

Implications of the Obesity Epidemic for Statin Therapy: Shifting Cholesterol Metabolism to a High Synthesis and Low Dietary Absorption State

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Abstract: Obesity and the metabolic syndrome are becoming one of the biggest health challenges of the 21st century. Cholesterol metabolism is significantly altered in both obesity and metabolic syndrome in that cholesterol synthesis is increased and absorption reduced and this has important implications for the treatment of lipid disorders in both obesity and the metabolic syndrome. In the present review we discuss these changes in detail especially in the context of a more standardized approach for cholesterol reduction like the TARGET LDL trial. Customized care is topical in lipidology as we strive to achieve LDL cholesterol and non-HDL cholesterol targets in every patient.

Key Words: Cholesterol, cholestanol, statins, obesity, metabolic syndrome, diabetes, ezetimibe.

INTRODUCTION: TRENDS IN THE INCIDENCE OF DIABETES AND OBESITY

Recent data from the Framingham Offspring study have shown that the incidence of type 2 diabetes in the 1990s has doubled when compared to the 1970s, with most of the absolute increase in diabetes incidence occurring in the obese as determined by body mass index (BMI) of greater than 30 [1] thus confirming previous secular trends found in the San Antonio Heart Study from 1987-1996 [2]. This is of particular concern since the most recent NHANES survey has shown roughly one third of American adults over the age of 20 to be obese with an even higher reported prevalence in females from minorities where prevalence is reported as 42% in Mexican American women and 54% in Non-Hispanic black women [3]. The prevalence of obesity is increasing in the United States whether it is measured with BMI [3] or waist circumference as a measure of central adiposity [4]. Other NHANES data have also shown that diabetes is a highly prevalent disorder that affects 9.3% of the US population assessed 1999-2002 with another 26% having impaired fasting glucose ('pre-diabetes') and a nearly double prevalence of diabetes in minorities [5]. The prevalence of *diagnosed* diabetes in 2000 was nearly 5 fold that observed in 1958 (4.4 vs 0.9%) [6]. Despite the poor predictive value of BMI in predicting hypertension, dyslipidemia, metabolic syndrome, diabetes or hard clinical end points when compared to waist circumference or waist-to-hip ratios [7-12], epidemiologic studies have confirmed that as societies, we are becoming fatter and more diabetes-prone [4, 13]. Waist circumference measurements may better quantify abdominal obesity which with insulin resistance drives cardiometabolic risk *via* metabolic and thrombotic derangements [14-18]. The

metabolic syndrome, a clinical label for this phenomenon, describes the clustering of central adiposity with impaired fasting glucose, elevated blood pressure, high triglyceride (TG) and low high density lipoprotein cholesterol (HDL-C) [16, 19, 20] and is associated with an increased risk of cardiovascular events [21-24] as reviewed in [25]. Weight loss can improve insulin resistance, reduce total cholesterol and improve many of the glucose and lipid abnormalities associated with this syndrome; these observations were made before the term metabolic syndrome was popularized and are consistent with obesity as being the driver of this syndrome [26, 27]. The two most commonly utilized definitions are listed in Table 1; other definitions include an insulin resistance criterion and are therefore less practical [28]. This constellation of cardiovascular risk factors has a significant prevalence even in individuals with normal BMI [29] which further attests to the paucity of measures such as BMI in predicting cardiovascular risk. The metabolic syndrome affects roughly a quarter of Americans above the age of 20 [30] and 44% of Americans over the age of 50 [31]. Indeed, 86% of diabetics in this age group have the metabolic syndrome compared to a prevalence of 26% among those with normal fasting glucose. Importantly, those with both metabolic syndrome and diabetes diagnoses had more insulin resistance than those with a diagnosis of diabetes without the metabolic syndrome suggesting that these entities should be considered separately despite sharing common features [31].

Intriguingly, in an NHANES III analysis, patients with diabetes but without the metabolic syndrome (14% of diabetics) did not have an increased risk of coronary heart disease [31]. This is consistent with analyses from the Strong Heart Study and the Multiple Risk Factor Intervention Trial (MRFIT) showing wide variation in the prevalence of cardiovascular disease in diabetics that depends on co-existing risk factors [32, 33] and counters previous reports that diabetes automatically carries the same prognosis as a coronary artery disease equivalent [34]. Further, the new International

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Table 1. ATP III and IDF Definitions of the Metabolic Syndrome [36, 37]

IDF Criteria (first criterion compulsory plus any additional 2)	ATP III Criteria (3/5 required for diagnosis)
Waist circumference >94 cm in Europid males or > 80cm in Europid females (with ethnic-specific values for other groups)	Waist circumference >102 cm in males or > 88cm in females
Triglycerides >1.7 mmol/l or specific treatment for lipid abnormality	Triglycerides >1.7 mmol/l
HDL cholesterol <1.03 mmol/l in a male or <1.29 mmol/l in a female or specific treatment for lipid abnormality	HDL cholesterol <1.03 mmol/l in a male or <1.29 mmol/l in a female
Blood pressure >130/85 or anti-hypertensive medications	Blood pressure >130/85 or anti-hypertensive medications
Fasting glucose >5.6 mmol/l or previously diagnosed type 2 diabetes	Fasting glucose >6.1 mmol/l

Diabetes Federation definition of the metabolic syndrome incorporates the use of different criteria for central adiposity by ethnic group and this better defines the at-risk waist circumference for an individual [16]. The diagnosis of metabolic syndrome is thought to encompass unmeasured risk factors e.g. a pro-inflammatory profile or impaired fibrinolysis typically associated with the risk factors accounted for in the metabolic syndrome criteria [15, 17, 35]. In the literature, less attention has been given to changes in cholesterol metabolism that occur with obesity and insulin resistance. The purpose of this paper is to discuss normal cholesterol metabolism, to discuss the alterations in this dynamic process as patients acquire features of the metabolic syndrome and then finally to discuss the implications for statin therapy in this higher risk subgroup of patients in the context of the ongoing TARGET LDL trial. Future publications by the TARGET LDL group will specifically address the association of a high cholesterol synthesizer/low cholesterol absorber state with features of the metabolic syndrome. These issues are highly relevant to clinicians who prescribe statin therapy given the increasing prevalence of abdominal obesity [4] and the metabolic syndrome [30].

NORMAL CHOLESTEROL METABOLISM

Human cholesterol levels are dependent on interrelated processes: its synthesis in the liver, endocrine organs, muscle and skin, absorption from the diet and excretion into bile. The balance between these processes varies between individuals in that some may have a relative large contribution of hepatic synthesis whereas others may have a high dietary absorption. Of the cholesterol absorbed in the intestines, about 75% is from biliary sources undergoing enterohepatic circulation whereas dietary sources account for about 25% [38]. While intestinal absorption of bile acids is essentially complete under normal conditions, cholesterol absorption in healthy adult volunteers is variable with 29-81% (mean 56%) being absorbed in the small intestine [39]. This range of variability has been observed in other studies where cholesterol absorption ranged from 25-75% [40-42]. The biochemical details of cholesterol biosynthetic and excretory pathways are covered elsewhere [43] and are not the focus of this article which is concerned with cholesterol metabolism at the organism level and its implications for lipid-lowering

therapy. However, to study cholesterol metabolism in large numbers of patients, a practical method of quantifying cholesterol metabolism is required.

Sterol balance, the gold standard for quantifying cholesterol synthesis, is labor intensive, involves fecal collection (typically over 3 days) and is impractical for studies with large numbers of patients [44]. Radioactive isotopes have been utilized to quantify cholesterol absorption but may persist in the circulation for a year [45] and while an advance has been the use of stable isotopes with cholesterol modified with carbon-13 or deuterium [39, 46-50] which are reproducible for a given individual [49], the most practical method for studying cholesterol metabolism in a large number of patients is the measurement of precursor sterols or absorption markers with gas liquid chromatography [51]. For this application, lathosterol, desmosterol or mevalonate can be used as measures of cholesterol synthesis and cholestanol or plant sterols, campesterol or sitosterol, can be used as measures of absorption.

Serum levels of cholesterol precursors such as desmosterol and lathosterol expressed as ratios to serum cholesterol are negatively correlated with fractional and absolute absorption of dietary cholesterol and positively related to overall cholesterol synthesis while serum levels of the plant sterols, campesterol and sitosterol, exhibited positive correlations with fractional and absolute absorption of dietary cholesterol but were inversely related to the overall cholesterol synthesis [44, 52-54]. Further, cholestanol, an absorption marker, is positively correlated with other markers of absorption e.g. campesterol and sitosterol and negatively correlated to markers of synthesis e.g. cholestanol, desmosterol or lathosterol and has been validated by the sterol balance technique [53-57]. Plasma lathosterol, a validated synthesis marker, has been shown to correlate with hepatic HMG-CoA reductase activity in liver biopsies obtained at time of cholecystectomy [58]. Unlike the plant sterols such as campesterol or sitosterol, cholestanol levels are not dependent on its ingestion since it is very poorly absorbed [56] and it is a metabolite of endogenous cholesterol [59]; hence it may theoretically be a preferable marker for use in epidemiologic studies [60] although a ratio of a cholesterol precursor to plant sterol may even be superior to either a marker of ab-

sorption or synthesis alone [44]. Despite this theoretical concern, sitosterol and campesterol are validated markers of cholesterol absorption [52, 54] and are probably accurate surrogates of cholesterol absorption even in vegetarians who, despite their diet, still only ingest relatively modest amounts of these sterols with 10-15% absorption of campesterol and 5-7% absorption for sitosterol [61]. However, recent evidence suggests that the quantities of plant sterols available in normal diets, as little as 150mg, have appreciable effects on cholesterol absorption ascertained by the effect of their extraction from ingested food on cholesterol absorption [62]. Like cholestanol, markers of synthesis such as lathosterol have also been shown to be accurate predictors of cholesterol metabolism whose accuracy is independent of diet [57]. Using these various techniques, the tight interrelationship between cholesterol synthesis and absorption has been demonstrated.

In subjects consuming a consistent diet, both fractional and absolute absorption of cholesterol is negatively associated with cholesterol synthesis [63]. This dynamic process responds to diet. A typical North American diet contains approximately 450mg of cholesterol per day of which 55% is absorbed while cholesterol synthesis on such a modest cholesterol diet is 11-13mg/kg per day [64]. Studies undertaken in the 1970s using sterol balance methodology demonstrated the suppression of cholesterol absorption and synthesis and an up-regulation of biliary secretion in response to ingestion of large amounts of cholesterol of 3g per day with up to 1g being absorbed but also showed considerable variability in the ability to downregulate fractional absorption and synthesis [65]. A later study of 9 subjects investigated the effect of less marked increases in dietary cholesterol from 250mg per day to 750mg per day and showed that serum cholesterol levels rose only in 3 of the 9 patients who were unable to suppress hepatic synthesis in response to response to the increased dietary load [66]; a finding similar to other small studies [67]. Reduced absorption efficiency and reduced cholesterol synthesis, which has been mechanistically tied to reduced HMGCoA Reductase activity [68, 69], are the major compensatory mechanisms to increased dietary intake; other mechanisms such as increased biliary re-excretion of cholesterol or increased fecal bile acids play minor roles in the compensatory process [64, 67] and an increase in bile acid synthesis is variable [70]. Subsequently, a report by McNamara and colleagues investigated the effects of changing dietary cholesterol and the quality of the fat from polyunsaturated to saturated in diets to determine which is the more important determinant of serum cholesterol. Again, when dietary cholesterol is increased from 250mg per day to 800 mg per day, reduced efficiency of cholesterol absorption and reduced hepatic synthesis were found but the main determinant of plasma cholesterol levels is the fat quality of meals [69], a finding confirmed by other studies [71]. Indeed, McNamara and colleagues showed while about two thirds of subjects can compensate for increased cholesterol intake, the more important and more consistent determinant of plasma total cholesterol and LDL cholesterol (LDL-C) levels was the dietary fat quality (saturated versus unsaturated) than the cholesterol content per se [41, 69, 72]. The molecular mechanisms for this observation have only been recently described [73]. This observation is consistent with earlier reports [70]

and with epidemiologic studies showing fat quality to be an important determinant of cardiovascular events whereas dietary cholesterol is not [72, 74-76]. Other studies have also shown that percentage of absorbed cholesterol is not related to total plasma cholesterol or LDL cholesterol [39]. An extreme example of the tight regulation of these processes is a case report of a man who eats 25 eggs (~5 grams of cholesterol) per day but has a normal plasma cholesterol; this man absorbed only 18% of dietary cholesterol compared to 55% in controls consuming a mean 220 mg of cholesterol per day [77]. Indeed, endogenous cholesterol synthesis decreases with increased dietary consumption and this is a *graded* response within the normal range of daily cholesterol consumption from 26-650mg [47, 48, 69, 78]. Highly responsive suppression of endogenous cholesterol synthesis is observed in the Masai of East Africa who have low serum cholesterol and low prevalence of atherosclerosis assessed by histology at necropsy despite a high fat, high cholesterol diet (composed chiefly of Zebu cattle milk, cow blood and occasional meat providing 66% of calories from fat and 600-2000mg of cholesterol/day) and high dietary cholesterol absorption [79]. Importantly, these compensatory changes occur *acutely* for a given meal [48] as described by Harwood *et al.* in 1987 by measuring HMG CoA reductase activity in blood mononuclear cells [80]. HMG CoA reductase activity was shown to have a diurnal rhythm with increased activity at night, fasting and cholesterol feeding were shown to inhibit its activity within 2 hours and plasmapheresis to deplete circulating cholesterol resulted in a rebound activation of HMG CoA reductase. On a molecular level, sterols decrease the transcription of HMG CoA reductase [81] and increase its degradation [82]. Whether a given patient mainly absorbs cholesterol ('absorber'), synthesizes cholesterol ('synthesizer') or shows an intermediate phenotype ('mixed') is important for lipid-lowering therapy [83].

THE CLINICAL SIGNIFICANCE OF VARIATIONS IN CHOLESTEROL METABOLISM

While individuals who are hyper-absorbers of cholesterol may not have markedly different lipid and lipoprotein levels to those who have a synthesizer phenotype [53], their response to statin therapy is suboptimal. The 4S trial protocol pre-specified an up-titration of the simvastatin dose from 20mg/d to 40mg/d in patients who failed to reach treatment total cholesterol (TC) below 5.2mmol/l at 6 weeks [84]; a similar titration was also employed in the IDEAL trial [85]. When the patients that required the up-titration of dose (poor responders) are compared to a subgroup of those that did not (good responders), differences in cholesterol metabolism emerge. The good responders had higher baseline levels of cholesterol synthesis markers and lower levels of absorption markers than those with a poor response [86]. In another substudy of the 4S trial, cholestanol was determined in 867 patients at baseline before randomization to placebo or simvastatin and the population stratified into quartiles of cholestanol:cholesterol ratio [55]. Those in the lowest quartile representing patients with more synthesis of cholesterol and less absorption of cholesterol had the greatest responses in serum cholesterol to simvastatin and the greatest reduction in the precursor sterols consistent with greater inhibition of cholesterol synthesis in patients who primarily synthesize

cholesterol despite more patients who are poor synthesizers of cholesterol having the dose of simvastatin increased. Conversely, simvastatin induced the greatest increases in plant sterols in patients with the highest quartile of cholestanol representing those that absorb the most [55]. Similar data have been reported with atorvastatin where 20 or 40mg/d (average 29mg) for one year increased campesterol by about 80% and reduced lathosterol by 50% and this reduction in cholesterol precursors correlated with the reduction in total cholesterol. Interestingly, as with simvastatin, patients who had higher baseline levels of cholesterol absorption markers had a poorer LDL-C response to atorvastatin [87]. These results showing changes in markers of cholesterol synthesis and absorption have been confirmed in statin intervention trials that used sterol balance and fractional absorption [88]. This rebound increase in cholesterol absorption with statin use may explain why a small proportion of treated patients have diminished response to statins on long term follow up [89]. Likewise, inhibition of cholesterol absorption has also been shown to produce rebound increases in cholesterol synthesis. Patients with inhibited cholesterol absorption e.g. those with gut resections or celiac disease have increased cholesterol synthesis as determined by sterol balance and increased levels of synthesis markers [90, 91]. This effect is also seen with pharmacotherapy; ezetimibe reduced fractional cholesterol absorption from 50% to 23%, a 54% ($p < 0.001$) reduction, and this effect was also confirmed by reductions in campesterol and sitosterol:cholesterol ratios by 41 and 34% respectively. Conversely, ezetimibe 10mg/d increased synthesis by 89% ($p < 0.001$) by sterol balance and also increased the validated surrogate of cholesterol synthesis, the lathosterol:cholesterol ratio, by 72% ($p < 0.001$) [92]. Likewise, stanol ester feeding to reduce cholesterol absorption decreases markers of absorption (cholestanol and plant sterols) while increasing cholesterol precursor sterols and the increase in the ratio of a precursor sterol:plant sterol correlated negatively with the LDL-C response to stanol ester feeding [93]. Indeed, the efficacy of sitostanol margarines which can inhibit cholesterol absorption by up to 65% [94] is dependent on pre-treatment serum campesterol concentrations; those with the highest concentrations have the greatest treatment-induced reductions in both campesterol and TC [95] and similar observations have been reported with phytosterol margarines where a high baseline campesterol to cholesterol ratio was consistent with enhanced LDL-C reduction [96]. Indeed, the reductions in TC correlated with those in campesterol [95] consistent with the efficacy of these agents being tied to their anti-resorptive action. Likewise, from a 4S subgroup, patients on statin therapy selected for a high baseline cholestanol:cholesterol (indicative of significant cholesterol absorption) had a 7% reduction in TC and a 12% reduction in LDL-C after being treated with sitostanol ester margarine whereas those with a low cholestanol:cholesterol had no significant reduction in TC or LDL-C [97]. In a similar study, a low lathosterol:campesterol ratio was also shown to predict benefit from sitostanol margarine; 13.8% ($p < 0.01$) LDL-C reduction for low ratio versus 4.3% (not significant) for high ratio [98]. The clinical significance of inhibiting cholesterol absorption should not be underestimated since near-complete inhibition of this pathway in type 2 diabetics with a combination of neomycin and stanol ester

margarine can decreased LDL-C by 37% [99]. The clinical significance of cholesterol metabolism for a given patient precludes these details being consigned to academic interest.

In an 868 subgroup analysis of patients enrolled in the 4S study, those in the highest quartile of cholestanol:cholesterol had no clinical benefit from simvastatin therapy and had a 2.2 fold increased risk of major cardiac events when compared to patients in the lowest quartile [100]. Hence the lack of serum lipid response in patients who have an 'absorber' phenotype does translate into an increased risk of having a hard clinical endpoint. Conversely, patients who are statin-hyporesponders i.e. absorbers would be expected to have enhanced responses to anti-resorptive agents. Indeed, in a group of hypercholesteremic patients receiving statin therapy, hyporesponders to statin therapy were hyper-responders to ezetimibe and this is consistent with the need to treat individual patients with different therapeutic modalities [101]. Indeed, the individual responses to ezetimibe ranged from 6-60% reduction in LDL-C and this contrasts with the *average* of 20% reported in population studies where no analysis of interindividual variation is undertaken [102]. The lack of clinical benefit in patients who do not have a lipid response to statin therapy in the aforementioned 4S subgroup is perhaps not surprising given that the clinical benefit of lipid-lowering therapies, including statins, cannot be dissociated from their effect on lipids in a meta-regression analysis of all lipid-lowering trials [103] and also the observation that, on meta-analysis, the clinical benefit of statin therapy is concordant with event rates at a given cholesterol level from cohort data [104].

An understanding of cholesterol metabolism can also be useful to guide the treatment of patients with familial hypercholesteremia in addition to 'regular' patients. In patients heterozygous for familial hypercholesteremia, higher plasma mevalonic acid, a surrogate for cholesterol synthesis [105] predicted a good response to statin therapy and those with a good response showed greater reduction in plasma mevalonic acid concentrations on treatment [106, 107]. Further, the E4 genotype, which has been associated with relatively greater absorption of cholesterol was more common in poor responders than good responders to statin therapy in heterozygous familial hypercholesteremia patients while the severity of the underlying mutation in the LDL receptor had no predictive value [107]. Given that measures of cholesterol metabolism are highly reproducible for a given individual, much of the variation within a population appears to be driven by different intrinsic physiology [49, 108].

THE GENETIC BASIS OF INTRINSIC DIFFERENCES IN CHOLESTEROL SYNTHESIS AND ABSORPTION

Genetic variation accounts for intrinsic variations in cholesterol metabolism. In a study of hypercholesteremic subjects who had cholesterol metabolism determined by sterol balance, measuring synthesis and absorption markers and fractional dietary cholesterol absorption, a familial association was found in the probands and their siblings [53]. Further, in a study of 28 monozygotic twins, genes explained about 50% of the variance in LDL-C reductions in response to dietary manipulation despite one twin running approximately 50km

per week more than the sedentary twin [109]. These studies suggest that a patient's cholesterol metabolism is an inherited phenomenon. Ample evidence supports this thesis.

Two Finnish studies have shown that individuals with the ApoE4/4 genotype have higher rates of cholesterol absorption than those with 3/3 or 3/4 genotypes and that the higher absorptive rates are associated with lower cholesterol synthesis rates in the liver [110, 111]. The E4 allele has a frequency of 15% in Caucasian populations and individuals with this haplotype have higher TC, LDL-C and apolipoprotein B (ApoB) levels than those with the E2 haplotype while the E3 haplotype is associated with an intermediate phenotype [112]. The E4 allele is more prevalent in Finnish populations and this is important in interpreting the many dietary intervention studies undertaken in this population by Miettinen [113]. Some previous studies have shown the high cholesterol absorber phenotype to be associated with higher TC, LDL-C and HDL-C [56, 63] but this is not a consistent finding [49]. Importantly, the E4 allele predicts greater reductions in LDL-C in response to low cholesterol, low fat diets than other alleles which is consistent with these individuals absorbing more cholesterol [114]. Further, the E4 genotype also predicts TC and LDL-C lowering which is typically 7.5-10% and 10-15% respectively in response to esterified stanol or sterol esters dietary supplements which decrease fractional cholesterol absorption more markedly in those with the E4 than the E3 genotype (9 vs 2%; $p < 0.05$) [61, 93, 115]. Conversely, a high cholesterol diet significantly increased TC only in patients with the E4 genotype and had a non-significant effect on those with an E2 phenotype [116] and this may partially explain why only some individuals have increased serum cholesterol when fed high cholesterol diets [112]. While carriers of the E4 genotype have greater responses to dietary intervention than E2 carriers, this is further modulated by multiple polymorphisms in numerous genes including ABCG8, ApoA-I, ApoA-IV, ApoC-III, ApoB [112, 117, 118] and others as systematically reviewed by Masson *et al.* [119]. The ABC transporter system, mutations of which can cause sitosterolemia, a disease where plant sterols are absorbed excessively [120-122], also plays a role in determining cholesterol metabolism phenotype. Indeed, polymorphisms of ABC5 and ABC8 which cause sitosterolemia have been shown to determine cholesterol metabolism determined by measuring synthesis and absorption markers of cholesterol [108, 123, 124]. Berge *et al.* suggest that 81-84% of variation in variability in plasma sterols (campesterol and sitosterol) can be attributed to hereditary factors [108]. Patients with sitosterolemia, an exceedingly rare condition, have an increased absorption of plant sterols, have increased serum cholestanol with reduced biliary excretion of plant sterols and cholestanol [122] while sitosterolemic livers have a severe deficiency of HMG-CoA reductase protein which is consistent with suppressed cholesterol synthesis [125] and a lack of response to statin therapy. Hence, polymorphisms of the ABC5 and ABC8 genes represent a continuum while sitosterolemia is an extreme phenotype. Also, polymorphisms in Niemann-Pick Type C1 Like1 (NPC1L1) protein which may be the target for the anti-resorptive agent ezetimibe [126, 127] also determine genetic variations in cholesterol absorption [128]. NPC1L1 knockout mice have about a 70% reduction in cholesterol absorption

compared to wildtype mice [127]. The clinical ramifications of polymorphisms in this gene are of great importance in determining the response of LDL-C to ezetimibe at its standard dose of 10mg per day [129]. One in eight subjects lack a common haplotype of NPC1L1 and these subjects had a 36% reduction in LDL-C at 12 weeks compared to 24% ($p < 0.01$) in subjects with the common haplotype. Another rare haplotype is present in ezetimibe non-responders [130]. Analysis of patients from the ezetimibe trials EASE and VYVA identified more polymorphisms and correlated these to a cholesterol absorption as determined by sitosterol [131]. Careful examination of data from the first human trial to characterize fractional cholesterol absorption with ezetimibe revealed wide variations in inhibition of cholesterol absorption [92]. Such variation cannot be attributed to different diets since ezetimibe inhibits cholesterol absorption and reduces LDL-C to a similar degree in vegetarian patients consuming 30mg/d of cholesterol [132] as in patients consuming a regular diet with 200-500mg/d of cholesterol [42]. This similar lipid-lowering efficacy in vegetarians is due to inhibition of absorption of biliary cholesterol.

Other polymorphisms affecting statin efficacy have been described. For example, polymorphisms in the HMG-CoA reductase gene were shown to influence the degree of LDL-C reduction in response to pravastatin [133]. Also, loss-of-function mutations of PCSK9 have been shown to occur at a frequency of 2.8% in African Americans and to be associated with 28% reductions in LDL-C with an 88% decrease in the incidence of coronary artery disease while a sequence variation in 3.2% of white subjects is associated with milder reductions in LDL-C and coronary artery disease incidence [134]. PCSK9 knockout mice have increased levels of LDL receptor expression and an enhanced response to statin therapy [135]. While such genetic variants may influence total body cholesterol metabolism, assessment of cholesterol metabolism was not undertaken by the investigators. Novel genetic regulators are being identified and it is likely that many remain to be discovered [136]. Measuring a patient's cholesterol metabolism not only integrates many of these genetic factors but also accounts for environmental influences on cholesterol metabolism.

ALTERATIONS IN CHOLESTEROL METABOLISM WITH DIABETES AND OBESITY

In a genetic study of cholesterol metabolism, families with high cholesterol synthesis and low absorption had a high BMI, low HDL-C and sex steroid binding globulin. Further, intra-family correlations were significant for synthesis and absorption markers (cholestanol) with a low absorption state being associated with higher triglycerides and fasting glucose [53]. This echoed the findings of a previous 868-patient subgroup analysis from the 4S study where patients with low cholesterol absorption (determined by low cholestanol:cholesterol) had lower HDL-C and tended to have a higher BMI (28 vs 25) and triglycerides than their counterparts with a high cholestanol:cholesterol [100]. Further, in a study of the influence of polymorphisms in ABC5 and ABC8 on cholesterol absorption, those in the lowest tertile of cholestanol:cholesterol were found to have higher BMI, plasma glucose, serum insulin and triglycerides and choles-

terol synthesis markers and lower HDL-C, campesterol and sitosterol than those in the highest tertile of cholestanol:cholesterol [123]. These studies are consistent with observations in the pre-statin era showing that increased body weight, before the current practice of measuring waist circumference, is associated with increased cholesterol synthesis as ascertained with various methods [57, 137, 138] and provided early clues of the changing cholesterol metabolism in response to phenotypic changes such as obesity and the dynamic response of this phenotype to weight loss [138]. Indeed, in the 1960s, cholesterol synthesis was observed to correlate with body weight but not plasma cholesterol concentration [139] and this finding has been confirmed by more recent studies [54]. Importantly, adipose tissue produces a variety of cytokines and hormones [140] but has poor cholesterol synthetic capacity and is not a significant source of increased cholesterol synthesis [64, 141, 142]. Liver biopsy specimens from obese patients have a greater activity of HMG-CoA reductase when compared to those from normal weight patients [143]. In a study of 63 Finnish volunteers, serum levels of cholesterol precursors, desmosterol and lathosterol (expressed as ratios of $\mu\text{g}:\text{mg}$ cholesterol), were negatively related to both the fractional and absolute absorption of dietary cholesterol and serum HDL-C and positively correlated with very low density lipoprotein cholesterol (VLDL-C) while serum levels of plant sterols, campesterol and sitosterol, exhibited positive correlations with fractional and absolute absorption of dietary cholesterol, and HDL-C, but were inversely related to overall cholesterol synthesis and VLDL-C [44]. The converse of these observations also apply to cholestanol, a marker of cholesterol absorption; in a study of 61 healthy Finnish volunteers, cholestanol correlated directly with HDL-C and inversely with VLDL-C and TG [56]. When quantitated absolutely without the use of surrogates, similar findings were found with fractional cholesterol absorption correlating negatively with BMI [63]. Obese individuals have higher rates of cholesterol synthesis and bile acid synthesis with a subsequently larger intestinal cholesterol pool; however, a simple dilution effect does not completely explain reduced absorption in these individuals [54]. These studies support the notion that as patients become more obese, more insulin resistant and acquire a high TG and low HDL-C, cholesterol absorption decreases and synthesis increases. In 2000, Miettinen suggested that low cholesterol absorption is a component of the metabolic syndrome [144].

Not surprisingly, the changes in cholesterol metabolism in type 2 diabetics are similar to those seen in obese individuals; namely a lowered cholesterol absorption efficiency, decreased absorption marker ratios [144-147] and increased levels of synthesis markers and cholesterol synthesis determined with sterol balance [145, 147-149]. Indeed, when obese subjects are studied (BMI>30), obese type 2 diabetics had lower cholesterol absorption than non-diabetic obese participants (29 vs 42% $p=0.01$) and had higher levels of cholesterol precursor sterols which correlated with serum insulin in both diabetic and non-diabetic participants [145]. However, body weight and insulin resistance also appear to modulate cholesterol metabolism independent of diabetes. When overweight diabetics with an average BMI of 31 were compared to normal-weight diabetics with an average BMI

of 24, the obese diabetics were found to have a lower fractional cholesterol absorption of 32 vs 40% ($p<0.05$) and higher cholesterol synthesis suggesting that obesity modulates cholesterol metabolism independent of diabetes [150]. This effect is reversible. In a study of overweight diabetics who were prospectively followed for 2 years, a 6kg weight loss (~6.4% weight loss), reduced serum glucose by 14% and TG by 25% but had no effect on serum TC or LDL-C [144]. Interestingly, this degree of weight loss which is of a similar magnitude to that achieved in the Diabetes Prevention Program [151], increased serum markers of cholesterol absorption which correlated with the degree of weight loss and increased fractional absorption of cholesterol by 28% from 30 to 38% ($p<0.01$) with a concomitant decrease in fecal sterols. More acute weight loss of 15.5kg (18% of body weight) over a 3 month period in obese type 2 diabetics has also been shown to markedly reduce precursor sterols:cholesterol ratios by ~20% and to increase cholestanol:cholesterol by 33% with the change in this ratio correlating with serum insulin during weight reduction [152]. Other studies also show that insulin resistance can modulate cholesterol synthesis even when fasting glucose is in the normal range. Indeed, blood glucose correlated directly with cholesterol synthesis markers and inversely with absorption markers even in non-diabetic individuals and this suggests cholesterol synthesis increases with graded increases in insulin resistance [153]. The association between insulin resistance and cholesterol synthesis was confirmed in a study of normoglycemic men who had insulin sensitivity determined with the hyperinsulinemic euglycemic clamp [154]. Cholesterol synthesis precursors were shown to correlate with fasting insulin concentration and to correlate negatively with the rates of insulin-stimulated whole-body glucose uptake independent of BMI [154]. Since insulin resistance is a driver of the metabolic syndrome, it is of no surprise that patients with the metabolic syndrome were shown to have higher cholesterol synthesis and lower absorption [155]. These findings extend to elderly individuals as shown in a 400 patient study from Finland where the mean age was 80 years old. Participants with the lowest quartile of cholestanol:cholesterol ratio had a higher incidence of diabetes, metabolic syndrome and components of the metabolic syndrome including a higher waist circumference, fasting TG, fasting glucose and lower HDL-C [156].

In contrast to type 2 diabetics, type 1 diabetics have higher cholesterol absorption and lower synthesis when compared to healthy controls [146, 157] or weight-matched type 2 diabetics [158] and this recapitulates the phenotype seen in streptozotocin-induced diabetes in rats [159] which is mechanically tied to reduced hepatic and intestinal ABC5 and ABC8 expression thereby mimicking the sitosterolemia phenotype that is partially reversible on insulin administration [160]. Markers of cholesterol absorption are especially elevated in poorly controlled type 1 diabetics and can be normalized with intensified insulin treatment [161]. Consistent with this is the observation that supplementation of the diet with 800mg of cholesterol per day has been shown to increase plasma LDL-C more in type 1 diabetics than in matched controls [162]. This may have therapeutic implications for lowering cholesterol in type 1 diabetics where males are at a 4 fold increased risk of cardiovascular events and females at an 8 fold increased risk [163].

IMPLICATIONS OF CHANGING CHOLESTEROL METABOLISM FOR STATIN THERAPY

Since statin therapy appears to be most efficacious in lowering plasma cholesterol and hard clinical endpoints in patients who mainly synthesize cholesterol and absorb less cholesterol, they may be more efficacious in obese patients with insulin resistance given that these phenotypic characteristics are associated with increased cholesterol biosynthesis. Further, one may expect statins to be more efficacious in relative terms in type 2 diabetics and in patients with the metabolic syndrome since these patients are often obese and insulin-resistant. However, since these patients are at increased absolute risk of cardiovascular disease, absolute residual risk would still be significant despite statin therapy in this group of patients. This section discusses evidence for increased statin efficacy in patients with features of the metabolic syndrome in the context of the increasing prevalence of obesity and type 2 diabetes.

Analysis of the 4S population by HDL-C and TG quartiles revealed variations in statin efficacy. The 458 patients who fell into *both* the lowest quartile of HDL-C (<1.0mmol/l) and highest quartile of TG (>1.80mmol/l) expectedly had more additional features of the metabolic syndrome (high BMI, hypertension, diabetes) than the 545 patients who were in both the highest quartile of HDL-C (>1.34mmol/l) and lowest quartile of TG (<1.11mmol/l) [164]. Patients with low HDL-C and high TG also had an increased prevalence of prior myocardial infarction and prior revascularization at baseline and the patients within this subgroup treated with placebo had a 36% 5-year event rate compared to 21% in patients with isolated increased LDL-C and patients in this subgroup had the greatest event reduction with statin therapy (HR 0.48; 95%CI 0.33-0.69). The major coronary event rate reduction was not significant in patients with an isolated high LDL-C and high HDL-C/low TG (HR 0.86; 95%CI 0.59-1.26) although the confidence intervals were wide. Importantly, the treatment-by-subgroup interaction for major coronary events was significant ($p=0.03$) and is concordant with this subgroup of patients receiving greater *relative* benefit from statin therapy than their high HDL-C/low TG counterparts [164]. Mechanistically, when these same patients were studied, the patients with the low HDL/high TG lipid profile were shown to have higher markers of cholesterol synthesis and lower markers of cholesterol absorption [165]. Further, patients with the isolated high LDL-C clustered in the previously discussed clinical non-responder subgroup of 4S defined by a high baseline cholestanol:cholesterol. This is concordant with a study using fluvastatin where patients with low HDL at baseline were found to have reduced angiographic progression of atherosclerosis and improved event-free survival than those with normal HDL-C [166].

However, not all studies have been consistent with the notion that patients with features of the metabolic syndrome or type 2 diabetes have enhanced responses to statin therapy either in terms of lipid-lowering efficacy or hard clinical end point reduction. Since type 2 diabetics have a high prevalence of the metabolic syndrome, examining diabetic subgroups may help answer the question regarding enhanced

statin efficacy in patients with features of the metabolic syndrome. Of the major statin trials, the Heart Protection Study (HPS) randomized large numbers of diabetic and non-diabetic patients with 90% of the diabetics enrolled being type 2 diabetics. Diabetic patients showed similar changes in lipids as the non-diabetic cohort and also had a similar reduction in clinical events [167]. However, baseline differences in the diabetic and non-diabetic cohorts were slight; the average HDL-C was 1.03 in both groups but the TG and BMI were slightly higher in the diabetic cohort being 2.3 mmol/l vs 2.0 mmol/l and 28.6 vs 27.2 respectively. Further, in a comparison of the non-diabetic patients enrolled in the 4S study, metabolic syndrome and non-metabolic syndrome were compared [168]; amongst the excluded diabetics, the prevalence of the metabolic syndrome was 61%. This study used BMI >30 to define abdominal obesity but otherwise adhered to the ATPIII criteria [36]. Importantly, the LDL-C lowering efficacy was not superior in patients with the metabolic syndrome although these patients had a higher TG and lower HDL than in patients without the metabolic syndrome [168]. For hard clinical end points, there was a non-significant treatment by subgroup interaction ($p>0.12$) for metabolic syndrome patients deriving more clinical benefit. However, 80% of the metabolic syndrome patients only had 3 of the metabolic syndrome criteria and the most prevalent criteria satisfied were for blood pressure, HDL-C and TG (61.4% of the patients with metabolic syndrome had these three criteria only); only 28% of the metabolic syndrome patients had a BMI>30 suggesting that this subgroup may have represented the milder spectrum of the phenotype. Further, the frequency of dose titration was not reported in this paper and it is possible that the patients without the metabolic syndrome had more frequent dose titration. While a retrospective analysis from WOSCOPS also found no interaction between the efficacy of pravastatin and the presence of the metabolic syndrome, this study did not characterize cholesterol metabolism in these subgroups and used BMI in lieu of waist circumference with an average BMI in the metabolic syndrome group of 28 [169]. However, the Measuring Effective Reductions in Cholesterol Using Rosuvastatin Therapy I (MERCURY I) trial assessed the efficacy of several statins in patients with and without the metabolic syndrome and found no enhanced lipid-lowering efficacy in metabolic syndrome patients [170]. Determination of cholesterol metabolism may have been useful in determining the extent of differences in cholesterol metabolism between these groups and the resultant effects on efficacy of statin therapy. However, the 4S analysis, like others from WOSCOPS, AFCAPS/ TexCAPS and TNT confirmed that patients with the metabolic syndrome are at higher absolute risk [171] and derive more absolute benefit from statin therapy with lower numbers needed to treat compared to the entire cohort [169, 172]. Unfortunately, in a similar recent publication from the TNT investigators, lipid lowering in patients with the metabolic syndrome was not compared to those without it [172].

It is interesting to note that the prevalence of metabolic syndrome in the 4S and the TNT studies, which recruited similar patients but were done about a decade apart, were 27% versus 56% consistent with the different metabolic profile of patients with coronary artery disease that are typically

being treated with statins today. Indeed, comparing the distribution of cholestanol values expressed as mmol of cholestanol:mol of cholesterol x100 in patients thus far enrolled in the TARGET LDL program (described below and see [83]) with those described by the 4S group reveals a shift in cholesterol metabolism to a high synthesizer, low absorber phenotype. Our patients are from a contemporary Australian population with coronary artery disease, peripheral vascular disease or ischemic stroke and the 25th, median and 75th percentiles of cholestanol expressed as a ratio to TCx100 were 74, 96 and 118 respectively compared to 107, 127 and 148 respectively described by the 4S investigators [100]; see Fig. (1).

However, the lack of difference in lipid-lowering efficacy of statins in metabolic syndrome patients may be related to the use of LDL-C instead of non-HDL-C to assess efficacy. Obese subjects have increased production of atherogenic LDL as measured by ApoB metabolism despite normal LDL-C [173]. Obesity and insulin resistance are associated with a reversible increase in rates of VLDL triglyceride and VLDL ApoB production [26, 174-176] and similar findings are found with the metabolic syndrome where VLDL ApoB secretion is inversely correlated to markers of cholesterol absorption [155]. In addition, cholesterol absorption markers have been shown to be negatively correlated with VLDL-ApoB pool size, the intermediate density lipoprotein (IDL) ApoB size and plasma TG which echo previous findings by Miettinen that cholesterol synthesis markers correlate with VLDL-C [44] and is consistent with the alterations in kinet-

ics of lipoprotein metabolism that occur in the metabolic syndrome [177]. With regard to statin therapy, phenotype-dependent efficacy is observed on components of non-HDL-C. In normotriglyceridemic individuals, statins have little impact on the concentration of large VLDL but as TG increases, statins reduce VLDL, chylomicrons, chylomicron remnants and small dense LDL numbers (assessed by LDL ApoB) in contrast to the phenotype-independent effects on IDL and LDL [178]. Indeed, kinetic studies have shown that the major effect of statins is to increase the clearance of VLDL particles [179, 180]. Hence, by typically only reporting LDL-C levels and largely ignoring non-HDL-C goals, statin trials may be missing the true effect of statins on the total number of atherogenic ApoB particles and phenotypic influences on statin efficacy. While kinetic studies are not practical in large scale clinical trials, non-HDL-C is simply calculated from a standard lipid panel and accounts for LDL-C, VLDL-C, IDL-C (and chylomicron remnants and lipoprotein a) and therefore provides a more accurate measure of the cholesterol in atherogenic particles. Arguably, non-HDL-C is of limited *additional* value in those with TG<200mg/dl because the VLDL-C is not substantially elevated [36, 181]. The ATP III report recommends non-HDL-C targets of 0.8mmol/l (30mg/dl) higher than the LDL-C goal as a secondary goal of treatment in patients with TG> 200mg/dl although the report acknowledges that non-HDL-C may be the superior predictor of cardiovascular risk than LDL-C and is highly correlated with total ApoB [36]. However, since hypertriglyceridemia affects 16% of the American population

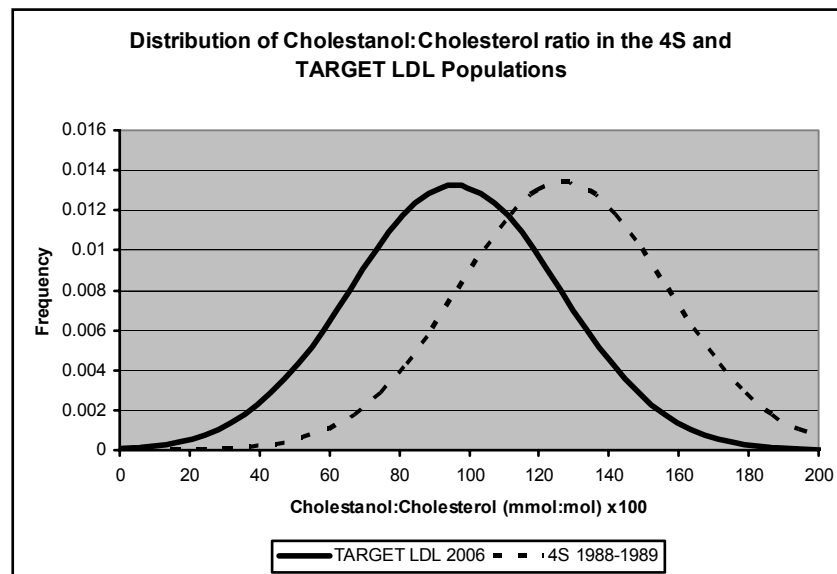


Fig. (1). Distribution of Cholestanol:Cholesterol ratios in the 4S and the TARGET LDL populations. The population being treated today show a different cholesterol metabolism phenotype than those recruited by the 4S investigators with a shift to a lower absorber and higher synthesizer state. Applying a finding from a 4S subgroup analysis showing lack of statin efficacy in participants in the highest quartile of cholestanol:cholesterol x100 i.e. > 148 mmol:mol [100] to our contemporary population would mean that only 5% of our patients would be 'non-responders'. However, unlike the 4S investigators, we are aiming for aggressive LDL-C targets of 1.8mmol/l (70mg/dl) and non-HDL-C of 2.6mmol/l (100mg/dl) and hence we have a sizeable proportion of patients who are 'inadequate responders' despite using a high dose, potent statin and anti-resorptive therapy is indicated to reach this target. It is not possible to differentiate the effects of diabetes and obesity from those of genetics in these disparate populations. The graphs assume a normal distribution and are based on population statistics which show a consistent inter-quartile range within each population. The standard deviations in the cholestanol:cholesterol ratios in the two populations were very similar; the main difference is in measures of central tendency.

and 37% of diabetics [182], non-HDL-C should be considered as a target for therapy in a sizeable proportion of the population where LDL-C may be less reliable for risk prediction [183] and correlates better than LDL-C with measures of obesity [184]. Indeed, non-HDL-C may become the principal lipid target given the trends in obesity, the proven superiority of non-HDL-C over LDL-C in risk prediction [183, 184] and the additional insights on statin mechanisms of action that its use might offer. While ApoB quantifies risk better than either non-HDL-C or LDL-C [183], this method is less practical and more costly. Unfortunately, major statin trials seldom report non-HDL-C.

Hence, there is a need to prospectively assess cholesterol metabolism in subjects enrolled in statin trials to determine correlations between features of the metabolic syndrome with serum markers of cholesterol metabolism and their impact on statin efficacy on *both* LDL-C and non-HDL-C targets. It may be that while the cholesterol 'synthesizer' phenotype is more common in patients with these diagnoses, assessment of cholesterol metabolism may be required to determine which of these patients will be hyper-responsive to statin therapy and can be managed with statin monotherapy to reach the current target of 70mg/dl. Also, just as cardiovascular risk has been shown to rise at lower levels of waist circumference in different ethnic groups, it will be important to describe differences in cholesterol metabolism in various populations. Current data on differences in cholesterol metabolism in different ethnic groups is very limited and caution must be exercised in interpreting data from these small numbers of patients. African Americans have been shown to have a higher fractional cholesterol absorption than whites (63 vs 55% $p=0.03$) [39] and the expected result of this is relatively reduced and a reduced response to statin therapy. African Americans have been shown to have slightly diminished responses to statin therapy compared to whites [185, 186] and similar responses to add-on ezetimibe (in EASE) as whites and Hispanics [187] but cholesterol metabolism and racial differences in thereof were not determined in any of these studies. Perhaps the minorities studied in the Antihypertensive and Lipid-Lowering Treatment to Prevent Heart Attack Trial (ALLHAT-LLT), where blacks, hispanics and other minorities comprised roughly 60% of the trial population, could be analyzed further for the purposes of hypothesis generation [188]. Studies performed in the 70s demonstrated different fractional cholesterol absorption in people of various nationalities; 28% in Tarahumara Indians (Mexico), 39% in Papua New Guinean Highlanders and 44-49% in Australians and Americans [189]. Cholesterol metabolism in indigenous peoples and ethnic minorities may require further evaluation in modern living conditions since these studies were undertaken in subjects consuming their traditional diets and exposure of Tarahumara Indians to a Western diet for as little as 5 weeks has been shown to increase LDL-C by 39%, TG by 18% and weight by 3.8kg (7%) [190]. Evaluating cholesterol metabolism prospectively in statin trials and relating this to phenotype and efficacy in reaching LDL-C and non-HDL-C targets is particularly important as developing nations become fatter since they acquire more metabolic disturbances at smaller degrees of weight gain [16].

THE NEED FOR CUSTOMIZED LIPID LOWERING THERAPY AND THE TARGET LDL TRIAL

The clinical relevance of a patient's cholesterol metabolism phenotype has arguably only become important as we aim to reduce cholesterol to lower levels than previously. LDL cholesterol was identified by the NCEP ATP reports as being the primary focus of cholesterol-reducing therapy and successive NCEP ATP reports have recommended successively lower LDL cholesterol goals for high risk patients [36]. An amendment to the ATP III report recommended that the target LDL in *very* high risk patients may be changed from 2.6mmol/l (100 mg/dl) to 1.8mmol/l (70mg/dl) in light of the Heart Protection Study (HPS) & PROVE-IT TIMI22 [191]. Subsequently, a meta-analysis of the four 'lower is better' trials which included PROVE-IT TIMI 22, A to Z, IDEAL and TNT confirmed that intensive lipid lowering therapy (mean LDL-C 75mg/dl) does indeed translate into a 16% relative risk reduction of coronary death or myocardial infarction ($p<0.00001$) compared to standard therapy (mean LDL-C 101mg/dl) [192]. In a provocative retrospective analysis by the PROVE-IT TIMI22 investigators, the benefits of lowering LDL-C to levels lower than current recommendations suggested further clinical benefit of achieving LDL-C of <40mg/dl without an increase in drug-related adverse events [193]. This is concordant with the log-linear relationship between LDL cholesterol and CAD risk in epidemiologic studies, and this relationship holds true at low LDL-C [191].

Hence, while lower LDL-C have translated into reductions in hard clinical endpoints, rates of achieving these levels are unsatisfactory. Maximal doses of statins often fail to achieve LDL-C targets [194] and there is marked variation in response to statins. For example, in a 6 month trial with simvastatin 80mg, the top 5% of responders had reductions in LDL-C of ~70% whereas the bottom 5% responders showed no change at all [113]. Increasing the rate of achieving LDL-C targets is the impetus for conducting the TARGET LDL trial that will treat patients according to their cholesterol metabolism phenotype [83]. In the Vytorin versus Atorvastatin (VYVA) study, a six week lipid lowering trial, only 36% of patients treated with atorvastatin 80mg reached a target LDL cholesterol of <70mg/dl [195]. This study also showed that only 64% of patients treated with simvastatin 80mg/ezetimibe 10mg (Vytorin) reach a target LDL cholesterol of <70mg/dl and hence Vytorin in place of statin monotherapy will not ensure that all patients reach the new optional target of <70mg/dl. The EXamination of Potential Lipid-modifying effects Of Rosuvastatin in combination with Ezetimibe versus Rosuvastatin alone (EXPLORER) trial, presented at the XIV International Symposium on Atherosclerosis is concordant with the inadequacy of monotherapy in achieving low LDL-C targets in the majority of patients. This trial showed that only 35% of patients will reach the 70mg/dl target with rosuvastatin 40mg monotherapy. However, there would be little need for a customized approach to lipid-lowering if the old target of 100mg/dl is used; VYVA showed that 85% of patients reach this target with atorvastatin 80mg, EXPLORER showed that 79% of patients will reach this target with rosuvastatin 40mg and hence the need for a customized approach to lipid lowering therapy is topical in contemporary

medicine as we strive to achieve lower LDL-C targets in all patients. Real world data from NCEP Evaluation Project Utilizing Novel e-Technology (NEPTUNE) II show that while 60% of patients with coronary artery disease or equivalents obtained an LDL-C < 2.6 mmol/l (100 mg/dl), only 27% of these patients reached a non-HDL-C of < 3.4 mmol/l (130 mg/dl) [196] and the number of high-risk patients reaching the aggressive LDL-C target of 1.8 mmol/l (70 mg/dl) was only 17.8%. The design of the TARGET LDL trial is described in Fig. (2) and the reader is also referred to Hoenig *et al.* [83] for further background.

A customized therapy approach based on cholesterol metabolism is certainly attractive in that it integrates multiple genetic polymorphisms in cholesterol metabolism and also incorporates the effects of phenotypic influences such as diet, obesity, insulin resistance, diabetes or even bowel surgery. However, this approach does not incorporate all of the polymorphisms involved in statin efficacy such as polymorphisms involved in drug metabolism. Cytochrome P450 3A4 (CYP3A4) metabolizes lovastatin, simvastatin and atorvastatin, Cytochrome P450 2C9 (CYP2C9) metabolizes fluvastatin while pravastatin, rosuvastatin and pitavastatin are not metabolized through the P450 system. In addition, Cytochrome P450 3A5 (CYP3A5) protein, which is only expressed in ~10% of Caucasian individuals results in reduced efficacy of lovastatin, simvastatin and atorvastatin; presumably due to enhanced drug metabolism [197]. Also, polymorphisms in the hepatocellular uptake transporter OATP1B1 which extracts statins from portal blood can influence the extent of statin-induced inhibition of cholesterol synthesis and *basal* concentrations of sterols prior to drug administration [198]. These are mere selective examples; numerous polymorphisms in the P450 enzymes and other genes in-

involved in drug disposition influence statin efficacy and safety and are reviewed elsewhere [178, 197]. Further, polymorphisms in apolipoprotein A-I and paroxonase influence the degree to which statins raise HDL-C and other polymorphisms in numerous genes, some of which are not obviously linked to cholesterol or drug metabolism, are putative predictors of statin efficacy [197, 199]. Theoretically, a pharmacogenomic approach to customized drug therapy could be used to guide lipid-lowering therapy. However, genetic analysis would be prohibitively expensive and could not account for undiscovered polymorphisms. Further, a genetic approach will also not reflect the effect of phenotype on cholesterol metabolism and therefore statin efficacy. Given the track record of cholestanol in predicting lipid-lowering efficacy and clinical efficacy of statin therapy in the 4S cohort, it is probably the best *current* method of customizing lipid-lowering therapy; or at least the most pragmatic.

PERSPECTIVE AND CONCLUSION

The importance of recognizing the changing cholesterol metabolism is of crucial importance as we treat cardiovascular disease resulting from the global obesity epidemic. The effect of cholesterol metabolism needs to be prospectively characterized in a randomized clinical trial; the TARGET LDL is such a large ongoing clinical trial. The higher prevalence of metabolic syndrome and cardiovascular disease observed in South Asians, African Americans and Hispanics highlights the need to describe cholesterol metabolism in different ethnic groups for effective lipid-lowering therapy [16, 200]. This increasing prevalence of cardiovascular disease in these minorities is related to urban living and the adoption of Western lifestyles and diet; for example, the prevalence of type 2 diabetes in urban India has increased

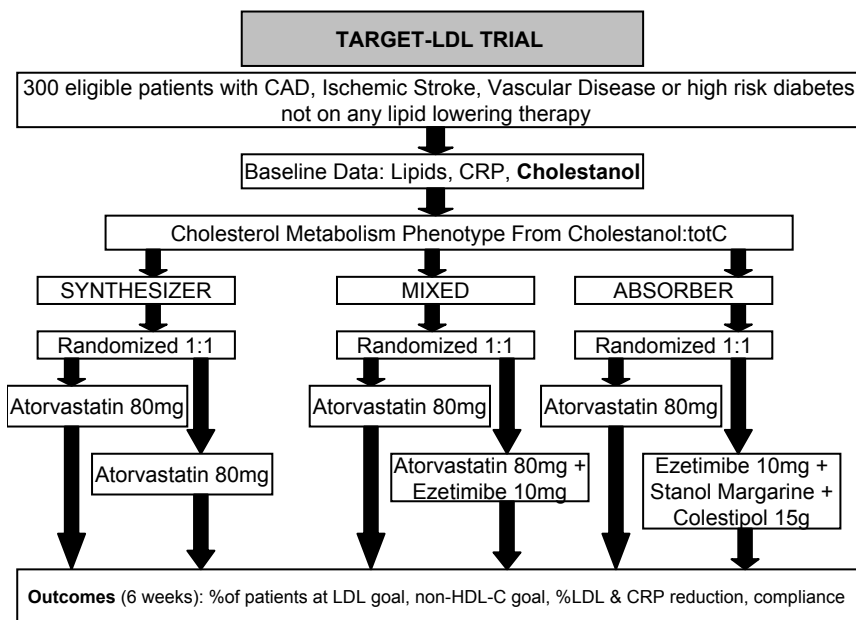


Fig. (2). The TARGET LDL trail design and outcomes. CAD: coronary artery disease, CRP: C-reactive protein, DL-C: Low density lipoprotein cholesterol, non-HDL-C: non-high density lipoprotein cholesterol.

from 3% in 1970 to 12% in 2000 [200]. Genetic factors are thought to contribute to increasing prevalence and incidence of diabetes in minorities according to the 'thrifty gene hypothesis' which offers an evolutionary explanation for enhanced resistance of European populations to diabetes who are thereby also protected from cardiovascular disease, menstrual irregularities and polycystic ovary syndrome during the reproductive years [201]. Indeed, the 'thrifty gene' is a likely contributor for the age-adjusted prevalence of diabetes in developed countries being 5% in those of European Ancestry compared to 40% or higher in Aboriginal populations and is concordant with greater metabolic risk at lower levels of waist circumference. The scale of the discrepancy in diabetes prevalence between ethnic groups is likely to grow by 2030 where the projected prevalence of diabetes is projected to increase the most in India, Asia, the Middle East, Latin America and Africa [202]. Although little formal evaluation of cholesterol metabolism in different ethnic groups has been undertaken in modern times, extrapolation from available data in other ethnic groups suggests that as diabetes-prone minorities gain weight and acquire a metabolic syndrome phenotype, they shift cholesterol metabolism from an 'absorber' to a 'synthesizer' phenotype. This may mean that such patients will have an enhanced serum and clinical response to statins although this is mere speculation. Hence, off-patent statin therapy will have a major role in the prevention and treatment of cardiovascular disease in the developing world where drug cost may be an issue. Several overdue statin trials in minorities include Investigation of Rosuvastatin in South-Asian subjects (IRIS) in Americans of South Asian origin and The Study Assessing rosuvastatin in the Hispanic Population (STARSHIP) in Hispanics [200]. An understanding of shifting cholesterol metabolism is also crucial for those practicing medicine in more affluent parts of the World. Indeed, as we aim for lower LDL-C targets, we will need to use combination drugs more often. However, knowledge of the patient's cholesterol metabolism and thus the optimal way to treat a given patient may reduce treatment cost, minimize side effects due to the unnecessary prescription of medications and, most importantly, increase the number of high risk patients at their target LDL-C of <1.8mmol/l (70mg/dl) and non-HDL-C of <2.6mmol/l (100mg/dl). This is the point that the TARGET LDL trial will have to prove [83].

CONFLICT OF INTEREST & FINANCIAL DISCLOSURES

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