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Review

Cholestanol: A serum marker to guide LDL cholesterol-lowering therapy

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Abstract

Statins have been the mainstay of lipid-lowering therapy since their introduction. However, as lower LDL cholesterol targets are sought, adjunct therapies are becoming increasingly important. Few patients reach new targets with statin monotherapy. We propose that the cholestanol:cholesterol ratio can be used to guide lipid-lowering therapy and result in greater numbers of patients reaching target LDL cholesterol. By determining whether a patient is mainly a synthesizer or absorber of cholesterol, customized regimens can be used and are expected to improve patient outcomes and minimize costs of treatment.

Keywords: LDL; Cholestanol; Cholesterol; Statins; Ezetimibe; Bile acid binding resins; NCEP ATPIII

Contents

1. Introduction: LDL cholesterol as a therapeutic target

Epidemiologic data from populations with and without coronary artery disease (CAD) have highlighted the importance of reducing LDL cholesterol in preventing both new-onset CAD and recurrent ischemic events [1–6]. Indeed, there is a log-linear relationship between LDL cholesterol and CAD risk, and this relationship holds true at low LDL cholesterol levels [7]. Not surprisingly, 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 [8]. A recent amendment to the ATP III report recommended that the target LDL in *very* high risk patients may be changed from 100 to 70 mg/dl in light of the Heart Protection Study (HPS) and PROVE-IT TIMI22 [7]. Also, more aggressive therapy is supported by the findings of the Treat To New Targets (TNT) trial where atorvastatin 80 mg reduced LDL cholesterol by 24 mg/dl more than atorvastatin 10 mg and reduced non-fatal myocardial infarction and stroke by 22% and 25%, respectively [9]. However, the mean LDL cholesterol in the high dose statin arm of TNT was 77 mg/dl; a level higher than the optional 70 mg/dl target specified in the ATP III update. In PROVE-IT TIMI22, only 43.9% of patients randomized to atorvastatin 80 mg reached dual targets of LDL cholesterol <70 mg/dl and C-reactive protein (CRP) <2 mg/l [10].

Reaching target LDL cholesterol levels is a problem; the ATP III report pointed out that only slightly more than half of the patients enrolled in secondary prevention studies reached an LDL cholesterol of <100 mg/dl. In 1997, data from the lipid treatment assessment program (L-TAP) showed

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that only 18% of CAD patients achieve an LDL cholesterol <100 mg/dl [11]. More recent data presented in 2004 from NCEP Evaluation Project Utilizing Novel e-Technology (NEPTUNE) II show that 57% of patients with CAD or CAD risk equivalents were at their ATP goal [12]. Two important points can be made from this discussion. Firstly, the use of higher potency statins in high doses has improved the percentage of CAD patients at target LDL cholesterol levels (100 mg/dl) although this percentage is still suboptimal. Secondly, there is increasing evidence that lower LDL cholesterol targets should be sought since lower levels have translated into reductions in hard clinical end points. However, if only 57% of CAD patients are at the ATP III target of 100 mg/dl, the proportion of very high risk patients that will reach a target LDL cholesterol of <70 mg/dl on typical doses of statins is likely to be even smaller.

The purpose of this paper is to highlight the factors that could influence the response to statin therapy and to discuss the concept of using the cholestanol:cholesterol ratio to guide LDL cholesterol-lowering therapy. Initiating therapy with a customized regimen will become increasingly important as lower LDL cholesterol targets are sought. Simply increasing statin dosages may not be a viable option since increasing doses typically only decrease LDL cholesterol by a further 6% [13] and may not be safe. The A–Z trial showed that high doses of statins increased the incidence of myopathy and elevated liver enzymes [14]. Likewise, the TNT trial showed higher rates of drug discontinuation and elevated liver enzymes in the high dose statin arm [9]. These safety concerns also apply to rosuvastatin which may have more adverse effects than older statins [15]. Importantly, even maximal doses of statins often fail to achieve LDL cholesterol targets [16] and therefore many patients will require adjunct LDL cholesterol-lowering therapy. This point is illustrated by the recently published vytorin versus atorvastatin (VYVA) study which showed that only 36% of patients treated with atorvastatin 80 mg reach a target LDL cholesterol of <70 mg/dl [17]. The VYVA study also showed that only 64% of patients treated with simvastatin 80 mg/ezetimibe 10 mg (vytorin) reach a target LDL cholesterol of <70 mg/dl and hence vytorin in place of statin monotherapy will not ensure that all patients reach the new optional target of <70 mg/dl. On meta-analysis, vytorin (80/10 mg) reduces LDL cholesterol levels by 59.7%, which is only slightly more than the 58.5% reduction with rosuvastatin 40 mg [18]. Therefore, neither the indiscriminate use of high dose, potent statins nor the indiscriminate use of vytorin in unselected patients can be expected to universally reduce LDL cholesterol levels to below the 70 mg/dl target.

2. Are all hypercholesteremic patients the same?

The efficacy of statin therapy in a given patient will depend on three factors: the type of statin used, extrinsic factors (e.g. time of drug administration, compliance and co-interventions) and intrinsic factors. This review will discuss these factors seriatim before focusing on intrinsic factors that determine response to LDL cholesterol-lowering therapy.

Different statins have different LDL cholesterol-lowering efficacies. The 2003 meta-analysis of statin trials by Law included 164 short-term placebo-controlled trials in patients treated with statins in a variety of clinical situations. The LDL cholesterol-lowering efficacy is expressed as the difference between the treatment and the placebo groups. This meta-analysis found that rosuvastatin 80 mg reduced LDL cholesterol by an average 108 mg/dl (58%), atorvastatin 80 mg by 102 mg/dl (55%), simvastatin 40 mg by 69 mg/dl (37%) lovastatin 40 mg by 68 mg/dl (37%), pravastatin 40 mg by 53 mg/dl (33%) and fluvastatin 40 mg by 50 mg/dl (27%) [19]. The PSCOP study showed that switching from pravastatin to simvastatin increased the number of patients achieving ATP goals and this translated into improved clinical outcomes. However, 31% of patients using simvastatin did not reach target LDL cholesterol again illustrating that changing to a more potent statin cannot be used as a universal strategy [20].

There are a number of extrinsic factors, mainly patient behaviors, which can modify the LDL cholesterol response to statin therapy. Compliance is a major issue as an Australian survey showed that about a third of all patients prescribed statins discontinue drug therapy within 6 months [21]. Poor compliance has also been documented in other populations [22,23] and improves if statin therapy is started in hospital [24,25]. A low fat and low cholesterol diet has additive effects on reducing LDL cholesterol [26–28] and can produce impressive LDL cholesterol reductions in the order of 30% [29]. Timing of statin administration is also important since cholesterol synthesis is highest when dietary intake is low [30]. Hence statins may be more effective if given in the evening rather than in the morning [31]; the exception is atorvastatin, which has a longer half-life [32]. Pharmacokinetic considerations can also explain heterogeneity in the response to statins. Statin metabolism occurs via CYP450 3A4 and 2C9 pathways [33], and polymorphisms in the CYP enzymes can affect statin efficacy [34,35]. Also, LDL cholesterol-lowering efficacy can be reduced if statins are given with CYP450 inducers, pravastatin being an important exception as it is not metabolized by these pathways [36]. However, even when these extrinsic factors are taken into account, there remain intrinsic differences in LDL-cholesterol response to statin therapy.

In a 6-month trial with simvastatin 80 mg, the top 5% of responders had reductions in LDL cholesterol of ∼70% whereas the bottom 5% responders showed no change at all [37]. What is the basis for these inter-individual differences? Plasma cholesterol is derived from two sources: exogenous (dietary) and endogenous (hepatic) [38]. Both types of cholesterol are absorbed in the intestine by a saturable transport mechanism [39]. Importantly, individual variability exists with regard to the proportion of cholesterol

Table 1
Classification of patients into three categories based on cholestanol:cholesterol ratio

	Synthesizer	Mixed	Absorber
Physiology	High rates of hepatic cholesterol synthesis and low rates of gastrointestinal absorption	Moderate rates of both hepatic cholesterol synthesis and gastrointestinal absorption	High rates of gastrointestinal cholesterol absorption and low rates of hepatic synthesis
LDL-cholesterol response to statins	Hyper-responders	Intermediate response to statins	Poor response
Cholestanol:cholesterol ratio	Low	Intermediate	High
Plasma levels of markers of cholesterol synthesis	High	Intermediate	Low
Proposed treatment strategy	Statin monotherapy	Statin + ezetimibe	Ezetimibe + bile acid binding resin + stanol margarine

from exogenous versus endogenous sources—some individuals are avid 'absorbers' whereas others are 'synthesizers' [37]. Hence individuals can be classified into three categories descriptive of their cholesterol metabolism: absorbers, synthesizers or mixed. Absorbers have decreased cholesterol synthesis because absorbed cholesterol reduces the activity of HMGCoA reductase [40], which catalyzes the rate-limiting step in cholesterol synthesis [41], and indeed an inverse relationship between cholesterol absorption and synthesis was observed in the 4S study [42]. The three categories are summarized in Table 1.

While the basis for this intrinsic variation is unclear, 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 [43,44]. Further, in hemodialysis patients, the 4/3 genotype was associated with higher LDL cholesterol than the 3/3 or 3/2 genotype [45]. Also, the E2 allele may be protective to the development of CAD whereas the E4 allele may be detrimental [46]. The ABC transporter system may also play a role in determining phenotype since polymorphisms have been associated with low-absorption [47]. Carriers of the E4 genotype have greater responses to dietary intervention than E2 carriers and this is further influenced by multiple polymorphisms in numerous genes including ABCG8, ApoA-I, ApoA-IV, ApoB [48,49]. Recently, polymorphisms in the HMG-CoA reductase gene were shown to influence the degree of LDL cholesterol reduction in response to pravastatin [50]. This was touted as a possible way to customize LDL cholesterol-lowering treatment. However, HMG-CoA reductase activity can be regulated by multiple factors and the genotype of this enzyme does not integrate whole body cholesterol metabolism. For example, the balance between synthesized and absorbed cholesterol can also be influenced by colorectal surgery [51] or liver disease [52]. Clearly, the use of genetic methods to customize lipid-lowering therapies would be very complex and expensive. New polymorphisms that impact the efficacy of lipid-lowering therapies are periodically described in the literature. Recently identified loss-of function mutations of PCSK9 have been shown to occur at a frequency of 2% in African Americans and to be associated with 40% reductions in plasma cholesterol [53]. PCSK9 mutant mice were found to have increased levels of LDL receptor and an enhanced response to statin therapy [54]. In addition, recent advances have been made in evaluating the variation in response to ezetimibe which interferes with the NPC1L1 gene product [55,56]. Single nucleotide polymorphisms in NPC1L1 are associated with differences in ezetimibe efficacy [57]. While a genetic approach to customizing lipid-lowering therapy may be appealing, genetic testing cannot account for unknown polymorphisms and cannot account for epigenetic and environmental factors.

3. The clinical utility of cholestanol in guiding therapy

Cholestanol is a sterol that differs from cholesterol by the absence of a double bond in the B ring and, in humans, is present in far lower concentrations than cholesterol [58]. Its metabolism in human tissues has not been fully characterized. Cholestanol and plant sterols are markers for cholesterol absorption [30,42,59,60] and its measurement requires gas–liquid chromatography. We propose that the cholestanol:cholesterol ratio can identify whether an individual is mainly an absorber, synthesizer, or has a mixed phenotype. Absorbers will have a high cholestanol:cholesterol ratio whereas synthesizers will have a low ratio. Importantly, this ratio integrates all known and unknown polymorphisms in lipid proteins, concomitant medical illnesses and dietary habits of an individual and can be used to predict response to statin therapy. Patients with a mixed phenotype are expected to show intermediate response to monotherapy and therefore initiation with statin and ezetimibe combination therapy is reasonable. However, patients who can be identified as either synthesizers or absorbers may be treated with statin monotherapy or ezetimibe, stanol margarine and a bile acid binding resin as appropriate. Synthesizers have greater responses to statins

[42,61]. Importantly, the 4S study showed that those with cholestanol:cholesterol ratios in the highest quartile had no clinical benefit from simvastatin. Further, in the treatment arm, those in the highest quartile had a 2.2-fold increased risk of coronary events compared to those in the lowest quartile of cholestanol:cholesterol ratio [62]. Hence the cholestanol:cholesterol ratio can predict LDL cholesterol response to a statin and can also predict clinical benefit from therapy.

In contrast to synthesizers, absorbers have poor responses to statins. An extreme example of the cholesterol absorber phenotype is homozygous sitosterolemia. This is a recessive condition caused by a mutation in an ABC transporter resulting in increased absorption of plant sterols and dietary cholesterol, decreased biliary excretion of sterols, xanthomas and premature CAD [63]. Statins are ineffective for this condition; treatments include bile acid binding resins, ezetimibe and ileal bypass surgery [64]. The lack of efficacy with statins is expected since cholesterol synthesis is inhibited in this condition [65]. Hence 'absorbers' are expected to show particular benefit with anti-resorptive agents and bile acid binding resins. Bile acid binding resins and ezetimibe have independent mechanisms of action and can be safely combined [66]. Ezetimibe localizes at the enterocyte brush border and selectively inhibits absorption of cholesterol from the intestinal lumen into enterocytes [67]. Bile acid binding resins bind bile acids and thereby interrupt their enterohepatic circulation. Hence the liver increases production of bile acids from cholesterol with a resultant decrease in hepatic cholesterol content and an increase in LDL receptor levels and hepatic LDL cholesterol clearance [68]. This combination is arguably the optimal initial therapy in the absorber phenotype where a poor response to statin therapy is expected. The addition of bile acid binding resins to ezetimibe to treat the absorber phenotype is not mechanistic i.e. bile acid binding resins do not inhibit cholesterol absorption. Rather, these patients show a poor response to statins and hence require adjunct therapies to ezetimibe which typically reduces LDL cholesterol level by the order of 20% [67]. Non-pharmacologic inhibitors of cholesterol absorption include plant sterols and stanols [69] and these therapies may have a role in treating patients with the absorber phenotype; however, pharmacotherapy is the focus of this paper.

A natural concern that may arise from treating synthesizers or absorbers with only one modality of therapy is the upregulation of the opposing pathway. The first human cholesterol absorption study of ezetimibe showed that the drug reduced cholesterol absorption by 54% and reduced LDL cholesterol by 20% [70]. The use of this drug was associated with a rebound increase in cholesterol synthesis but this could theoretically be inhibited with a statin. Conversely, the use of statins is associated with a rebound increase in cholesterol absorption [42,71,72]. 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 [73]. However, despite theoretical up-regulation of opposing pathways, it is still a reasonable strategy to initiate therapy with one modality of therapy where appropriate and to assess LDL cholesterol response. Mixed therapy can always be used later if targeted therapy does not reach target LDL cholesterol.

The utility of the cholestanol:cholesterol ratio is especially important in the context of an expanding number of adults to be treated as lower target LDL cholesterol is sought. The application of the ATP III guidelines results in a treatment population of 36 million patients, an increase of 21 million from the ATP II guidelines [74]. By placing patients into three categories: synthesizers, mixed phenotype and absorbers, optimal therapy can be delivered and costs of therapy minimized. While blanket therapy with statin and ezetimibe increases the proportion of patients who reach their NCEP target [17,75,76], customizing therapy is an attractive option from patient outcome and economic viewpoints.

While ideally every patient would have a cholestanol: cholesterol ratio to determine his or her phenotype before initiating therapy, clinical clues can offer limited guidance. For example, whether a patient is an absorber or synthesizer is genetically determined and a significant intra-family correlation has been found [77]. Hence, if a close family member of a patient had a particularly good response to a synthesis inhibitor (statin) or an absorption inhibitor (ezetimibe), it would be reasonable to use the same therapy initially for that patient. Also, the synthesizer phenotype is associated with obesity and features of the metabolic syndrome indicating that statins may be the optimal treatment in such patients [47,62,77–79]. Indeed, in a study of normoglycemic men, insulin resistance was found to be associated with increased cholesterol synthesis and decreased cholesterol absorption as assessed by cholesterol absorption markers and levels of cholesterol precursors [80]. Variation also exists among type 2 diabetics; with increasing body weight as assessed by body mass index being associated with increased serum levels of markers of cholesterol synthesis [81]. Further, patients in the 4S study that had a high triglyceride-low HDL lipid profile (typical of the metabolic syndrome) tended to have greater reductions in cholesterol with simvastatin than in patients with isolated high LDL cholesterol [82]. This is concordant with a study using fluvastatin where patients with low HDL at baseline were found to have greater angiographic and clinical response than those with normal HDL [83]. Interestingly, weight loss in obese type 2 diabetics was found to decrease markers of cholesterol synthesis [84]. Conversely, type 1 diabetics show a synthesizer phenotype when compared to weight-matched type 2 diabetics [85]. While these observations may be of peripheral utility in guiding therapy, the cholestanol:cholesterol ratio is superior in determining a patient's phenotype and allows a more refined prediction of response to statins.

Table 2
Practical considerations in using cholestanol:cholesterol ratio to customize lipid-lowering therapy

Population	The population used to define the phenotypes should be a population where aggressive therapy to target LDL cholesterol levels of <70 mg/dl is an option as recommended by the ATP III guidelines (please see text) The population, at baseline should be free of lipid-lowering therapies that can interfere with a true baseline determination of cholestanol:cholesterol ratios
Data analysis	After determination of baseline cholestanol:cholesterol ratios, diminishing efficacy of statin monotherapy needs to be demonstrated as the baseline cholestanol:cholesterol ratios increase. This would allow the thresholds for the intermediate reponders (mixed phenotype) and the hyporesponders and non-responders (absorber phenotype) to be defined Such thresholds can then serve as the basis for a clinical trial where customized therapy is compared to statin monotherapy
Advantages	If this concept is proven, an increased number of patients would reach target LDL cholesterol levels and this would be expected to improve clinical outcomes This approach could minimize the cost of therapy in that rational drug therapy takes precedent over empirical adjunct therapy Patients who would not benefit from statins would not be prescribed statins. This would spare patients the cost of statins and any possible adverse effects The cholestanol:cholesterol ratio is a one-off test undertaken at baseline and can be performed (in Brisbane) for 80 Australian Dollars
Disadvantages	The determination of cholestanol:cholesterol ratio requires gas–liquid chromatography and the expertise of trained technicians The test cost of 80 Australian Dollars may seem expensive when off-patent statins are available

4. Practical considerations in using cholestanol to guide lipid-lowering therapy

Customizing lipid-lowering therapy is of importance only for high-risk patients where low LDL cholesterol targets are sought (<70 mg/dl). This point is highlighted by the previously mentioned VYVA data. While only 36% of patients reached LDL cholesterol levels <70 mg/dl on atorvastatin 80 mg, 85% of patients reached the <100 mg/dl target with this dose [17]. Therefore, customized therapy is only justified when aggressive therapy is mandated since higher targets are largely attainable with statin monotherapy. Hence, a population cohort to derive cholestanol:cholesterol ratio definitions for the three proposed categories should only include patients where aggressive therapy is an option as defined by the ATP III amendment. Such patients could include patients with established CVD and multiple major risk factors (especially diabetes), poorly controlled risk factors, metabolic syndrome or patients with acute coronary syndromes. There is little point to sampling from low risk populations since treatment to levels of LDL cholesterol as low as 70 mg/dl is not supported by current guidelines and almost all of these patients can reach ATP III target LDL cholesterol levels with statin monotherapy. Another factor that will influence the cholestanol:cholesterol ratios that define the three categories will depend on the statin used. Indeed, as statin dose and potency increase, the number of patients that can be treated with statin monotherapy increases. A further caveat in utilizing the cholestanol:cholesterol ratio to customize lipid-lowering therapy is that cholesterol metabolism is a dynamic process. As previously discussed, statin therapy causes a rebound increase in cholesterol absorption and anti-resorptive agents such as ezetimibe cause a rebound increase in cholesterol synthesis. Hence in deriving data from a high risk cohort to define the phenotypes, the cohort should be free of lipid-lowering therapies including statins, ezetimibe, bile acid binding resins and stanol margarines which may preclude obtaining a true 'baseline' cholestanol:cholesterol measurement. In practice, this will limit this approach to *newly diagnosed* patients only and cannot be applied to patients already on lipid-lowering therapy without further population sampling. Practical considerations regarding the use of cholestanol to guide lipid-lowering therapy are discussed in Table 2.

The choice of using cholestanol to customize therapy is based on the experience of the 4S investigators. Markers of cholesterol synthesis include squalene, cholestenol, desmosterol, and lathosterol [59]. These markers of cholesterol synthesis are inversely related to markers of absorption which include the plant sterols, campesterol and sitosterol. Serum cholestanol has been shown to positively correlate with serum plant sterols (campesterol and sitosterol) and fractional cholesterol absorption [86]. Further, plasma cholestanol levels are inversely correlated with cholesterol synthesis as assessed by serum precursor sterols: desmosterol and lathosterol [42,86]. Hence cholestanol is a reasonable marker for the customization of therapy since its plasma levels reflect both dietary absorption and hepatic synthesis. While several of these markers may theoretically be used to customize therapy, cholestanol has the advantage of predicting clinical outcomes in the previously mentioned 4S subgroup of 868 patients [62] and hence its proposed use

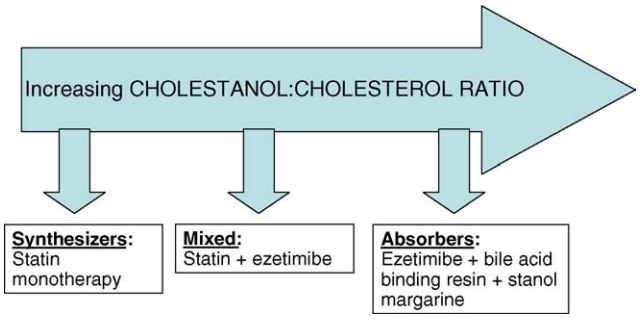

Fig. 1. Categorization of hypercholesteremic patients and suggested first-line therapies.

to guide lipid-lowering therapy is based on experience rather than scientific reasoning.

5. Conclusion and perspective

In summary, the cholestanol:cholesterol ratio can be used to classify patients into three categories (see Table 1) before commencing LDL cholesterol-lowering therapy. Those with an 'absorber' phenotype will have a high cholestanol:cholesterol ratio, 'synthesizers' will have low values and 'mixed' patients will have intermediate values. As discussed in this article, absorbers might be best treated with ezetimibe, stanol margarines and bile acid binding resins whereas synthesizers may be best treated with statin monotherapy (see Fig. 1). Those with a mixed phenotype are likely to require treatment with both therapeutic modalities; e.g. a statin and ezetimibe. Such guided therapy may increase the number of patients reaching their ATP III goals and minimize the costs of health care, and needs to be tested in a prospective randomized clinical trial. Such a trial, the TARGET-LDL trial is underway in Brisbane, Australia.

Perhaps one of our more controversial suggestions is that patients with an absorber phenotype forego statin therapy *initially* and be treated with ezetimibe, stanol margarine and a bile acid binding resin. A popular opinion is that all patients should be treated with a statin because this class of drug has purported pleiotropic effects. Yet in the 4S substudies, the statins were of no use in lowering LDL cholesterol or improving clinical outcomes in the absorber phenotype. If these pleiotropic effects were to translate into meaningful clinical end points, even patients with an absorber phenotype should show clinical benefit. Further, the Law meta-analysis quantified the effects of statins on LDL cholesterol and ischemic heart disease (IHD), and with regard to IHD events, the outcomes of 58 studies were similar *regardless of the method used* to decrease LDL cholesterol (fibrates, resins, niacin, statins or dietary change) after standardization was undertaken for absolute reductions in LDL cholesterol and duration of treatment [19]. Hence, to our knowledge, there is currently no evidence for the clinical relevance of pleiotropic effects of statins. Indeed, we challenge those who believe that statins have clinically relevant pleiotropic effects to undertake a placebo-controlled trial that recruits patients with an absorber phenotype. If these pleiotropic effects are of any clinical relevance, there should be dissociation between the extent of LDL cholesterol-lowering and hard clinical end points.

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