] performed a study comparing cholesterol homeostasis surrogates amongindividuals with and without metabolic syndrome. Subjects with metabolic syndrome hadhigher plasma concentrations of cholesterol synthesis surrogates and lower concentration ofabsorption markers than controls. Notably, differences in the absorption markers, but not inthe synthesis markers, disappeared when adjusted for differences in waist circumferencebetween groups. Among cholesterol homeostasis surrogates, increased plasma squaleneconcentration (marker of synthesis) was the best predictor of the presence of metabolicsyndrome. The

evidence for higher cholesterol synthesis and lower cholesterol absorption inpatients with metabolic syndrome has been supported by other studies as well [41-43]. Cof?? n et al. [44] took these observations further. In a multivariate analysis of 674dyslipidemic patients and 361 healthy subjects, they showed that a 1-SD increase in thesitosterol-to-cholesterol ratio (reflecting increased cholesterol absorption) was associatedwith reduced risk of all features of metabolic syndrome as well as with a reduction in the riskof having metabolic syndrome as a whole. Individual associations were stronger with visceralobesity and weaker with high blood pressure. These observations are concordant with thefinding that patients with low plasma HDL-C concentrations vs. those with high HDL-C arecharacterized by relatively higher endogenous cholesterol synthesis and lower cholesterolabsorption [45]. Proposed mechanisms underlying the perturbed cholesterol homeostasis in metabolicsyndrome vary. Some investigators suggested that abdominal obesity may be the primaryfactor responsible for the perturbed cholesterol homeostasis in metabolic syndrome [46], while others have suggested insulin resistance per se may be responsible for thesemetabolic changes [47]. In order to shed some light on this discussion, both viewpoints willbe discussed. 2. 4. Abdominal obesity and cholesterol homeostasisAbdominal obesity is one of the key etiological features of metabolic syndrome. Waistcircumference is the key variable used to characterize abdominal obesity in metabolicsyndrome [48, 49]. It is well known that excess abdominal fat and predominantly visceral fatis associated with cardiometabolic features similar to the ones present in metabolicsyndrome, including high plasma concentrations of triglycerides

[19]. High levels oftriglycerides are the result of an increased secretion of triglyceride-rich VLDL particles fromthe liver and the small intestine, which in turn favours the accumulation of small dense LDLparticles [50]. Together, abdominal obesity and high triglycerides predict the presence of ahighly atherogenic metabolic triad comprising increased insulin and apoB concentrationsand small, dense LDL particles [51]. High apoB and small dense LDL are features not alwaysspecifically associated with plasma LDL cholesterol concentrations [9, 13] and thus mayreflect perturbed whole-body LDL homeostasis. Adipose tissue is no longer recognized simply as an energy storage organ, with now wellestablishedendocrine functions contributing to the release of free fatty acids and a widerange of pro- and anti-inflammatory cytokines [52]. Free fatty acids released by visceral fatcells enter the blood stream to be directed into the liver, thereby interfering there with lipidmetabolism and stimulating the synthesis of cholesterol [17]. Peltola et al. [53]demonstrated that the degree of visceral fat (VAT) determined by computerizedtomography in 109 normoglycemic subjects predicted a higher estimated cholesterolsynthesis (as measured by plasma squalene concentrations). This association between VATand cholesterol synthesis was independent of variations in subcutaneous fat, insulinsensitivity and plasma triglycerides. Estimates of cholesterol absorption correlatednegatively with various indices of obesity including levels of visceral fat and positively withinsulin sensitivity. Interestingly, none of the cholesterol absorption or synthesis markers wasassociated significantly with the amount of subcutaneous fat or with insulin secretion. Basedon this, they have suggested that visceral obesity may be more important to

cholesterolhomeostasis than subcutaneous fat [53]. They have also suggested that squalene synthesisin itself in fat cells may contribute to the detrimental effects of abdominal obesity oncholesterol homeostasis. The strong association with the degree of visceral abdominalobesity may explain why cholesterol synthesis is increased in metabolic syndrome. Thereduced cholesterol absorption in abdominally obese subjects may simply be counterregulating the increase in cholesterol synthesis seen in these individuals to maintain wholebody cholesterol homeostasis. These observations are challenged by data having shown that increased synthesis anddecreased absorption of cholesterol was dependent on liver fat content rather than on bodyweight [54]. Indeed, plasma surrogate markers of cholesterol synthesis in patients with nonalcoholicfatty liver disease (NAFLD) were shown to be significantly higher while markers forcholesterol absorption were significantly lower than in non-NAFLD control subjects. Thesedifferences remained highly significant after adjustment for sex and body mass index. Patients with NAFLD also had higher visceral adipose tissue levels measured by computedtomography than controls [54]. However, and surprisingly, authors have not controlled forthis important difference between patients when assessing the impact of NAFLD onsurrogates of cholesterol homeostasis. Although it is tempting to conclude based on thesedata that liver fat, rather than obesity per se, is a key determinant of whole cholesterolbalance, the specific role of visceral fat in this process through its impact on free fatty acids, triglyceride and apoB metabolism remains to be more definitely established. 2. 5. Insulin resistance and cholesterol homeostasisThe data associating abdominal

obesity and insulin resistance and the molecular as well asphysiological mechanisms underlying this association are undisputable. It is beyond thescope of this paper to review this evidence (see Bergman et al. [17], Scaglione et al. [46] andHajer et al. [55] for reviews). Researchers have suggested that insulin resistance per se mayplay a key role in the regulation of whole-body cholesterol homeostasis, independent ofobesity. Simonen et al. [56] have shown that directly measured cholesterol absorption waslower in obese patients with type 2 diabetes than in body-weight matched controls, whilecholesterol synthesis was higher. Plasma blood glucose concentrations were positivelycorrelated to cholesterol synthetic rate in both groups. These results suggested thatcholesterol homeostasis is perturbed in diabetes, and thus in insulin resistance states, independent of obesity alone since the two groups were matched for body weight. Asindicated above, body weight and body mass index are not specific markers of intra-abdominalvisceral fat, which was not measured in this study by Simonen et al. [56]. Theextent to which insulin resistance state and type 2 diabetes would have predicted aperturbed cholesterol homeostasis independent of variations in visceral fat levels was notaddressed. Hoenig et al. [57] showed that the quantitative insulin sensitivity check index (QUICKI)correlated negatively with percent LDL-C reduction in 66 high-risk vascular patients treatedwith atorvastatin 80 mg for 6 weeks. Insulin-resistant patients had higher levels ofcholesterol synthesis markers and lower levels of absorption markers and the correlationbetween QUICKI and percent LDL-C response to statin was no longer significant whenadjusted for variations in markers of cholesterol homeostasis. It was suggested that insulin resistantpatients might have

superior LDL-C responses to statin therapy, partly due to theirhigh baseline cholesterol synthesis. These observations are to some extent supported by a series of data from cross-sectionalstudies. In their study of NAFLD patients, Simonen et al. [54] have found positiveassociations between serum fasting insulin and cholesterol synthesis markers and negativeassociations with cholesterol absorption markers. However, these associations were notindependent of liver fat accretion in these patients. Peltola et al. [53] reported a negativecorrelation between cholesterol synthesis markers and whole-body glucose uptake, hencesuggesting that a higher degree of insulin sensitivity is associated with lower endogenouscholesterol synthesis. However, as indicated above, the association between visceral fat andestimated cholesterol synthesis in this study was independent of concurrent variations ininsulin sensitivity [53]. Pihlajamaki et al. [58] showed that the degree of insulin resistancemeasured by hyperinsulinemic-euglycemic clamps in 72 healthy normoglycemic men wasassociated with increased cholesterol synthesis and to a lesser degree with decreasedcholesterol absorption. Associations were significant even after adjustment for theconfounding effect of BMI. However, there was a significant difference in waistcircumference between insulin-sensitive and insulin-resistant groups. Paramsothy et al. [59]have shown that cholesterol absorption markers were highest in lean insulin-sensitive menand women, whereas cholesterol synthesis markers were highest in lean insulin-resistantand obese insulin-resistant patients. Although insulin-sensitive and insulin resistant leansubjects were matched for body mass index, the latter group had high levels of intraabdominalvisceral fat. Authors did not discuss

the extent to which this may have affectedthe association between insulin resistance and cholesterol homeostasis. Therefore, it cannotbe concluded from these studies that insulin resistance affects cholesterol homeostasisindependent of abdominal visceral obesity. Gylling et al. [47] investigated the link betweeninsulin resistance and altered cholesterol homeostasis in 781 participants with variousdegrees of insulin resistance (normoglycemia, impaired fasting glucose, impaired glucosetolerance, and type 2 diabetes). The authors reported that peripheral insulin sensitivityevaluated by the Matsuda index was inversely related to the lathosterol/sitosterol ratio inthe entire population independently of BMI, suggesting that peripheral insulin resistancemay up-regulate cholesterol synthesis independent of overall obesity. However, variations inwaist circumference were also an independent predictor of the plasma lathosterol/sitosterolratio, an integrated marker of whole body cholesterol homeostasis. Again, it cannot be ruledout from these data that the association between insulin resistance and cholesterolhomeostasis is fully independent of obesity, particularly abdominal obesity. Nevertheless, ithas been proposed that hyperinsulinemia as seen in insulin resistance states may up17regulate the expression of SREBP-1c, a transcription factor that stimulates the synthesis offatty acids and the production of VLDL particles [60]. On the other hand, SREBP-2, anothertranscription factor up-regulating de novo cholesterol synthesis, does not appear to beaffected by hyperinsulinemia or hyperglycemia. Further research is therefore required toshed more light on these potential mechanisms and on how each one relates to abdominalobesity and insulin resistance respectively.