Cholesterol and triglyceride standardization procedures have been used extensively and continuously since the 1950s. Definitive and Reference Methods, as well as primary and secondary standards, have been developed and maintained as the basis for evaluating the accuracy of results by various methods in many laboratories. But, although standardization efforts for apolipoprotein A-I and B measurements have been reported in detail in the scientific literature, much less has been reported in the area of total and lipoprotein cholesterol and triglyceride standardization efforts. Standardized cholesterol and triglyceride concentrations, determined in multiple large epidemiological and clinical studies, have been instrumental to the National Cholesterol Education Program panels that have assessed the lipoprotein values associated with risk of coronary disease, and have determined the cutpoints that are now used extensively by physicians to guide diagnosis and treatment of individual patients.

The editorial in Clinical Chemistry by Sniderman and Cianflone [1] highlighted some of the potential advantages of measurement of apolipoproteins in clinical practice, most notably in its referral to the articles by Contois et al. on apolipoprotein (apo) A-I and apo B reference intervals [2, 3]. The improvements in apo A-I and apo B measurements in the past few years have been immense, thanks in great part to work by the IFCC Committee on Apolipoproteins, chaired by Santica Marcovina. This Committee developed World Health Organization (WHO)-IFCC International Reference Materials for apo A-I and apo B, which are now used internationally by manufacturers to set assay calibration [4–6]. The editorial by Sniderman and Cianflone, however, also indicated, mistakenly, that similar standardization of lipid and lipoprotein lipids does not exist.

Although much has been written during the past few years regarding the progress in apolipoprotein measurement and standardization, it was clear from the editorial that public knowledge and understanding is lacking regarding international standardization of the lipid constituents of lipoproteins. In fact, the Lipid Standardization Program (LSP) of the Centers for Disease Control and Prevention–National Heart, Lung and Blood Institute (CDC-NHLBI) has provided standardization for lipid and lipoprotein cholesterol and triglyceride since 1957 in the US and in countries throughout the world [7]. So that all may have a complete understanding of the importance of the standardization programs that exist for the cholesterol and triglyceride constituents of lipoproteins, we describe here the programs that are available.

CDC-NHLBI LSP

Definitive and Reference Methods, purified primary standards, and serum reference materials have been devel-

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1 Jean Mayer USDA Human Nutrition Research Center on Aging at Tufts University, 711 Washington St., Boston, MA 02111.
2 Pacific Biometrics Research Foundation, Seattle, WA.
3 H.S. Raffaele, Milan, Italy.
4 Rotterdam University Hospital, Rotterdam, The Netherlands.
5 Washington University School of Medicine, St. Louis, MO.
6 State Laboratory of Hygiene, Madison, WI.
7 Osaka Medical Center for Cancer and Cardiovascular Diseases, Osaka, Japan.
8 Institute of Biochemistry, Glasgow Royal Infirmary, Glasgow, Scotland, UK.
10 Centers for Disease Control and Prevention, Atlanta, GA.
*Author for correspondence. Fax 617-556-3103; e-mail mcnamara_li@hnrc.tufts.edu.

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11 Nonstandard abbreviations: apo, apolipoprotein; LSP, Lipid Standardization Panel; CDC-NHLBI, Centers for Disease Control and Prevention—National Heart, Lung and Blood Institute; CHD, coronary heart disease; NCEP, National Cholesterol Education Program; and CRMLN, Cholesterol Reference Method Laboratory Network.
developed and coordinated at the CDC as standardization resources for national and international standardization programs [7], in cooperation with NIST, NHLBI, AACC, NCCLS, and WHO. Reference materials have also been developed and evaluated in cooperation with NIST and the College of American Pathologists, and in consultation with manufacturers of diagnostic reagents. The CDC-established Reference Methods for measuring total cholesterol, HDL-cholesterol, LDL-cholesterol, and triglyceride [8] are used to set reference values for the serum pools used for LSP standardization. These frozen reference pools, stable in storage at −50 °C to −80 °C, are then used to transfer values assigned by the Reference Methods to research and epidemiological laboratories, as points of reference in the evaluation of coronary heart disease (CHD) risk. They have provided the basis for standardization of cholesterol and triglyceride analyses in >30 clinical trials conducted by the NHLBI [7], and in multiple US and international clinical trials, such as the West of Scotland Prevention Study [9] and the CARE Study [10]. International standardization of cholesterol and triglyceride measurements has also been provided to laboratories supporting WHO projects and to international epidemiological central laboratories under WHO sponsorship, through the WHO Collaborating Center for Reference and Research in Blood Lipids established at the CDC.

This long-term standardization activity has documented the reliability of these Reference Methods, which are tied to the NIST Definitive Methods for cholesterol [11] and triglyceride [12]. Thus, national and international standardization of serum lipid and lipoprotein measurements has been accomplished through CDC, NHLBI, and WHO collaboration, and comparable results are obtainable throughout the world because of these standardization efforts.

**CHOLESTEROL REFERENCE METHOD LABORATORY NETWORK**

With the advent of enzymic assays and alternative assay configurations such as dry chemistries, the effects of matrix interactions have required the use of fresh serum comparisons to ensure transfer of accuracy within National Cholesterol Education Program (NCEP)-specified guidelines [13–16]. For this purpose, in 1988, the CDC initiated the Cholesterol Reference Method Laboratory Network (CRMLN) to increase standardization support for manufacturers and clinical laboratories. The CRMLN comprises laboratories in the US, Canada, Europe, and Asia that are all tightly standardized to the CDC (see Appendix). The standardization protocol developed by the CRMLN for total cholesterol is now recognized as a model for standardization of other clinical chemistry analytes. The use of fresh specimens in analytical systems affected by matrix effects ensures accurate results on patients’ specimens and comparability of results nationally and internationally. Manufacturers that participate in the program follow the NCCLS EP9 protocol [17], which involves comparing test methods with the Reference Method for 40 fresh sera. Comparisons that meet precision and accuracy requirements set by the CRMLN receive a certificate of traceability. A list of current certificate holders is maintained within the AACC homepage on the World Wide Web (http://www.aacc.org/standards).

When establishing the fresh serum certification program, the CRMLN decided that the top priority was certification of manufacturers’ systems, because the accuracy of each manufacturer’s analytical system would impact on measurements in thousands of clinical laboratories. If clinical laboratories used the analytical systems as directed by their respective manufacturers, traceability to the Reference Methods could thus be transferred.

In addition to the program offered to manufacturers, however, an alternative program is available directly to clinical laboratories, which provides for a six-sample comparison and a certificate when requirements are met. The comparison program for clinical laboratories is less intensive than the manufacturers’ protocol, for two reasons. First, because in most cases the manufacturer would have already completed the certification program on the system, the clinical laboratory comparison would simply confirm that the system was working as intended. In cases of heterogeneous systems, confirmation that the various entities work to produce accurate patients’ results is obtained. The second reason was clearly one of economics: Clinical laboratories would generally not have the resources to afford the costs of a 40-sample comparison. To maximize the statistical power of evaluating clinical laboratories’ six-sample comparisons, however, these laboratories are required to analyze each sample in duplicate on each of 3 days, whereas manufacturers must analyze each sample only once, in duplicate.

The CRMLN maintains direct traceability to the CDC through a rigorous standardization program. CRMLN laboratories perform the Abell–Kendall Reference Method for cholesterol [18], enzymic methods for triglyceride that are standardized to the chromotropic acid Reference Method at the CDC [19], and a designated comparison method for HDL-cholesterol that is tied to the HDL-cholesterol Reference Method [20, 21]. The CRMLN laboratories are surveyed monthly to ensure that their analytical performance meets tightly defined specifications. Efforts by the CRMLN to help improve analytical performance of cholesterol and triglyceride measurements in clinical laboratories through the manufacturers is succeeding, as detailed in reports of improvement in results from proficiency testing programs [22].

Historically, LDL-cholesterol values, derived in most laboratories by mathematical calculation, have generally been assumed to be standardized whenever total cholesterol, HDL-cholesterol, and triglycerides are standardized. More recently, however, in conjunction with NCEP performance guideline development [13–16], it was determined that within-limit performance of the latter three analyses did not ensure within-limit performance of the
first. For that reason, the NCEP Working Group on Lipoprotein Measurement recommended that methods be developed that would allow actual LDL-cholesterol measurements to be made in clinical laboratories, and that those methods replace mathematical calculations of LDL-cholesterol. Methods suitable for clinical laboratory measurement of LDL-cholesterol have begun to be developed and to be compared with the Reference Method [23–25] and must now be added to lipid standardization programs. An unofficial standardization program instituted by Pacific Biometrics Research Foundation (Seattle, WA) in 1993 to standardize LDL-cholesterol determinations among laboratories performing beta-quantification (ultracentrifugation) has been expanded to include those results derived by other methods of measurement. The CDC has recently initiated the first steps of a similar program that would transfer traceability of the CDC Reference Method among CRMLN and LSP laboratories for eventual use in LDL-cholesterol certification protocols.

**NCEP CUTPOINTS: DERIVATION AND UTILIZATION**

The Definitive and Reference Methods for lipoprotein cholesterol and triglyceride measurements that have been accepted by the NCEP Working Group on Lipoprotein Measurement [14–16] were developed at NIST and CDC, and have withstood the challenge of time as basic points of reference. The isolation procedures required in the LDL- and HDL-cholesterol Reference Methods, which have been developed and maintained at the CDC, have been clearly documented for validity with respect to the assessment of risk for CHD [26–29]. Standardized cholesterol and triglyceride measurements are the foundations for assessment of CHD risk. The relationships between total, LDL-, and HDL-cholesterol and CHD risk, prevention, and treatment have been documented extensively. Moreover, physicians and patients have become educated as to what analyte concentrations are desirable and what concentrations pose a risk of disease. These standardized lipid measurements can also be used in evaluating the relative importance of other potential markers. For example, in the studies by Contois et al. [2, 3], the recommended cutpoints for apo A-I and apo B, for determining CHD risk, were derived in part from the corresponding NCEP cutpoints already established for lipid and lipoprotein cholesterol concentrations. In addition, because standardization of lipids and lipoproteins is accomplished through Reference Methods that are traceable to NIST Definitive Methods, with use of primary standards and reference calibrators, the critical problem of accurate protein concentration determination of a surrogate standard, such as narrow-cut LDL for apo B [27], is obviated and there is agreement on acceptable reference methods—unlike the situation for apo B [28].

In conclusion, standardized cholesterol and triglyceride measurements provide long-standing basic characteristics for estimating CHD risk. The standardization programs allow direct comparison among the results of many studies, separated by time, country of origin, age, gender, and ethnicity. Because many of the differences in concentration are small, it is important to have, and to document, accuracy and precision limits so that significant associations can be evaluated. Lipid standardization programs provide this ability. Therefore, lipoprotein cholesterol and triglyceride values compiled from national and international trials in CDC-NHLBI-standardized laboratories are the basis by which the NCEP Adult Treatment Panel and other advisory committees throughout the world have classified desirable, borderline, and high-risk lipoprotein values. In the future, use of standardized lipid and apolipoprotein measurements will, we hope, provide even more detailed information for assessment of CHD risk and evaluation of individual dyslipidemic patients.

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**References**


Appendix: List of CRMLN Laboratories

IN THE US
Jean Mayer USDA Human Nutrition Research Center on Aging at Tufts University
711 Washington St.
Boston, MA 02111
Contact: Judith R. McNamara, MT
(617) 556-3104
mcnamara_li@hnrc.tufts.edu

Wadsworth Center for Laboratories and Research
Empire State Plaza
Albany, NY 12201
Contact: Robert Rej, PhD
(518) 473-0117
(518) 473-2900
bobrej@wadsworth.org

University of Washington
Northwest Lipid Research Laboratories
2121 N. 35th St.
Seattle, WA 98103
Contact: Santica Marcovina, PhD
(206) 685-3331
(206) 685-3279 fax
smm@u.washington.edu

Washington University School of Medicine
Lipid Research Center
660 S. Euclid Ave.
St. Louis, MO 63110
Contact: Thomas G. Cole, PhD
(314) 362-3516 phone
(314) 362-7657 fax
thomcole@imgate.wustl.edu

Wisconsin State Laboratory of Hygiene
E. 465 Henry Mall
Madison, WI 53706
Contact: David Hassemer, MS
(608) 833-1770, ext. 102 phone
(608) 833-2803 fax
hassemer@clia.slh.wisc.edu

OUTSIDE THE US
Canadian Reference Laboratory (1996) Ltd.
307–2083 Alma St.
Vancouver, BC V6R 4N6 Canada
Contact: David W. Seccombe, MD, PhD
(604) 222-1879 phone
(604) 222-0134 fax
7361.1047@compuserve.com
The measurement of apo B provides critical information that is complementary to that provided by the plasma and lipoprotein lipids for the assessment of coronary risk and the choice of appropriate pharmacological therapy. Why then is this measurement not in more widespread clinical use? I suggest two explanations. First, against the evidence, there is a lingering perception that problems persist in its measurement in routine clinical practice. Far from this being the case, however, the measurement of apo B has met every reasonable standard of laboratory precision and reliability to allow its widespread introduction in clinical laboratories. The second impediment is that the introduction of new tests has become subject to the authority of consensus conferences, a new approach to medical decision-making.

In response to the above article by McNamara et al., who wrote in response to the editorial by Cianflone and myself, published in Clinical Chemistry last year [1], it appears that the word “standardization” means something different to them than it does to us. Words do matter, and they may be slippery creatures indeed. To illustrate this, let me first list various meanings that another important word, hypercholesterolemia, has taken on and then return to the word under discussion: standardization. In addition, I believe that a much more
important question should be addressed; namely, if the measurement of apo B is as important as we have stated, why is it not in more widespread use? Trying to answer this question may shed light on some of the most important influences on decision-making in modern medicine, influences that have largely escaped common view and criticism, and it is on this issue I will particularly focus.

I am a cardiologist, and coronary artery disease dominates my discipline. The initial clinical study by my colleagues and I involving apo B was published in 1980 [2]. We reported, as did Avogaro et al. before us [3], that many patients with coronary disease had apo B concentrations that were disproportionately increased compared with the serum concentrations of total or LDL-cholesterol. But attention then—and since—as McNamara et al. make clear, has centered almost exclusively on total and LDL-cholesterol, not on apo B. Unfortunately, the fact of the matter is that most patients with coronary disease do not have markedly high cholesterol concentrations. Indeed, for the most part, their concentrations of total and LDL-cholesterol are indistinguishable from those in individuals who do not have coronary disease [4–7]. In my opinion, that is the reason why the “normal” limits for cholesterol have decreased successively, first from the 95th to the 90th percentile [8], thence to the 75th [9], and after that all the way to the 50th percentile in my country, Canada [10]. Some very reputable people say that virtually everyone in Western Society is hypercholesterolemic in comparison with societies in which vascular disease is rare—and, of course, in an important way, they are right. But where does that leave those of us who try to diagnose and treat individuals from Western societies?

By definition, hypercholesterolemia used to be the exception in coronary patients, but by changing the definition, it became the rule—the difficulty being, of course, that as sensitivity increased, specificity plummeted. Changing the definition created two true, but contradictory, realities. The first is that for large groups, risk undoubtedly increases as cholesterol concentrations mount [11]. By contrast, for individuals, because of the enormous overlap of values, unless total or LDL-cholesterol is markedly increased, little prognostic information of value is obtained. The clinical difficulty that this situation creates should have meant that new tests developed to yield additional information as to risk would be greeted with enthusiasm. That was what I thought would be the case with apo B. I was wrong.

Our editorial [1] dealt with the merits of measuring apo B. The above paper by McNamara et al. deals with whether the measurements of plasma triglyceride, HDL-cholesterol, and LDL-cholesterol are standardized. Although I recognize and respect the considerable efforts made by many to improve the accuracy and precision of the measurements of the plasma and lipoprotein lipids, I differ from McNamara et al. on the meaning of “standardization.” For me, as a clinician, it means that the test measures accurately what it purports to measure and that if any laboratory uses any approved manufacturer’s reagents in an approved fashion, that laboratory will get the same answer as any other laboratory that does the same. By that definition, the determinations of both cholesterol and apo B have been “standardized.”

But nothing in life or medicine is that simple. In the case of cholesterol, a Definitive Method exists to measure it; apo B values, on the other hand, are assigned by comparison with a primary preparation whose mass has been determined by amino acid composition. In the hierarchy of analytical precision, the measurement of cholesterol would seem ahead of apo B. Indeed, it is, but not as far as one might initially assume, given that the cholesterol Reference Methods against which the manufacturer’s products are calibrated have a bias when compared with the Definitive Method. That is to say, the everyday “reference methods” for neither cholesterol nor apo B are Definitive Methods. Perhaps another word would help us here: “harmonization,” by which is meant the process that ensures that different manufacturers’ products give the same answers in the everyday world; measurements of both cholesterol and apo B meet that standard. But harmonization has a weak sound to it; standardization has a stronger, more scientific ring to it, even though its definition in the Concise Oxford Dictionary, “obtain by analysis specific value of (solution etc.) for purposes of comparison,” comes very close to harmonization. Whatever word we choose—standardization or harmonization—must not obscure the fact that both cholesterol and apo B can be accurately and precisely measured in clinical laboratories.

However, that is not yet the case for HDL- and LDL-cholesterol and triglyceride. To be sure, primary Reference Methods (not Definitive Methods) for HDL-cholesterol and triglyceride have been developed by the CDC, but they have not yet been fully implemented, even by the CRMLN laboratories. In my view, considering the measurements of HDL-cholesterol and triglyceride as being in the same category as cholesterol and apo B measurements would demand an elasticity in the word “standardization” that the Mad Hatter might approve of, but I do not. Moreover, just how many laboratories in the world are approved by use of this standard of comparison, and how are we clinicians to know which is which as we review their reports? My understanding is that even in North America only a relatively small number of the total are “approved.” And even for those that are, compliance in the US, for example, is certified only at 6-month intervals.

Indeed, it was because the measurements of apoproteins had been truly standardized [12–15] that we felt the studies of Contois et al. [16, 17] were particularly important. Unfortunately, recognition of this achievement is not as widespread as it should be, with the view persisting even amongst some eminent authorities that reliable measurement of apoproteins can still be achieved only in research laboratories. Even McNamara et al., while ac-
knowledging—indeed, even applauding—the standardization of apoprotein measurement at the beginning of their article, seem to question it at the end, evidence of just how deeply old doubts are lodged and how difficult they are to dispel. Meanwhile, while I continue to disagree with McNamara et al. on the issue of standardization, my views are similar to theirs on the too frequent inaccuracies involved in determination of LDL-cholesterol. To overcome this problem, they appear to suggest that direct measurement of LDL-cholesterol should rapidly be adopted. On this score, I believe that although the methods proposed to date are promising, they have not been fully evaluated and, in any case, they are far from being uniformly standardized.

Now let me use this opportunity to address the even more important issue: Why is measurement of apo B not routine? That was the major thrust of our editorial [1]: Measurement of apo B provides critical information, complementary to that obtained from measuring the lipoprotein lipids, information that is essential for accurate clinical diagnosis and the right choice of therapy. The normolipidemic patient may have an increased number of LDL particles but if apo B is not measured, that will be missed. In normolipidemic individuals with an increased apo B, the risk of cardiovascular disease is increased to the same extent as in persons with type II hyperlipoproteinemia [18]. The same is true for the hypertriglyceridemic patient with an increased apo B [18]. Moreover, how can therapy be intelligently selected for the hypertriglyceridemic patient if apo B is not known [19]? Indeed, is any therapy required for hypertriglyceridemic patients if apo B is not increased [19]? Packard and colleagues [20] reported that the commonest dyslipoproteinemia associated with coronary disease is mild hypertriglyceridemia, low HDL-cholesterol, and increased numbers of small, dense LDL particles. Genest et al. showed that familial hyperalphaproteinemia can also be associated with an increased apo B concentration [21]. Surely these diagnostic advantages should not be restricted to research laboratories, now that apo B assays have been standardized.

Assume for a moment that measurement of apo B does add important information to the characterization of vascular risk and the choice of therapy. Why then is it available in so few laboratories? I suggest it is because decision-making in medicine has changed radically in the past few years. There is little difficulty generating a lobby for a test where payment is assured, but it is virtually impossible to obtain support for a test for which payment is not already in place. I am not arguing that anyone can, or should, do tests for nothing. But what criteria are being used to judge whether a test will be paid for and who are applying the criteria?

Much of our decision-making has been turned over to consensus conferences, typically small groups of individuals who consider questions of the moment and then issue guidelines on how these should be managed. The consensus or guidelines are promulgated as the formal views of whatever group chose the members of the panel. The acronym of the meeting becomes the author and the authority of the report, eclipsing the identities of the individuals who actually constructed it. Much good has occurred by this route, but the limitations of the process have evaded scrutiny.

In a word—dare I say it—the process is unstandardized. Indeed, no approach I am aware of has been accepted so broadly with so little analysis of its contents and methods. From 1992 to January 1997, the Medline Database and the Health Star database list 913 consensus conferences, 141 from the NIH alone. As for guidelines, 3286 are indexed as publication types. I have not reviewed each citation, but unquestionably a number will be retrieved both as a guideline and as a consensus statement. As well, some have been published more than once. But even halving the numbers still leaves a remarkable growth industry. Moreover, the conclusions of these conferences can carry enormous consequence, both for the practice of medicine and for the economics of the practice of medicine. In some areas, many millions of lives may be affected and the allocation of many millions of dollars determined by a process not subject to peer review.

We, the readers, are usually told little or nothing about the total base of information considered, how it was analyzed, how often the members met, and for how long. The strength of evidence is often weighted, but how were these decisions reached? In general, no minority reports are presented. What then does the word “consensus” mean? How often is what comes out determined by who went in? How often does the need to achieve “consensus” mean that such gatherings become meetings of the like-minded?

Regarding lipid analyses, I believe it is fair to say that the NCEP guidelines [9, 22] drive the process around the world. Each cycle has begun with an NCEP report, after which other groups and countries respond. The reports differ, in some ways importantly, but overall they are also very similar. With respect to the last NCEP conference [22], I would be the first to point out that, at that time, important information about apo B was missing. Most critically, the measurement of apoproteins had not been standardized; moreover, clear-cut results from a large prospective study favoring apo B determinations were not available. Both of those requirements have now been met [12–15, 23]. But does that mean that nothing will change until this group meets again? And what if they say apo B measurements are not clinically useful? Given the authority the process has acquired, does this mean any change is put on hold indefinitely?

Delay is not neutral. The strategies that lower LDL-cholesterol concentrations lower apo B. The strategies that lower LDL-cholesterol and apo B save lives and reduce the need for bypass surgery and angioplasty [24–27]. Because small, dense LDL particles contain less cholesterol than normal LDL particles do, apo B is a better marker of LDL particle number than is LDL-cholesterol.
Using apo B, therefore, does not change the LDL argument; it merely extends it. Bluntly put, by not measuring apo B, we are not treating a large number of people who could otherwise have been helped.

What about cost? No one can deny the thrust of governments everywhere to control medical costs. Who believes governments’ first priority is now quality of care rather than cost of care? In the US, the situation is even more complex with, for the first time, healthcare being organized into large economic units for which the ultimate goal is profit. Given this reality, how likely is it that new tests and therapies will be introduced as early as possible? In this case, however, without developing the argument here, I believe measurement of apo B will save money as well as lives. As already noted, the key problem with the cholesterol algorithm is that unless values are markedly increased, cholesterol is a poor marker of individual risk. Many patients must be treated when only a few will benefit. Apo B now, and other markers soon [29, 30], will markedly improve our ability to recognize the truly high-risk individuals so that fewer need be better treated and overall cost will be less.

In this Counterpoint, I have ranged far from the narrow issue of standardization that prompted it. I have addressed issues, not individuals, but I am aware of the risks that criticism holds for the critic. Misinterpretation of motive can divert attention from the message to the messenger. Even so, I must express concern about the inherent risks in what seems to me to be a powerful, unseen, and dangerous trend to excessive concentration of decision-making in modern medicine. I am also concerned about the dangers of excessive commercialization of the practice of medicine. I am concerned that we are at risk of losing sight of who we are and why we do what we do. If that occurs, we will lose the essence of what we are, a profession committed to the best care of each life entrusted to us.

References


