Winter 2006 Volume XX, No. 1

The Fats of Life

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Dan Hoefner (hoefned@LabCorp.com) produced this issue of *The Fats of Life*.

This, the Winter 2006 issue of FATS, represents six full years posted on the aacc.org website. Reflecting on the changes that have occurred in the past six years is both interesting and enlightening. In 1999, the FATS was still being printed in hardcopy and mailed out to members, presenting considerable logistical challenge and expense. We were of course formatting the FATS electronically, but the electronic file for each issue had to be delivered to a printer. Then we had to receive by mail a set of mailing labels covering then current division members. The printed copies were then picked up and with the labels delivered to a mailing service. The mailing service delivered the folded, stapled and stamped hardcopies to the Post Office and each copy made its slow way by truck, train, ship or air to each Division member throughout the world. How quaint! And difficult to believe now that was only six years ago.

And I think back to 1990 when I first began editing FATS, taking over from Nader Rifai who succeeded the first Editor, Don Wiebe. Don, by the way, deserves the credit for the highly appropriate name, FATS OF LIFE. At that time, I received all the papers; we still had not come to the term "content," by snail mail. Communication with contributors was by phone or snail mail. We were not yet using email then and for many of us I believe it is difficult to remember a time when we were not conducting most of our communication over the Internet. We had to hand-deliver the formatted copy, very rudimentary at that time, to a printer who typeset the issue for printing. In these early years, we were limited in the number of articles that could be accommodated, because the production process was so laborious.

In the Internet era now, we communicate with authors electronically. Articles arrive as email attachments, are edited and formatted to the new esthetically pleasing FATS. And, as soon as we are satisfied with the content, the electronic copy is posted directly to the website and immediately available to the membership. The cost of printing and mailing has been eliminated and considerable time saved in making the material available.

The AACC national office has proposed additional enhancements. Our Division Information Officer, Dan Hoefner, should soon be able to post content directly to the aacc.org website rather than going through National Office staffers. And they have promised a search function that will allow efficient searching of article by search terms such as subject and author.

I want to also recognize the tremendous contributions by Dan Hoefner to the publication of the FATS. Dan, after substantially improving the LVDD Division site on the National website, then volunteered to take over organizing and formatting the Newsletter. Dan has made major improvements to the design and format and has been for the past year receiving all the content, checking and formatting and posting the completed FATS to the National site. Most of our Division members are probably unaware of Dan's role not only in driving the improvements, but also in saving thousands of dollars each year in production costs, funds that can now be diverted to other programs such as the Division dinner during the Annual Meeting. And, Dan has also begun helping with content; for example, in this issue he arranged the article on the fatty acid binding protein.

Russ Warnick, Editor
The Fats of Life

As we approach the end of 2005, I would like to wish you all a very Happy New Year. I hope that 2006 will bring you success in all your professional and personal endeavors. It had been a privilege for me having served you as the Chair of the Lipoprotein and Vascular Disease Division for the past two years. Fortunately for me, the LVDD has a talented management team and thanks to the help of both the current management team and the past Chairs, I had the opportunity to learn while performing my job as the Chair of our Division.

I would like to thank all of you who helped me in the last two years, and thank all of you who came to the division dinner meetings, management meetings, and other activities. I look forward to serving the Division as Past Chair and continue to contribute to our division where and when possible.

The LVDD will continue the tradition of our successful annual dinner meeting during the 2006 AACC annual meeting. Currently, we are actively planning for this important activity of the Division.

Beginning in January, Joe McConnell will take over as the new Chair and Gyorgy (George) Csako as the chair-elect. Having worked with both of them, I know the division will be in good hands. I hope to see all of you again in our division's dinner meeting this coming August! Please feel free to contact me or Joe McConnell if would like to make any comments or suggestions about the LVDD or are interested in serving as an officer for the LVDD in future years.

Regards,

Michael Y. Tsai
Past Chair, Lipoproteins and Vascular Diseases Division, AACC

Urinary L-FABP: A Novel Biomarker for Renal Disease and Its Role in the Diagnosis and Prognosis of Chronic and Acute Kidney Disease

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Chronic kidney disease affects 20 million adults in the US, and another 20-40 million are at risk for developing the disease during their lifetime. The mainstays of screening and testing for renal disease are serum creatinine and urinary protein tests. While the standards of care today, these tests have a number of shortcomings. Neither test can accurately diagnose the type of renal injury present. Serum creatinine is a poor marker of early kidney disease. Also, both are indirect measures of glomerular filtration using distantly produced substances, rather than a reflection of the state of the renal tissue. (1) What is needed are new biomarkers produced in the kidney in response to disease or other chronic or acute assault. In this paper, we describe a novel renal protein biomarker that has undergone recent extensive clinical testing and validation in Japan, but has yet to make news in the US and Europe.

L-FABP And The Tubular Response To Injury

What are FFAs?

Fatty acids are a source of energy, membrane lipids, and a number of lipid mediators in all living cells. In the circulation, FFAs are bound to protein, such as albumin (2), filtered through the glomeruli, and then reabsorbed with albumin into the proximal tubules. (3) In the cell, these FFAs are subject to oxidation, yielding fatty acid peroxides that are toxic to the cell.

What are FABPs?

Fatty acid binding proteins (FABPs) are a group of intracellular proteins of 14 kDa, belonging to the lipocalin family. FABPs play a key role in the binding and trafficking of fatty acids and some other hydrophobic molecules, as shown in Figure 1. FABPs help transport FFAs to organelles such as mitochondria and peroxisomes for oxidation. It has also been suggested that FABPs exert an influence on the activity of peroxisome proliferator-activated receptor (PPAR) by transporting ligands to the nucleus, thereby influencing transcriptional regula-tion of genes involved in the proliferation and differentiation of cells, and the metabolism of lipids and carbohydrates. (4)

Where is L-FABP produced?

Several isoforms of FABPs have been identified including liver, heart, adipocyte, and intestinal types. (5,6) In the human kidney, two types of FABP have been identified: liver type FABP (L-FABP) that is expressed in the proximal tubule, and heart type FABP (H-FABP), produced in the distal tubule. A number of studies have shown that FABPs are associated with tissue ischemia and damage in their organ of production. For example,

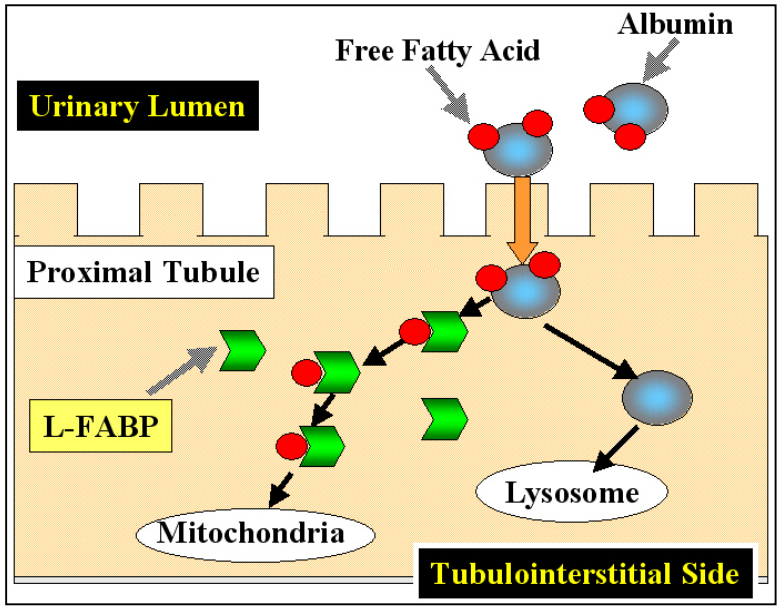

Figure 1. Physiological Role of L-FABP. Free fatty acids (FFAs) are bound to serum albumin, filtered through glomeruli and reabsorbed into the proximal tubule along with albumin. FFAs cause up-regulation of L-FABP gene expression. L-FABP, a carrier protein of 14.4 kDa expressed in the proximal tubule, plays a role in the intracellular transport of FFAs to mitochondria and/or peroxisomes for metabolism.

I-FABP, produced in the small intestine, has been reported to increase in the circulation during acute arterial thrombosis. (7) During the acute phase of acute myocardial infarction, H-FABP, the FABP type produced in the heart, is released from damaged ischemic cardiac muscle and appears in the blood. (8) Similarly, L-FABP expression and secretion into urine is increased during tubulointerstitial damage in the kidney. Kamijo (9) used transgenic mice containing the human L-FABP (hL-FABP) gene to study the effects of FFAs on hL-FABP expression. They found that hL-FABP expression in the transgenic mouse kidney was more upregulated, and urinary hL-FABP excretion increased to a greater extent, upon injection of FFA replete BSA, versus BSA alone. (9)

How is L-FABP related to kidney disease?

Tubulointerstitial injury has been implicated in playing a key role in chronic kidney disease (CKD) progression. (10-17) Urinary protein excretion is generally accepted as the major aggravator of tubulointerstitial damage and the best independent predictor of progression to end-stage renal disease. (10,11, 18-20) However, in massive proteinuria, FFAs are also overloaded to the proximal tubule. These induce inflammatory factors, including macrophage chemotactic factors, which in turn cause tubulointerstitial damage. (21)

A number of studies in kidney proximal tubular cells in animals or in culture have shown that increased uptake of fatty acids causes apoptosis and tubulointerstitial cell damage. (22-25) In multiple experimental proteinuria models, studies have shown that FFAs bound to albumin, rather than the albumin protein alone, have a predominant role in tubulointerstitial inflammation and progression of kidney disease. For example, Kamijo and collaborators showed that bovine serum albumin (BSA) loaded with FFAs injected intraperitoneally into mice caused severe tubulointerstitial damage as compared to FFA-depleted BSA, which caused only minor damage. (22) Thus, L-FABP is an endogenous marker of kidney health, whose synthesis and secretion into the urine increases with assaults to the tubulointerstitial cells, such as proteinuria and ischemia.

Rationale for the diagnostic use of L-FABP

Figure 2 shows a proposed mechanism for L-FABP's relationship to tubulointerstitial damage. FFAs that are overloaded in tubulointerstitial cells during massive proteinuria, are oxidized in the mitochondria and lysosomes, thus overloading the cells with lipid hydroperoxides. These molecules with reactive oxygen species damage the cell membranes. L-FABP binds these oxidized molecules and escapes through damaged cell membranes into the urinary lumen. Increased damage and assault leads to increased synthesis of L-FABP in the cell, and increased excretion of L-FABP into the urine.

Traditional tests of kidney function, using proteinuria, albuminuria, and creatinine clearance, are all based on the distant production of an endogenous marker and its subsequent filtration, secretion, and/or resorption by the kidney. Newer tests for substances such as alpha 1 microglobulin, N-GAL, and cystatin C are also based on a similar concept. In all of these conventional tests, multiple variables can produce different results among different

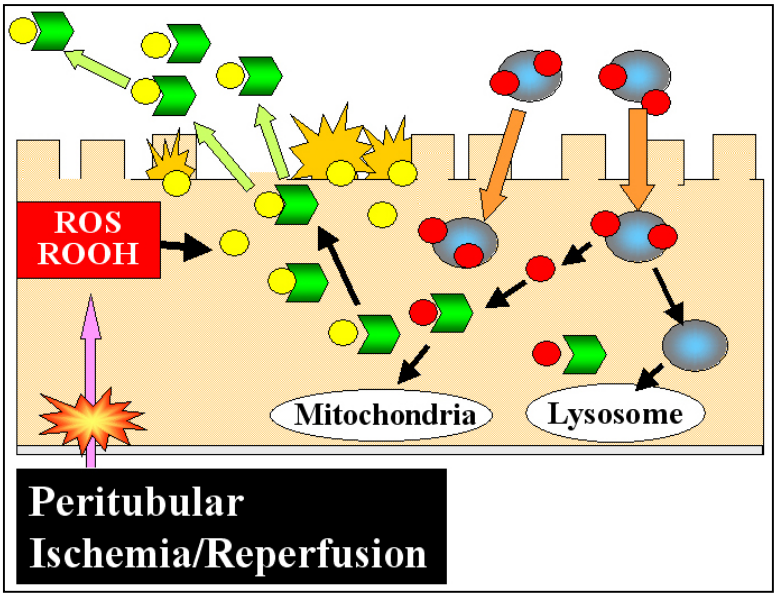

Figure 2. Rationale for Diagnostic Use of L-FABP. Lipid hydroperoxides (i.e. ROOH/ROS) are accumulated in proximal tubules during renal ischemia/reperfusion and other assaults to proximal tubules. L-FABP is excreted from the proximal tubules into urine by binding these cytotoxic lipids, and escaping through damaged cell membranes. The greater the assault, the greater the synthesis and secretion of L-FABP.

patients, including sex, age, muscle mass, etc. Tests using measurement of infused tracers eliminate the analyte production variation among individuals, but these tests are costly and cumbersome. All of these tests have estimated kidney function, estimated indirectly by how these substances travel through the kidney, as a goal. Furthermore, by the time function is impaired, disease may have been progressing for some time. Finally, all of the tests mentioned above mainly measure glomerular injury, even though many believe that tubulointerstitial changes correlate better with renal function. (22,26)

Many laboratories have been searching for new, endogenous biomarkers for kidney health to complement current tests of kidney function. The goal of these efforts is to gain a window on the health of the organ itself by measuring a protein produced in the kidney, that reflects the organ's relative health and/or injury, as for example, troponin C is used as an indicator of injury to heart muscle. (1) We believe that L-FABP is a strong candidate to become a new and sensitive endogenous biomarker of kidney health and disease, due its synthesis and secretion by the kidney during disease and other assaults. L-FABP will not likely replace conventional functional testing, but will complement it. We believe the urinary L-FABP assay provides physicians with a more sensitive, precise, and earlier measure for diagnosis and prognosis, than possible with current methods.

Principle of measurement

The procedure described here is a 2-step sandwich enzyme-linked immunosorbent assay (ELISA). L-FABP standard or urine samples are first treated with pretreatment solution, and transferred into an anti-L-FABP antibody immobilized plate containing assay diluent and incubated. During this incubation, L-FABP in the reaction solution binds to the immobilized antibody. After washing, the conjugate reagent is added as secondary antibody and incubated, thereby forming a sandwich of the L-FABP antigen between the immobilized antibody and conjugate antibody. After incubation, the plate is washed and a substrate for enzyme reaction is added. The color then develops according to the L-FABP antigen quantity present. The optical density is measured using a microplate reader and a calibration curve is prepared based on the obtained optical density, thereby determining the L-FABP concentration. (27)

CLINICAL RESULTS TO DATE

To date, urinary L-FABP testing has undergone recent extensive clinical research and evaluation, mainly in Japan, where this assay was developed. In 2005, eleven articles about L-FABP testing were accepted for publication in international peer-reviewed journals. The following reviews the highlights of clinical evaluation and use to date, as summarized in Table 1.

Establishing Normal Values

To establish normal values, Ishimitsu and colleagues measured L-FABP in the first morning urine of 908 people undergoing annual physical examinations. (28) Among 150 healthy subjects, urinary L-FABP was found to average 3.6 ± 0.2 mg/g creatinine. L-FABP values were significantly higher in patients with hypertension, diabetes mellitus, and chronic hepatitis. In regression analysis, L-FABP correlated better with α1-microglobulin than albumin, supporting the notion that L-FABP is a marker of renal tubular injury, rather than of glomerular leakage.

Chronic Kidney Disease

Type 2 diabetes

A number of investigators have looked at urinary L-FABP in type 2 diabetes, with the following results. Suzuki et al. (29) examined L-FABP levels in 356 patients with type 2 diabetes with a mean age of 63 years. Among them, 216 patients were normalbuminuric, and 64, 46, and 30 were classified as microalbuminuric, having clinical albuminuria, or having renal failure, respectively. L-FABP levels were found to be significantly associated with the stage of diabetic nephropathy. (29)

Nakamura and colleagues measured urinary L-FABP in 58 type 2 diabetics and compared the

Table 1. Summary of Recent Clinical Studies Examining the Utility of Urinary L-FABP Testing

Disease Type	Population	n*	Key Outcomes	Ref.
Normal	Annual physical exam subjects	908	• Average urinary L-FABP concentration in normal patients is 3.6 ± 0.2 μg/g creatinine • Urinary L-FABP was significantly higher in patients with hypertension, diabetes, and chronic hepatitis	28
Chronic kidney disease	Type 2 diabetes	356	• Urinary L-FABP increases significantly with advancing stages of diabetic nephropathy	29
	Type 2 diabetes	78	• Urinary L-FABP increases significantly with advancing stages of diabetic nephropathy • Pitavastatin treatment decreases urinary L-FABP	27
	Type 2 diabetes	108	• Pioglitazone treatment decreases urinary L-FABP	30
	Chronic glomerular disease	120	• Urinary L-FABP was significantly correlated with urinary protein, alpha-1 microglobulin, and serum creatinine • Urinary L-FABP was a better predictor of disease progression than urinary protein alone	31
	Chronic glomerular disease	48	• Urinary L-FABP can level used to identify in advance those patients whose renal disease progressed • Urinary L-FABP was a better predictor of disease progression than urinary protein alone	4
	Autosomal dominant polycystic kidney disease (ADPKD)	40	• Urinary L-FABP was significantly higher in patients with ADPKD versus normal volunteers • Candesartan treatment significantly reduced urinary L-FABP at 3 and 6 months versus placebo	33
	Focal glomerulosclerosis (FGS) and minor glomerular abnormality	41	• Urinary L-FABP was significantly higher in FGS patients, but not higher in those with minor glomerular abnormalities • Among FGS patients, urinary L-FABP was significantly higher in drug resistant patients versus drug responders	34
	IgA nephropathy	50	• Urinary L-FABP was significantly higher in patients with IgA nephropathy versus normal volunteers	35
Acute kidney disease	Contrast medium induced nephropathy (CMIN)	66	• Prior to angiography, urinary L-FABP was significantly higher in patients who eventually developed CMIN versus those patients who did not	36
	Septic shock	90	• Prior to treatment, urinary L-FABP was significantly higher in treatment non-survivors versus both survivors and healthy subjects • Among all treated patients, drug treatment significantly reduced urinary L-FABP in survivors, whereas it had little effect on urinary L-FABP in non-survivors	40
	Living donor kidney transplant recipients	12	• L-FABP levels in first urine from newly transplanted kidneys as correlated with measured peritubular blood flow and ischemic time of the organ during transplant	41
	Pre-term neonates	40	• Urinary L-FABP was approximately ten-fold higher in pre-term neonates versus normal adults and was: o Negatively correlated with gestational age and birth weight o Positively correlated with NAG and 8-OhdG levels	42

*Total subjects, including normal volunteers

results to 20 healthy age-matched volunteers (group E). They divided the 58 diabetics into four groups, as follows: 12 patients without nephropathy (group A); 20 patients with microalbuminuria (group B); 14 patients with macroalbuminuria and normal renal functions (group C); and 12 patients with chronic renal failure, but not yet undergoing hemodialysis (group D). Urinary L-FABP was found to be 6.2, 19.6, 26.8, and 52.4 mg/g creatinine in groups A-D, respectively. Twenty randomly selected group B patients then received 1 mg/day pitavastatin (group B1) or placebo (group B2) for 12 months. Group B1 showed a significant decrease in both urinary L-FABP and urinary albumin excretion (UAE) at 6 and 12 months, whereas there was little change in either parameter in group B2. (27)

Nakamura and colleagues also looked at the effect of pioglitazone on urinary L-FABP and UAE in 68 type 2 diabetics with microalbuminuria, and 40 normal volunteers. Pioglitazone, a PPARγ agonist, reduced both urinary albumin excretion and urinary L-FABP after 6 and 12 months of treatment, whereas glibenclamide, voglibose, or nateglinide had no effect on either parameter. The authors concluded that pioglitazone ameliorates both glomerular and tubulointerstitial damage in type 2 diabetics due to the reduction in UAE and urinary L-FABP, respectively. (30)

Chronic glomerular disease

Studies in non-diabetics have shown similar positive results for urinary L-FABP as both a diagnostic and prognostic marker of chronic renal disease. Kamijo and collaborators studied 120 patients with non-diabetic glomerular disease for between 15 and 51 months. A number of parameters were measured at baseline, and after the study period. Using stepwise regression with urinary L-FABP as a dependent variable, it was found to be correlated with urinary protein, urinary α1-microglobulin, and serum creatinine.

The patients were divided into 3 groups: mild proteinuria (urinary protein less than 1 g/g creatinine), moderate (1 to 3 g/g creatinine), and heavy proteinuria (>3 g/g creatinine). Urinary L-FABP was significantly different between all three groups. The mild proteinuria group was then divided into two groups: those whose renal disease progressed, and those who did not. Urinary L-FABP, serum creatinine, urinary protein, and mean blood pressure were all higher at the start of follow up in the progressors, but only urinary L-FABP was significantly different. Thus, the authors suggest that while urinary protein is widely used to predict the risk of renal disease progression and the risk of dialysis, urinary L-FABP may in fact be a better prognostic marker. (31)

In another study, Kamijo and colleagues studied 48 patients with non-diabetic chronic kidney disease for one year. They measured several clinical markers, including urinary L-FABP and urinary protein, every 1–2 months. At the end of one year, they divided the group retrospectively into progressors and nonprogressors, using change in creatinine clearance. Initial creatinine clearance was the same in both groups, but urinary L-FABP was significantly higher initially in the group that progressed versus the group that did not (111.5 vs. 53 mg/g creatinine, $P<0.001$). Urinary L-FABP was more sensitive than urinary protein in predicting the progression of CKD. However, urinary protein showed greater specificity than did urinary L-FABP. Over time, the progression of CKD tended to correlate with changes in urinary L-FABP, but not urinary protein. (4) In a later report of the same study, the authors showed that serum L-FABP was not correlated with urinary L-FABP. (32)

Polycystic kidney disease

Nakamura and colleagues studied L-FABP in 20 patients with autosomal dominant polycystic kidney disease (ADPKD) and 20 age-matched health volunteers. Urinary L-FABP was found to be significantly higher in ADPKD patients. The ADPKD patients were randomly divided into a treatment group receiving candesartan cilexetil (an angiotensin II receptor antagonist) and a placebo group. Candesartan significantly reduced the urinary L-FABP in the treatment group at 3 and 6 months. However, serum creatinine, BUN, 24-hour creatinine clearance, and blood pressure showed little change. The authors proposed that

elevated urinary L-FABP is associated with ADPKD, and that candesartan mitigates this effect. (33)

Focal glomerulosclerosis
Nakamura and colleagues also studied L-FABP in 17 patients with focal glomerulosclerosis (FGS) and 24 patients with minor glomerular abnormalities. They found that urinary L-FABP was significantly elevated in the group with FGS, but not in those with minor glomerular abnormalities. Furthermore, urinary L-FABP was significantly higher in a group of 8 drug-resistant FGS patients versus those who responded to drug treatment. Thus, L-FABP may be used as an aid to differentially diagnose patients with FGS, and as a prognostic marker for the likely success of drug treatment in those patients. The drug resistant group was later treated with a course of LDL apheresis, and urinary L-FABP re-measured. L-FABP was found to be significantly lower after LDL apheresis, perhaps due to the treatment ameliorating the tubulointerstitial lesions associated with FGS. (34)

IgA Nephropathy
Nakamura et al. studied L-FABP retrospectively in 30 patients with microscopic hematuria without proteinuria, and 20 age-matched normal volunteers. All patients had undergone renal biopsy for differential diagnosis between IgA nephropathy and thin basement membrane nephropathy. Urinary L-FABP was significantly higher in the patients diagnosed with IgA nephropathy when compared to both normal volunteers and those diagnosed with thin basement membrane nephropathy. The authors suggested that L-FABP may be a useful noninvasive tool for differential diagnosis in these patients. (35)

Acute Kidney Disease
In addition to chronic kidney disease, a number of researchers have looked at urinary L-FABP in acute renal disease.

Contrast medium-induced nephropathy
Patients undergoing imaging studies using contrast agents are at significant risk for acute renal failure (ARF). Nakamura and colleagues studied urinary L-FABP in 66 patients undergoing non-emergency coronary angiography or imaging using a non-ionic, low-osmolar type media (i.e., low risk for contrast nephropathy). All patients had their urinary L-FABP measured prior to the administration of the agent. Thirteen of 66 patients had significantly high L-FABP, and all 13 ended up with contrast medium-induced nephropathy. L-FABP remained significantly elevated at 24 and 48 hr, and for 14 days after, even when serum creatinine had normalized. The authors suggested that urinary L-FABP could be a valuable predictive marker for contrast medium-induced nephropathy. (36)

Septic shock
In Japan, patients with severe sepsis may be treated with hemoperfusion through a polymyxin B-immobilized (PMX-F) adsorbent column. Numerous reports show this to be safe and effective. (37-39) Nakamura and colleagues measured urinary L-FABP in 40 patients with septic shock, 20 ARF patients without septic shock, and 30 healthy volunteers. Of the 40 treated with PMX-F, plasma endotoxin levels were significantly reduced, 28 survived, and 12 died. Average pre-treatment urinary L-FABP levels were significantly greater in the non-survivors (2880 mg/g creatinine) versus survivors (1420 mg/g creatinine), or the healthy subjects (4.2 mg/g creatinine).

Urinary L-FABP levels in ARF patients without septic shock (120 mg/g creatinine) were significantly lower than those in both surviving and non-surviving septic shock patients. Among the survivors, PMX-F treatment significantly reduced urinary L-FABP levels from 1420 to 240 mg/g creatinine. However, PMX-F treatment had little effect on urinary L-FABP in the non-survivors (2880 vs. 2460 mg/g creatinine). The authors suggested that urinary L-FABP levels may be used to reflect the clinical prognosis of patients with septic shock, and also to monitoring the effectiveness of treatment. (40)

Living donor transplant
Sugaya and colleagues studied L-FABP in the urine of kidneys after living donor transplant. They

measured peritubular capillary blood flow using an intra-vital CCD imaging system. They also recorded kidney ischemic time, i.e., the time between clamping of the renal artery prior to removal from the living donor to the time urine first drained from the new kidney in the recipient. Urinary L-FABP, NAG, β_2-microglobulin, and α1-microglobulin were all measured in the newly transplanted kidney.

Urinary L-FABP levels were correlated with the inverse of the peritubular capillary blood flow, becoming detectable at blood flow speeds of less than 1mm/second. Urinary L-FABP was also shown to be highly correlated with the total ischemic time of the kidney. These results are consistent with earlier studies in transgenic mice showing that human L-FABP expression and secretion are increased under hypoxia. (41)

Pre-term neonates

Tsukahara and colleagues looked at urinary L-FABP in 40 pre-term neonates born with gestational ages of between 23 and 35 weeks. They found that urinary L-FABP levels were approximately ten times higher than adult values during the 30-day period after birth during which measurement occurred, and did not drop significantly during that period. The levels were negatively correlated with both gestational age at birth and birth weight. Urinary L-FABP was positively correlated with NAG (a marker for proximal tubule cell injury) and 8-OhdG (a marker for systemic oxidative stress) levels. Proximal tubular damage and oxidative stress may have a causative role in the high L-FABP levels observed. (42)

CURRENT STAGE OF DEVELOPMENT

Hewitt and colleagues have described the potential uses for, and sequential development phases of, new kidney biomarkers. (1) The clinical studies described above show that the urinary L-FABP has potential as a biomarker for: early diagnosis and prognosis, progression and regression, intervention, and as a drug surrogate endpoint. These studies also show that data for urinary L-FABP have now been published for the first four of five phases of biomarker development (i.e., *i.* preclinical discovery and confirmation; *ii.* clinical assay development and validation; *iii.* retrospective longitudinal studies; *iv.* prospective screening) and is ready to enter the final phase (i.e., *v.* disease control). The test is currently undergoing additional clinical trials in Japan to augment a planned regulatory filing. The L-FABP assay is now also in the hands of US researchers who are confirming similar utility in US clinical studies. CMIC Co., Ltd. is currently looking for business development partners for the urinary L-FABP test in the US and Europe.

SUMMARY AND CONCLUSIONS

Better and earlier tests for the diagnosis of kidney disease, the prognosis for treatment, and for surrogate markers for therapeutic effect, are all needed in the clinical laboratory. Primary care physicians and nephrologists need better tools to identify patients at risk for kidney disease, make the diagnosis earlier, and then monitor treatment. While current tests are based on estimated renal function, there is an unmet medical need for renal biomarkers that are produced in the kidney and whose level directly reflects kidney health. L-FABP, an intracellular protein involved in fatty acid metabolism, is a clinically useful biomarker easily measured in urine using a sensitive new ELISA. Urinary L-FABP has been shown to correlate with renal disease state, diagnosis, prognosis, and therapeutic effect in a number of studies of both chronic and acute renal disease. The product is in preparation for regulatory filing in Japan, where all published studies have occurred to date, but is now also gaining interest in the US and Europe.

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Therapeutic lifestyle changes remain the cornerstone of therapy in lowering low-density lipoprotein cholesterol (LDL-C) and the management of cardiovascular disease (CVD). Sufficient clinical evidence exists regarding the safety and efficacy of phytosterols and phytostanols, in lowering LDL-C, in primary and secondary prevention. Their incorporation into foods like, margarine, yogurt, or beverages, is well accepted and tolerated. These will be discussed in this brief review.

Cardiovascular diseases are the leading cause of morbidity and mortality in the United States. Several epidemiological, pathological and clinical studies have shown a significant positive correlation between increased levels of plasma lipids, particularly, total cholesterol, and LDL-C, and the incidence of CVD in humans (1). The cholesterol-lowering effects of dietary plant sterols (phytosterols) have been studied since the 1950s, and that of plant stanols (phytostanols) was first reported in 1986 (2,3). Since then, phytosterols/stanols have become well-known dietary adjuncts that effectively lower cholesterol without any symptomatic side effects. To this end, the Adult Treatment Panel (ATP III) of the National Cholesterol Education Program (NCEP) recommended the addition of plant sterols/stanols (2g/day) to the diet, as part of the therapeutic lifestyle changes dietary guidelines (4). The US Food and Drug Administration also issued a health claim stating that foods containing plant stanols and stanol esters may reduce the risk of CAD (5).

Plant sterols differ from cholesterol only in the structure of their side chain, whereas saturated sterols, termed stanols, lack the Δ5 double bond in their B-ring (Figure 1). Edible oils, seeds and nuts have a high content of plant sterols, the major ones being sitosterol, campesterol, and stigmasterol. The Western daily diet contains about 100-300 mg plant sterols and 20-50 mg plant stanols. Plant sterols and stanols exert their hypocholesterolemic effects possibly by interfering with the uptake of both dietary and biliary cholesterol from the intestinal tract in humans. In vitro and in vivo studies have shown no difference between phytosterols and phytostanols in reducing cholesterol incorporation into mixed micelles, and have shown that both sterol and stanol esters, have similar cholesterol-lowering effects through micellar competition. Most published studies have reported that phytosterols /stanols exhibit their cholesterol lowering effects at the dose range of 2-3 g/day, and a single study

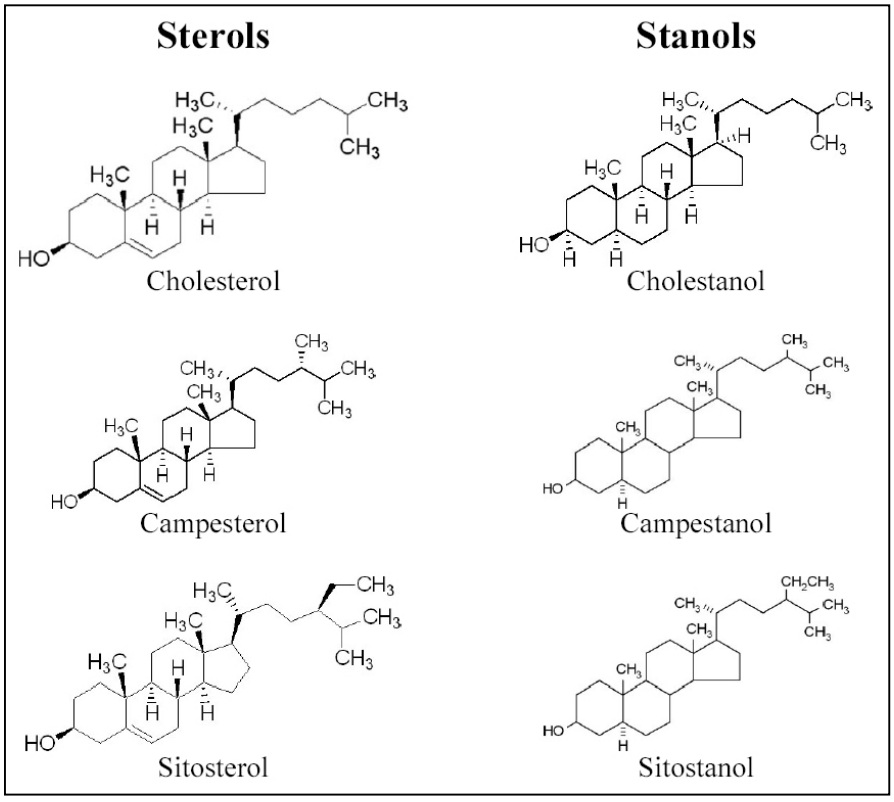

Figure 1. Structure of Plant Sterols and Stanols.

has demonstrated equivalent LDL-C lowering when administered either as single or divided doses on a daily basis (6).

The addition of plant sterols/stanols to the diet, through incorporation in fat-based foods, such as margarine, low-fat milk beverages and yogurt, has been associated with a significant reduction in serum total cholesterol and LDL-C in children, healthy normocholesterolemic and hypercholesterolemic adults, and Type 2 diabetics (7,8,9,10). Katan et al. (2003) performed a meta-analysis of 41 clinical trials, showing that the intake of 2 g/day of stanols or sterols (added to margarine, mayonnaise, olive oil, or butter), reduced LDL-C by 10-15%; intake of foods low in saturated fat and cholesterol and high in stanols or sterols, reduced LDL-C by 20%; and, additive effects were reported (16%-20% additional LDL-C lowering) by combining sterol or stanol intake with statin medication (7). In a randomized, double-blind, crossover study in 26 normocholesterolemic men, Richelle et al. (2005) reported a 60% decrease in cholesterol absorption, following a 1-week supplementation of low-fat milk-based beverage with free sterol or sterol esters (2.2 g sterol equivalents in 600 mL milk/day), compared to the control group (8). Mensink et al. (2002) have previously shown a 13.7% LDL-C lowering using esterified stanols (3 g/day) in low-fat yogurt, in 60 normocholesterolemic adults (9). In the first study examining the efficacy of plant sterols incorporated into non-fat matrices, we showed a significant 7.2% decrease in total, and a 12.4% decrease in LDL-C, in 72 mildly hypercholesterolemic subjects, following an 8-week supplementation of sterol-fortified orange juice (2 g sterol in 480 mL orange juice/day), compared to a placebo consuming sterol-free orange juice. The strategy of supplementing juices/beverages with plant sterols is very attractive, especially because it is also an excellent source of other micronutrients and antioxidants, such as ascorbate (provides the recommended daily allowance), folate, and other flavonoids; in addition to being often consumed at breakfast, it does not provide an additional source of fat, as do other phytosterol products (10). No significant effects of sterol/stanol supplementation on high-density lipoprotein (HDL) cholesterol, and triglycerides levels have been reported (9, 10). As a novel dietary strategy in lowering serum lipids and inflammation, both of which increase the risks for CAD, Jenkins et al. postulated plant sterols (1.0 g/1000 kcal), as an integral component of the "portfolio diet." The combination of phytosterols, almonds, soy protein, and viscous fibers, was shown to be equally effective as statin therapy in reducing LDL-C and C-reactive protein, a biomarker of inflammation, in hyperlipidemic adults (11). However, the active ingredient responsible for the lowering of LDL-C was not identified in this study.

An important issue that has been raised regarding the efficacy and safety of phytosterol/stanol consumption, is the concomitant decrease in plasma levels of fat-soluble vitamins, particularly, tocopherols and carotenoids, as a result of a decrease in their lipoprotein carrier molecules. A meta-analysis of 10 to 15 trials have shown that plasma levels of vitamins A, D and E, including alpha carotene and lycopene, were not affected by stanols or sterols. Beta-carotene levels underwent a decline, but it was not associated with adverse health outcomes (7). In this regard, Noakes et al. have demonstrated that an increase in consumption (≥5 servings) of high-carotenoid fruits or vegetables, like carrots, pumpkins, apricots, spinach, or broccoli, could effectively prevent the decline in plasma carotenoid concentrations accompanying phytosterol/stanol supplementation (12). In our study, with a beverage containing phytosterols (2 g/day), we failed to observe any significant reductions in vitamins E and beta carotene (unpublished data).

Yang et al. (13) showed convincingly that dietary plant sterols disrupt cholesterol homeostasis by affecting the ABC transporters, ABCG5 and ABCG8. Recently, Plat et al. also examined the mechanisms of cholesterol lowering effects of plant sterols and stanols, illustrating two distinct pathways: effects on mixed micellar composition and liver X receptor (LXR) gene activation. The authors demonstrated an increased expression of ATP binding cassette transporters (ABCA1) in

fully differentiated Caco-2 cells, which regulate cellular cholesterol levels, by transporting cholesterol back into the intestinal lumen. The LXR-activating potential of various plant sterols/stanols were positively correlated with ABCA1 mRNA expression (14).

Thus, as outlined in Table 1, plant sterols and stanols have a great potential in cardiovascular risk management, and present evidence is accumulating to promote their use for lowering LDL-C levels, as a first line of therapy as well as adjunctive therapy in patients needing a higher dose of lipid-lowering drug. Further investigations should focus on their incorporation into more commonly consumed low-fat, nutrient-dense foodstuffs, their affordability by the target population, and their effects on biomarkers of oxidative stress and inflammation, other than plasma lipids.

Table 1. Summary of cholesterol-lowering efficacy of plant sterols/stanols

- 2 g/day effectively lowers LDL-cholesterol as primary therapy
- They exert additive effects in combination with low-fat foods and statin therapy
- Are well-accepted as part of daily diet when included in margarine, butter, mayonnaise, yogurt, orange juice
- Are well-tolerated by all groups (children, type 2 diabetics, hypercholesterolemic adults)
- May act through micellar disruption

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Omega-3 fatty acids (FA; eicosapentaenoic and docosahexaenoic acids, EPA+DHA) are believed to be cardioprotective based on experimental, epidemiologic and interventional studies. Low intakes and depressed blood levels have both been associated with increased risk for coronary heart disease (CHD) mortality, especially sudden cardiac death. Typically, no adjustments have been made for serum lipid and lipoprotein levels in these studies despite the fact that supplementation with omega-3 FA is known to lower serum triglycerides (TG) levels (1). Hence, the extent to which a protective effect of increased blood omega-3 FA levels is mediated through effects on serum lipids is not known. The purpose of this study was to determine the relationship between a variety of omega-3 FA biomarkers, especially the red blood cell (RBC) EPA+DHA level [the Omega-3 index (2)], and plasma lipid and lipoprotein levels.

Methods

Studies Included: In this study, we compared blood levels of lipids and lipoproteins with those of omega-3 FA in six separate studies conducted at our center (Table 1). The lipid parameters included were total cholesterol, low density lipoprotein cholesterol (LDL-C), high density lipoprotein cholesterol (HDL-C) and triglycerides (TG). In all cases, samples were from either baseline or placebo periods, and all were fasting. Depending on the study, the omega-3 FA (EPA+DHA) content was measured in one of three different lipid depots: serum phospholipids (PL), RBCs or whole blood.

We examined these relationships in 2 ways: 1) we determined the correlations *within each study* between serum lipids and omega-3 FA (as measured in the depot examined in that study) and then statistically pooled the correlations into a single overall estimate, and 2) we converted all non-RBC omega-3 FA values into an estimated Omega-3 Index and then calculated a single pooled correlation utilizing individual data from all six studies simultaneously.

The latter approach required that the relationship between the Omega-3 Index and both serum PL and whole blood be established. Accordingly, a separate study was conducted in a group of 50 fasting samples drawn randomly from a lipid clinic population. The EPA+DHA proportions were measured in the serum PL, whole blood and in RBCs, and regression equations were derived so that the latter (the Omega-3 Index) could be estimated from either of the former two.

Laboratory Methods. Blood for lipids and lipoproteins analysis was drawn into serum separator tubes. Serum total cholesterol, triglycerides and HDL-C were measured enzymatically in either our research laboratory using a Cobras Fara II (Roche), as described previously (3), or in the hospital clinical laboratory. LDL-C concentrations were determined by the Friedewald equation (4), and if triglycerides were greater than 400 mg/dL, LDL-C was not estimated.

Fatty Acid Analysis. The FA composition of serum PL was measured by combined thin layer chromatography and gas chromatography as previously published (6). The FA composition of whole blood and of RBCs was measured in 50 μL aliquots, which were placed into a test tube and dried under a stream of nitrogen. The dried samples (whether whole blood, RBC or the isolated serum PL fraction) were treated with boron trifluoride (14%) in methanol and incubated at 100°C for 10 minutes. The FA methyl esters thus generated were extracted by addition of equal volumes of water and hexane. After brief shaking and centrifugation to separate layers, the hexane layer was transferred and evaporated under nitrogen. The FA methyl esters were reconstituted in hexane for analysis by gas chromatography (GC;

Shimadzu GC-9 or 14, Shimadzu Corporation, Columbia, MD) using a capillary column (SP-2560, 100 m, 0.25 mm internal diameter, 0.2 μm film thickness from Supelco, Bellefonte, PA). FA were identified by comparison with known standards, and the FA composition was reported as weight percent of total FA present.

Table 1. The studies

Study	n	Patient Characteristics	Source
1 (3)	45	Age 61±13 yrs; BMI 28±4. All patients had one of the three following dyslipidemia patterns using these cut-point definitions: ↑ TG: >150 mg/dL; ↓ HDL-C: <40 mg/dL (men) or <45 mg/dL (women); ↑ LDL-C: >160 mg/dL. *Atherogenic Dyslipidemia* (n=15) ↓ HDL, ↑ TG, and ↔ LDL-C *Isolated Hypercholesterolemia* (n=15) ↔ HDL-C and TG, ↑ LDL-C *Combined Dyslipidemia* (n=15) ↓ HDL-C, ↑ TG and LDL-C	Serum phospholipids
2	57	Ages 44±14 yrs; BMI 25±3. TG 150 – 300 mg/dL	Red Blood Cells (RBC)
3	76	Acute Coronary Syndrome patients. Age 46±5 yrs; BMI 32±6	Whole blood
4	103	Age-, sex-, and race-matched controls for Study 3. Age 47±5; BMI 29±8	Whole blood
5 (5)	120	Healthy Volunteers. Age 48±15 yrs; BMI = 26±5	RBC
6	29	Age 50±13 yrs; BMI 31±3. TG 150-500 mg/dL; HDL-C < 40 (men) and < 50 mg/dL (women)	Serum Phospholipids

Statistical Analysis. As described above, we used two different approaches to evaluate the relationship between omega-3 FA levels and serum lipids. In the first, we computed Pearson correlations for omega-3 FA vs. the TG, HDL-C and LDL-C values in each of the 6 studies using whatever omega-3 metric that was measured in each study. We then pooled the correlation coefficients across studies to estimate the average correlation. Pooled estimates were obtained by first transforming the individual correlations to an approximate normal scale (7), then taking a weighted average with weights equal to the inverse of the variances of the individual estimates (8). The resulting pooled estimate was then back-transformed to the scale of the correlations.

In the other approach, EPA+DHA values in serum phospholipids and in whole blood were first converted into an estimated Omega-3 Index, and then all data from all studies were pooled together and Pearson correlation coefficients calculated for each lipid fraction against the Omega-3 Index.

Results

Data from a total 430 patients were available for this analysis. In order to combine all non-Omega-3 Index values into the same metric for comparison with serum lipid values, the following equations were derived from an analysis of 50 samples:

Omega-3 Index =
Whole blood EPA+DHA x 0.91 + 2.73 (r=0.86)
Omega-3 Index =
Serum PL EPA+DHA x 0.73 + 3.64 (r=0.75)

When pooling Pearson correlation coefficients from the six studies, similar results were observed. Significant correlation coefficients (with 95% confidence intervals) were seen between blood omega-3 FA levels an all three major lipid fractions of interest: TG, r = 0.14 (0.05, 0.23); HDL-C, r =

0.16 (0.06, 0.25); and LDL-C, r = 0.12 (0.03, 0.22). However, as in the other analytical approach, the proportions of variance in these lipid fractions attributable to variations in omega-3 levels were very small, from 1.4% to 2.6% (Table 2).

Discussion

Blood levels of omega-3 have been inversely linked to reduced risk for cardiovascular events (9-11), as have increased intakes of these FA (which would naturally increase blood levels) (12;13). Whether the observed benefits are mediated through changes in serum lipids and lipoproteins or independent of them is not known. This study is the first to examine this question.

We found that in a group of 430 subjects, the proportion of variation in serum lipids that was attributable to variations in omega-3 levels was 10% or less, usually under 3%. Hence, even though some of these relationships were statistically significant (owing in part to the large sample size), they are not likely to be of clinical significance, particularly considering that the relationships with LDL-C and HDL-C were in the opposite direction as one might predict for cardioprotection. In other words, higher omega-3 levels were associated with slightly higher LDL-C and slightly lower HDL-C levels.

Limitations. This study had a number of limitations. First, converting one metric into another based on regression equations (with correlation coefficients of 0.75 and 0.86) necessarily introduces variability into the analysis, which could contribute to the apparent lack of meaningful relationships between blood omega-3 and serum lipids. Second, the 45 serum samples from Study 1 had been frozen (at -75ºC) for up to 4 years, and so some degradation could theoretically have taken place. However, others have demonstrated stability of such samples for up to 12 years (14). Third, about 25% of the patients in studies 3 and 4 were taking lipid-lowering drugs. These would clearly have reduced lipid [(but not omega-3 (15)] levels and perhaps obscured a relationship with blood omega-3 FA. The especially small contribution of omega-3 levels to the variability in LDL-C seen in these studies compared to the others suggests that this may have been the case. Even without these two studies, the relationships with lipids were quite weak, and as noted above, omega-3 levels were positively associated with LDL-C and thus highly unlikely to explain why higher omega-3 levels are beneficial.

Based on these findings, the inverse association between blood omega-3 FA content and risk for CHD cannot be explained by a more favorable lipid profile in subjects with higher omega-3 FA levels. These data support the hypothesis that blood omega-3 FA are an independent marker of risk for CHD mortality.

Table 2. Proportion of Variation in Lipid/Lipoprotein Levels Attributable to Omega-3 Fatty Acids

Study	n	Sample type	TG	HDL	LDL
1	45	Serum Phospholipid FA	6.28%	4.03%	10.54%
2	57	RBC FA	2.79%	4.65%	6.80%
3	103 (99)*	Whole Blood FA	1.79%	2.76%	0.04%
4	83 (76)	Whole Blood FA	4.19%	5.14%	0.03%
5	120 (116)	RBC FA	0.03%	0.15%	1.49%
6	29	Serum Phospholipid FA	7.18%	4.32%	3.49%
Pooled	437 (422)		2.0%	2.6%	1.4%

* Values in parentheses are numbers for LDL which are lower because LDL could not be calculated for samples with TG >400 mg/dL

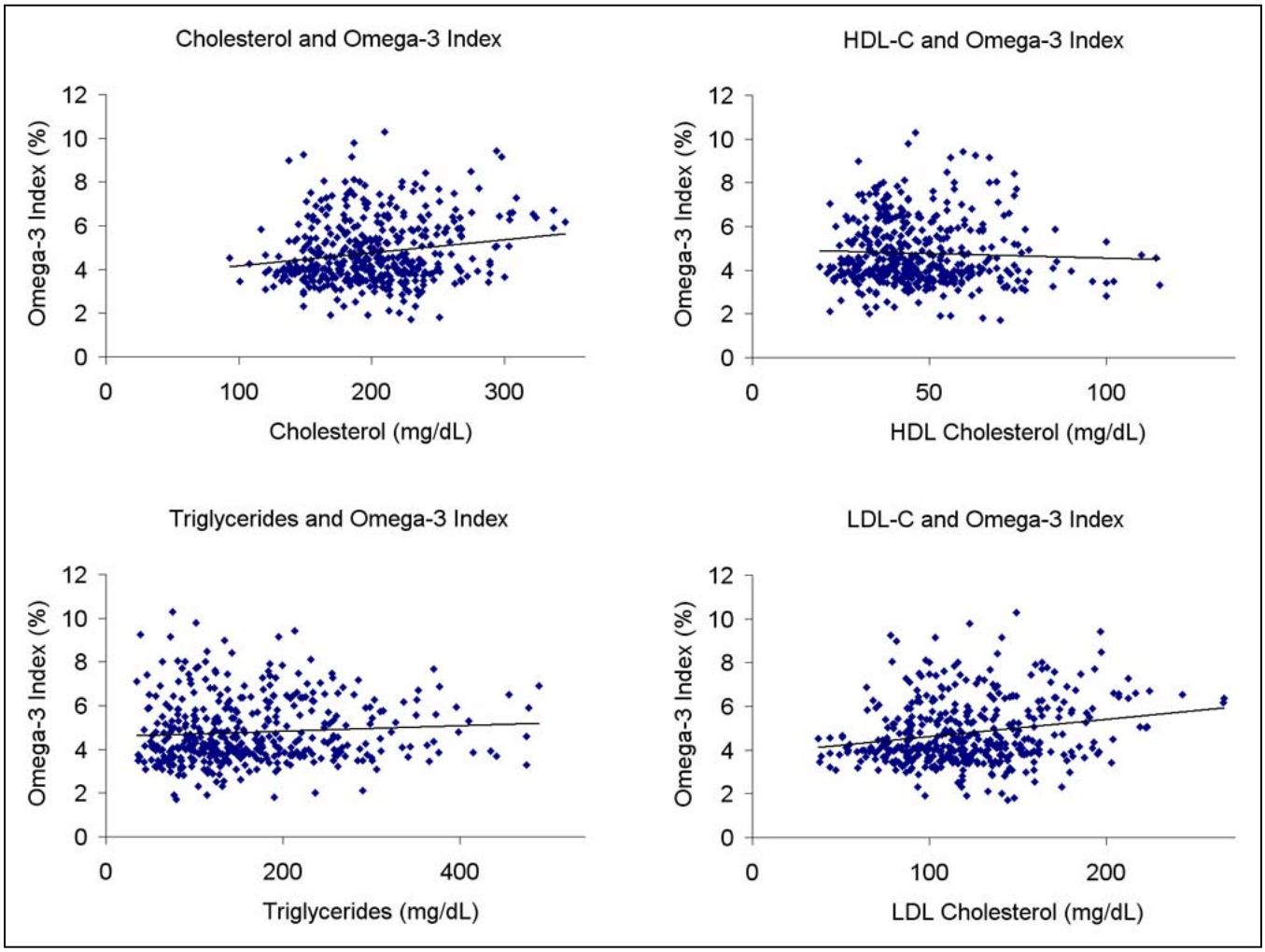

Figure. Relationships between serum lipids and the Omega-3 Index pooling all subjects from all studies (n=437). The Omega-3 Index was measured directly (n=177) or estimated from serum PL EPA+DHA (n=74) and from whole blood EPA+DHA (n=186) and then plotted against serum cholesterol, triglycerides, LDL-C and HDL-C.

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An International Study Examining the "Trueness" and Precision of Lipid Measurements in 27 Countries

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Clinical trials that are international in scope typically rely upon clinical laboratories that reside within the host country for the provision of laboratory tests that are used to monitor safety and, in some cases, to monitor the therapeutic efficacy of the drug under study. Often, the sponsors of such studies will assume that there is little variation in the test results as generated in the different countries allowing them to merge the data at the end of the study without consideration of this potential source of error.

We recently had the opportunity to conduct a pre-assessment of the "quality of lipid testing" as part of a large international lipid lowering drug trial. The pre-assessment study involved 27 clinical laboratories from the same number of countries. The sponsor had selected these laboratories on the basis of their reputation as preferred laboratories for the testing of lipids in each country. The pre-assessment study examined the measurement of total cholesterol (TC), LDL cholesterol (LDL), HDL cholesterol (HDL), and triglycerides (TG) in these countries. The National Cholesterol Education Program (NCEP) total error (TE) performance goals for lipid measurements by clinical laboratories were used for assessing performance and qualifying a laboratory's participation in the trial.

Methodology

Prior to their participation in the study, each of the laboratories provided information on their lipid measurement systems through a detailed registration process. Each of the 27 laboratories was shipped a common set of commutable human serum test samples (n=5). These matrix-insensitive samples had been collected without alteration from normal and dyslipidemic subjects. Samples were pooled to obtain lipid levels that covered the clinical range of interest for the trial.

Each of the participating laboratories was instructed to measure total cholesterol, LDL (calculated), HDL, and TG in each of the samples in triplicate, on each of three days. The Canadian External Quality Assessment Laboratory (CEQAL), which operates a Reference Method laboratory that is a member of the Cholesterol Reference Method Laboratory Network (CRMLN), assigned the lipid target values to these samples. Subsequently, the test results from each laboratory were used to calculate the laboratory's bias, CV (%) and TE (%) for the measurement of the different lipid fractions. The performance of each laboratory was assessed on the basis of %TE for the measurement of TC, LDL, HDL, and TG (9%, 12%, 13% and 15% respectively).

Results

The performance data (precision and total error) for the laboratories from each of the countries are presented in Figures 1 - 4. When ranked on the basis of TE, the laboratory in Peru had the best overall performance (see Figure 5).

Sixty-three percent of the laboratories had one or more of their lipid measurement systems operating outside of the total error budgets as recommended by the NCEP guidelines. In some a single analyte was at fault and in others two or more analytes were involved. The worst performance was seen for HDL cholesterol. The majority of the laboratories that failed the NCEP total error performance goals were able to achieve the performance goals for precision. In most of these cases it was a

lack of trueness (as reflected in % bias) that was responsible for their failure. In a few other cases, the failure was due to both imprecision and inaccuracy. On the basis of these data, 10 laboratories (37%) were deemed to have satisfied the performance criteria for inclusion in the clinical trail, 16 of the laboratories were asked to recalibrate their test systems to the CRMLN-CDC accuracy base, and one laboratory was rejected on the basis of unacceptably high imprecision.

Conclusions

The sponsors of clinical trials assume that a laboratory's accreditation status attests to the accuracy and consistency of the test results that it generates. In general, it is not fully appreciated that test results can and do vary significantly within and between laboratories and that this variation may be of sufficient magnitude to have a significant impact on the costs, outcome and conclusions that may be drawn on the basis of the laboratory data from a clinical trial.

In the present case, a pre-assessment study using fresh human serum samples was used to prequalify clinical laboratories for participation in a clinical trial that had been designed to examine the lipid lowering effect of a new drug. In spite of their reputation and accreditation status, 63% of the labs failed to meet the NCEP performance goals for the measurement of lipids. These failures were mainly due to calibration errors. Identifying and correcting the calibration problems at baseline facilitated the standardization of test results between the participating laboratories and enhanced the overall value of the clinical trial.

The sponsors of clinical trials should employ an auditing system that is independent of the contracted laboratory's quality management process for monitoring the quality of testing that is being provided.

Codes for the 27 laboratories are: 1, Peru; 2, Turkey; 3, Australia; 4, Pakistan; 5, Singapore; 6, United Arab Emirates; 7, S. Africa; 8, Jordan; 9, Panama; 10, Thailand; 11, Chile; 12, Israel; 13, Tunisia; 14, Korea; 15, Lebanon; 16, Philippines; 17, Malaysia; 18, Saudi Arabia; 19, Taiwan; 20, Indonesia; 21, India; 22, Mexico; 23, Hong Kong; 24, Guatemala; 25, Argentina; 26, Morocco; and 27, Kuwait.

Figure 1

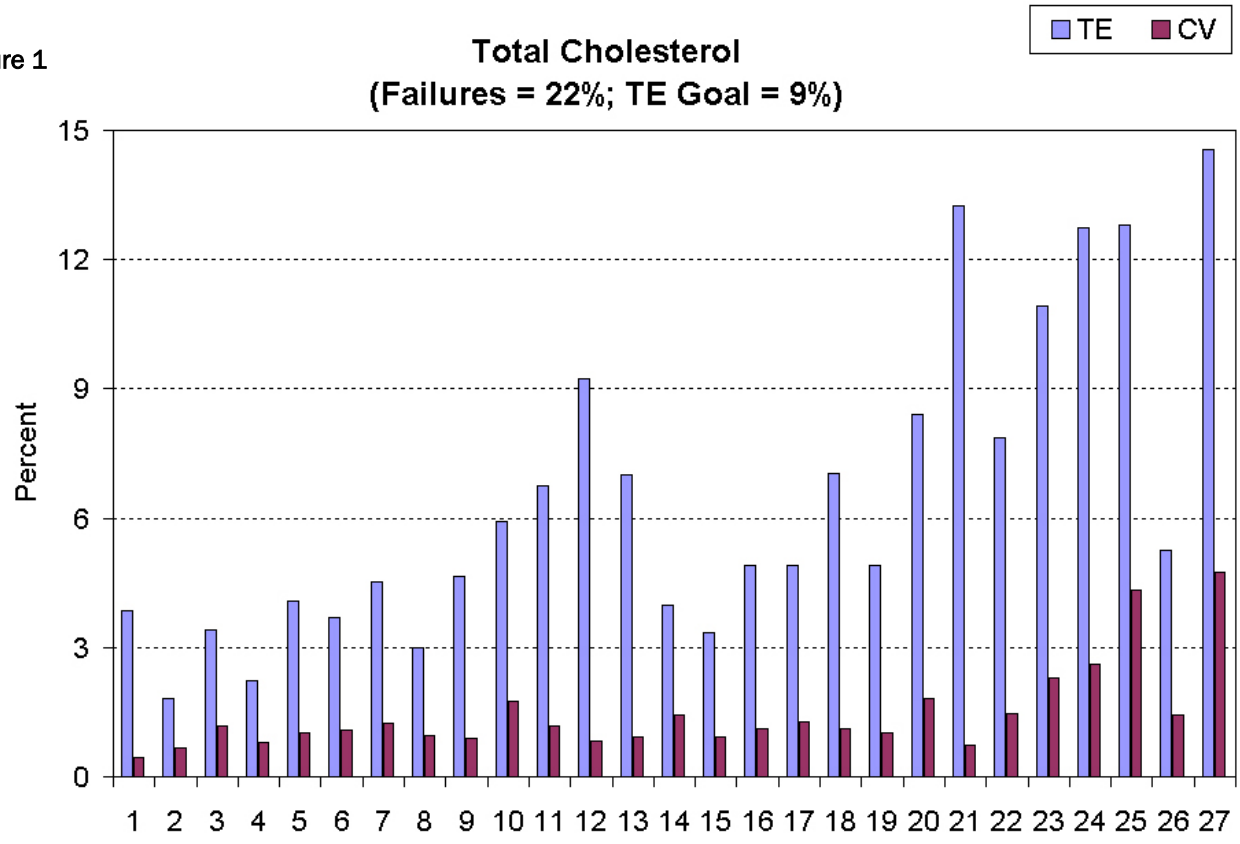


Figure 2

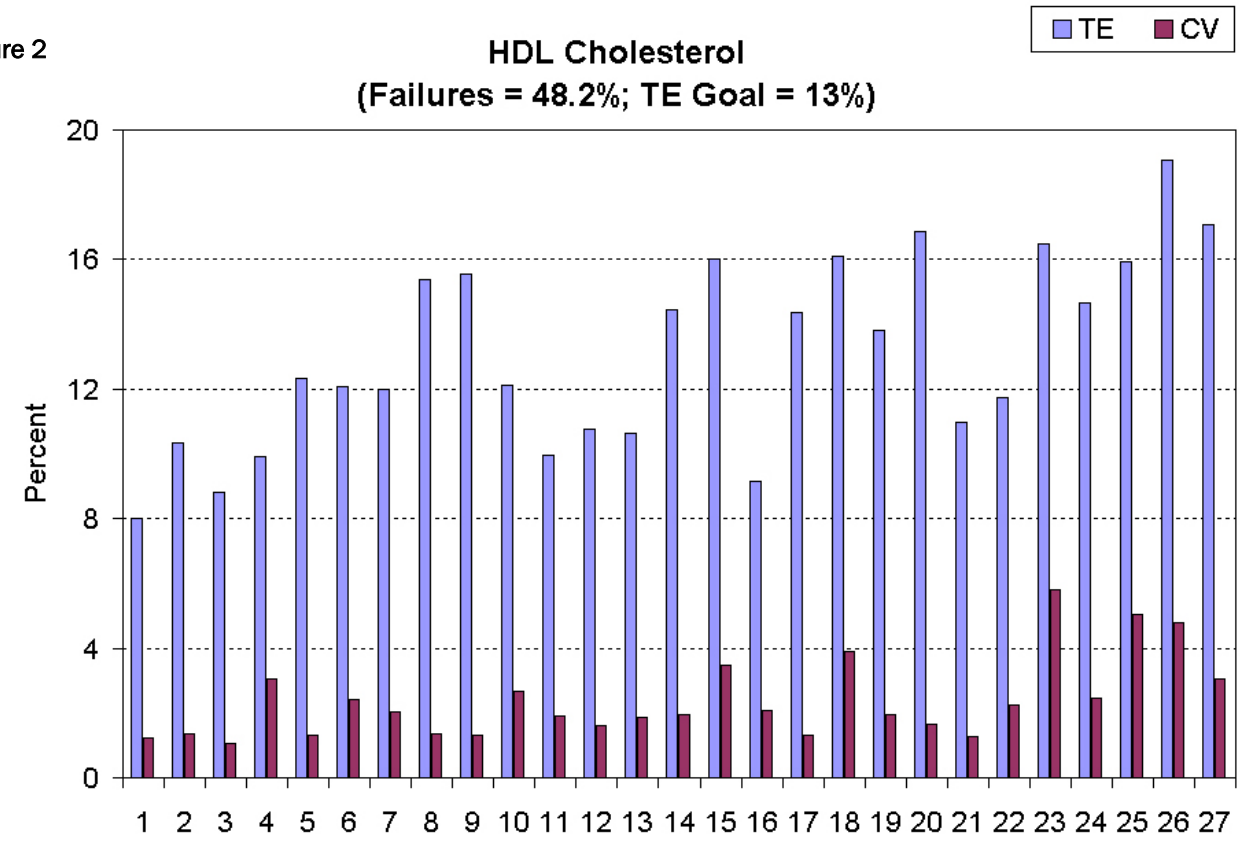


Figure 3

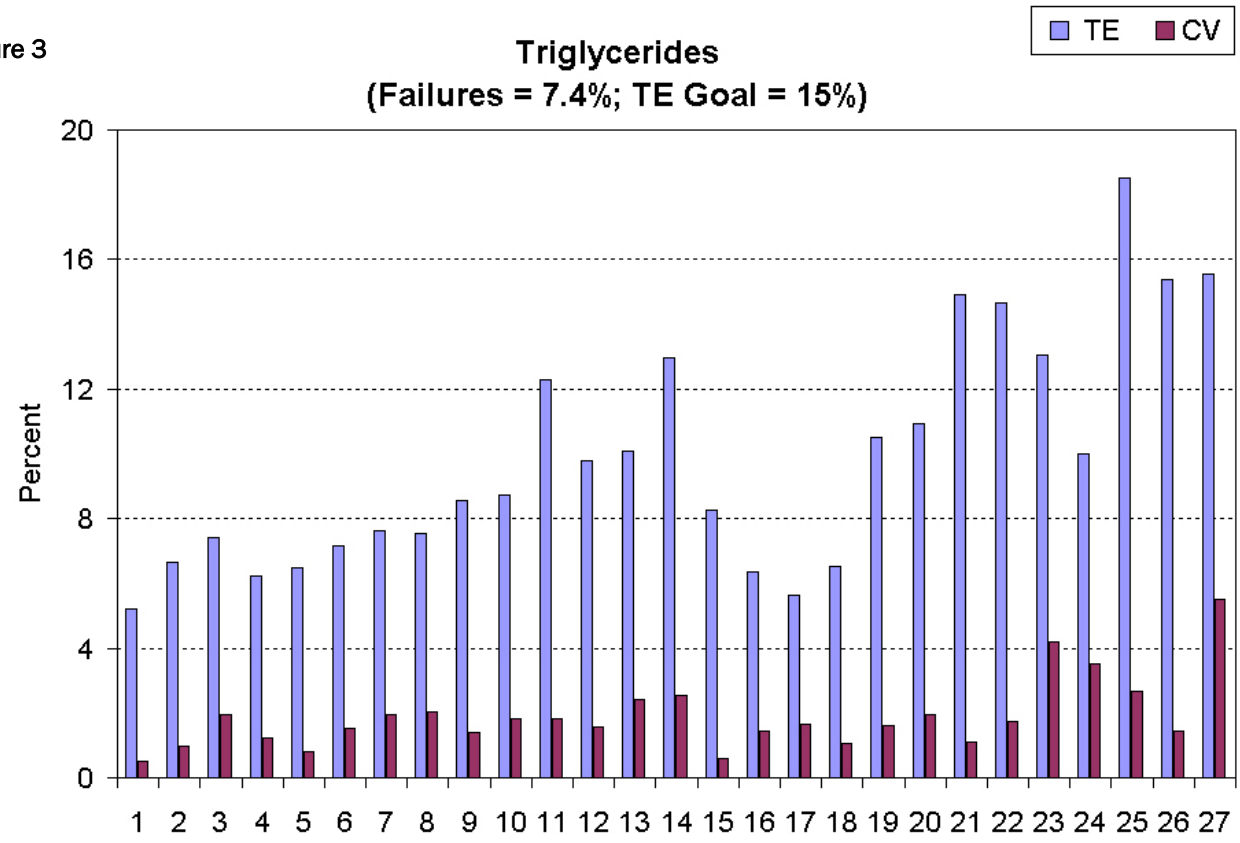


Figure 4

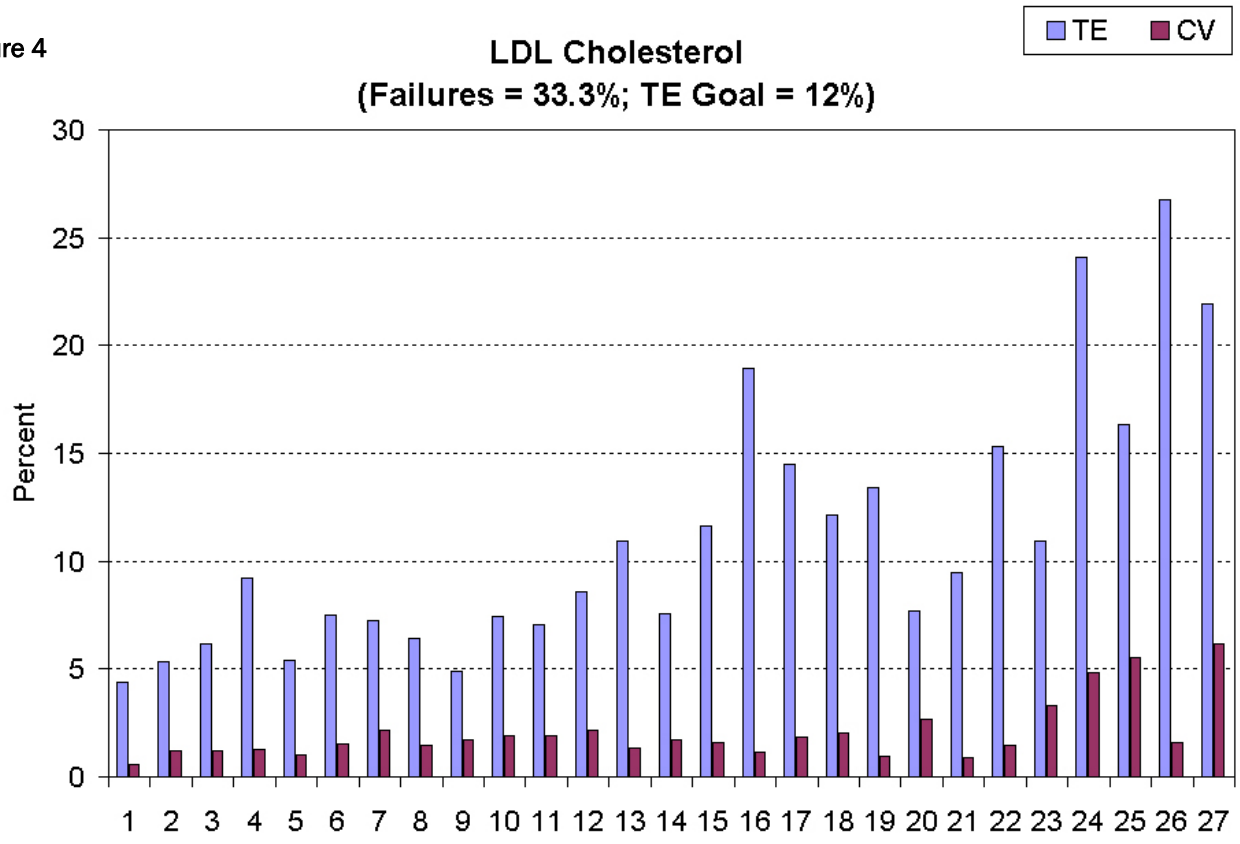
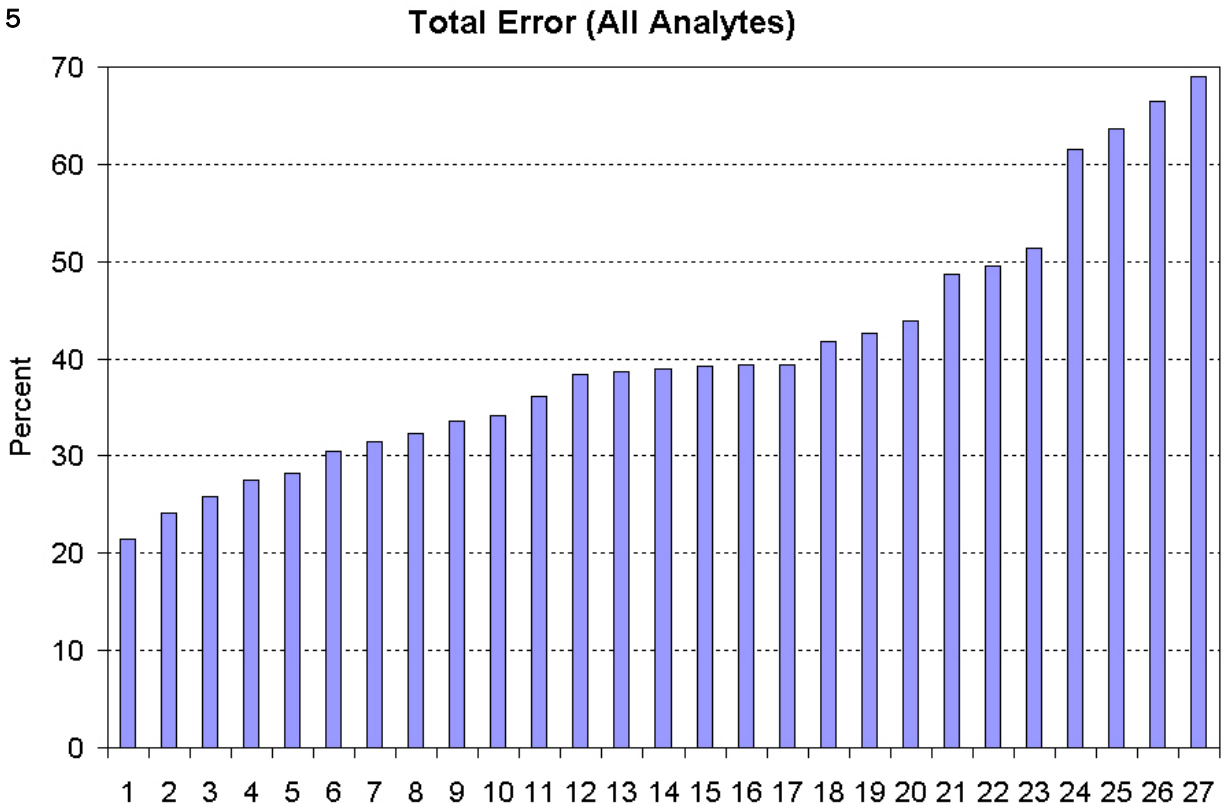


Figure 5



By Gyorgy Csako, M.D.

Title: Aspirin resistance and atherothrombotic disease.
Authors: Mason PJ, Jacobs AK, Freedman JE.
Journal: *J Am Coll Cardiol.* 2005 Sep 20; 46(6): 986-93.
Comments: This is an excellent review on aspirin resistance as it relates to cardiovascular disease prevention. Atherosclerotic plaque rupture or fissuring and subsequent occlusive or subocclusive thrombus formation are major causes of acute coronary syndromes and other cardiovascular diseases which, in turn, are responsible for more than two million hospitalizations and ~30% of all deaths in the U.S. each year. Platelets play a critical role in the pathophysiology of atherothrombosis, and aspirin is by far the most commonly used antiplatelet agent. Several clinical trials have demonstrated the efficacy of aspirin in secondary prevention of myocardial infarction, stroke, and cardiovascular death; and aspirin also may be justifiable for primary prevention. However, the clinical benefits vs. the risk of side effects (primarily bleeding complications) are not always as obvious in primary than secondary prevention. Another challenge is that, despite documented benefits, the absolute risk of recurrent vascular events among patients taking aspirin remains relatively high—estimated to be 8% to 18% after two years. At least part of this failure might be related to therapeutic resistance to aspirin. At the present, there are no formal diagnostic criteria and validated method(s) for the measurement of clinically important aspirin resistance. Nevertheless, aspirin resistance may affect 5% to 45% of the population and, given the prevalence of cardiovascular disease, its potential impact is large. The mechanism of aspirin resistance is uncertain and may include a combination of factors that affect platelet function. For instance, behavioral habits (e.g., non-compliance, tobacco smoking), pharmacodynamic factors (e.g., dose response, duration of therapy and drug interactions), biological factors (e.g., aspirin-insensitive thromboxane A_2 biosynthesis, alternate platelet activation pathways, etc.), and genetic mutations (e.g., COX-1 gene) and/or polymorphisms (e.g., glycoprotein IIb/IIIa receptor [Pl^{A2}]) may contribute to differences in aspirin responsiveness. Apparently, further studies are needed to establish the biological mechanism, diagnosis, population prevalence, clinical relevance, and optimal therapeutic intervention for aspirin resistance.

Title: Prediction of type 2 diabetes mellitus with alternative definitions of the metabolic syndrome: the Insulin Resistance Atherosclerosis Study.
Authors: Hanley AJ, Karter AJ, Williams K, Festa A, D'Agostino RB Jr, Wagenknecht LE, Haffner SM.
Journal: *Circulation.* 2005 Dec 13; 112(24): 3713-21.

and

Title: Critical questions about the metabolic syndrome. (editorial)
Authors: Greenland P.
Journal: *Circulation.* 2005 Dec 13;112(24):3675-6.
Comments: The metabolic syndrome continues to generate a great interest by clinicians, researchers, health-policy agencies and even by the lay literature. This syndrome is highly prevalent and, in addition to predicting cardiovascular disease (CVD) morbidity and mortality, it is strongly associated with the development of type 2 diabetes mellitus (DM), itself an important risk factor for CVD. The authors of this article compared the ability of various metabolic syndrome criteria (including those recently proposed by the International Diabetes Federation), markers of insulin resistance (IR) and inflammation, and impaired glucose tolerance (IGT) in the prediction of DM and to determine whether various proposed modifications to the National Cholesterol Education program (NCEP) metabolic syndrome definition improved predictive ability. They examined 822 subjects in the Insulin Resistance Atherosclerosis Study aged 40 to 69 years who were nondiabetic at baseline. After 5.2 years, 148 individuals had developed DM. IGT, metabolic syndrome definitions, and IR markers all significantly predicted DM, with odds ratios ranging from 3.4 to 5.4 (all $P<0.001$), although there were

no significant differences in the areas under the receiver operator characteristic (AROC) curves between the definitions. Modifying or requiring obesity, glucose, or IR components in NCEP-defined metabolic syndrome did not significantly alter the predictive ability of the definition under AROC curve criteria (all $P>0.05$). Similarly, although IR and inflammation variables were significantly associated with incident DM when included in multivariate models with NCEP-defined metabolic syndrome (all $P<0.01$), expanding the definition by adding these variables as components did not significantly alter the predictive ability of the definition under AROC curve criteria (all $P>0.05$). The authors concluded that, even without requiring the use of oral glucose tolerance testing or measures of IR or microalbuminuria, the International Diabetes Federation and NCEP metabolic syndrome definitions predicted DM at least as well as the World Health Organization definition. Modifications or additions to the NCEP metabolic syndrome definition had limited impact on the prediction of DM.

In the accompanying editorial, Greenland pointed out that, although many articles are being published on the metabolic syndrome, because of the remaining uncertainties, three critical questions require urgent attention if the construct of this syndrome is to continue to be a focus of clinical practice or medical research. The first question is whether consideration of this syndrome, by whichever definition, provides a better understanding of the cause or pathogenesis of atherosclerotic CVD than those currently used. The second question is whether using the metabolic syndrome as a construct improves prediction of future risk of CVD compared with various alternatives. The third critical question is whether identification of the metabolic syndrome improves patient outcome compared with other available ways of identifying and treating CVD risk. While this is a key question to both clinicians and patients, it has not yet received any attention to date.

Title: Metabolomic identification of novel biomarkers of myocardial ischemia.

Authors: Sabatine MS, Liu E, Morrow DA, Heller E, McCarroll R, Wiegand R, Berriz GF, Roth FP, Gerszten RE.

Journal: Circulation. 2005 Dec 20; 112(25):3868-75. Epub 2005 Dec 12.

Comments: Recognition of myocardial ischemia is critical both for the diagnosis of coronary artery disease and the selection and evaluation of therapy. Recent advances in proteomic and metabolic profiling technologies may offer the possibility of identifying novel biomarkers and pathways activated in myocardial ischemia. Nuclear magnetic resonance (NMR) spectroscopy, mass spectrometry, and liquid chromatography appear to be the most promising techniques for assessing a large set of metabolites. The authors of this work report novel application of metabolomics to acute myocardial ischemia by identifying novel biomarkers and distinct clusters of related metabolites of ischemia. For the study, blood samples were collected before and after exercise stress testing from 36 patients. Of these, 18 patients demonstrated inducible ischemia ("cases") and 18 patients did not ("controls"). Plasma was fractionated by liquid chromatography, and metabolite profiling was performed with a high-sensitivity electrospray triple-quadrupole mass spectrometer. Lactic acid and metabolites involved in skeletal muscle AMP catabolism increased after exercise in both cases and controls. In contrast, there was significant discordant regulation of multiple metabolites that either increased or decreased in the cases but remained unchanged in controls. Functional pathway trend analysis with the use of novel software revealed that six members of the citric acid pathway were among the 23 most changed metabolites in the cases (adjusted $P=0.04$). Furthermore, changes in six metabolites, including citric acid, differentiated cases from controls with a high degree of accuracy ($P<0.0001$; cross-validated c-statistic=0.83). In addition to discovering novel metabolomic markers for myocardial ischemia, this study showed the general feasibility of identifying new disease markers with metabolomic approaches.

Title: Oxidized phospholipids, Lp(a) lipoprotein, and coronary artery disease.

Authors: Tsimikas S, Brilakis ES, Miller ER, McConnell JP, Lennon RJ, Kornman KS, Witztum JL, Berger PB.
Journal: *N Engl J Med.* 2005 Jul 7;353(1):46-57.

and

Title: A role for oxidized phospholipids in atherosclerosis.
Authors: Berliner JA, Watson AD.
Journal: *N Engl J Med.* 2005 Jul 7; 353(1):9-11. Erratum in: *N Engl J Med.* 2005 Oct 27; 353(17):1869.
Comments: According to our current understanding, proinflammatory oxidized low-density lipoprotein (LDL) may be the unifying link between lipid accumulation and inflammation in the vessel wall of atherosclerotic coronary arteries. Lipoprotein(a), or Lp(a), is an LDL-like particle containing a unique apolipoprotein, apo(a). Increased plasma levels of Lp(a) have been claimed to predict coronary artery disease (CAD), but the underlying mechanisms by which Lp(a) contributes to the pathogenesis of atherosclerosis are poorly understood. Recent studies showed that proinflammatory oxidized phospholipids are strongly associated with Lp(a) in human plasma. This suggested the presence of oxidized phospholipids on apo B-100-containing lipoproteins such as Lp(a) which may explain, at least in part, the atherogenic properties of Lp(a). In the present study, the authors investigated whether levels of oxidized LDL measured with the use of monoclonal antibody E06 reflect the presence and extent of obstructive CAD, defined as a stenosis of >50% of the luminal diameter. The levels of oxidized LDL and Lp(a) were measured in a total of 504 patients immediately before coronary angiography. Levels of oxidized LDL were reported as the oxidized phospholipid content per particle of apolipoprotein B-100 (oxidized phospholipid:apo B-100 ratio). Measurements of the oxidized phospholipid:apo B-100 ratio and Lp(a) levels were skewed toward lower values, and the values for the oxidized phospholipid:apo B-100 ratio correlated strongly with those for Lp(a) ($r=0.83$, $P<0.001$). In the entire cohort, the oxidized phospholipid:apo B-100 ratio and Lp(a) levels showed a strong and graded association with the presence and extent of CAD (i.e., the number of vessels with a stenosis of >50% of the luminal diameter) ($P<0.001$). Among patients 60 years of age or younger, those in the highest quartiles for the oxidized phospholipid:apo B-100 ratio and Lp(a) levels had odds ratios for CAD of 3.12 ($P<0.001$) and 3.64 ($P<0.001$), respectively, as compared with patients in the lowest quartile. The combined effect of hypercholesterolemia and being in the highest quartiles of the oxidized phospholipid:apo B-100 ratio (odds ratio, 16.8; $P<0.001$) and Lp(a) levels (odds ratio, 14.2; $P<0.001$) significantly increased the probability of coronary artery disease among patients 60 years of age or younger. In the entire study group, the association of the oxidized phospholipid:apo B-100 ratio with obstructive CAD was independent of all clinical and lipid measures except one, Lp(a). However, among patients 60 years of age or younger, the oxidized phospholipid:apo B-100 ratio remained an independent predictor of CAD. Thus, the authors concluded that circulating levels of oxidized LDL are strongly associated with angiographically documented CAD, particularly in patients 60 years of age or younger. Further, the data suggest that the atherogenicity of Lp(a) indeed may be mediated in part by associated proinflammatory oxidized phospholipids.

In an accompanying editorial, Berliner and Watson provide an elegant review for the possible role and pathomechanism of oxidized phospholipids in atherosclerosis. They point out that both *in vitro* and *in vivo* data are consistent with the pathogenicity of oxidized phospholipids in atherosclerosis. Phospholipids are ubiquitous molecules that are important to the structural integrity of cells and lipoprotein particles under physiologic conditions. Upon oxidation, however, they are taken up by scavenger receptors on macrophages, are recognized by the innate immune system, and can promote inflammation. Thus, oxidized phospholipids may not only be diagnostic markers of CAD, they may also represent a potential target for therapeutic intervention.

Title: Twenty-year trends in serum cholesterol, hypercholesterolemia, and cholesterol medication

use: the Minnesota Heart Survey, 1980-1982 to 2000-2002.

Authors: Arnett DK, Jacobs DR Jr, Luepker RV, Blackburn H, Armstrong C, Claas SA.

Journal: *Circulation.* 2005 Dec 20; 112(25):3884-91. Epub 2005 Dec 12.

Comments: Coronary heart disease (CHD) is the single largest cause of death in the U.S., leading to >1 of every 5 deaths in the U.S. In 2002, ~13 million Americans had CHD. In 2005, the estimated direct and indirect costs of CHD were in excess of $142 billion. CHD and its prevention clearly represent important health concerns. Reduction of hypercholesterolemia, a major risk factor for CHD, is a well-established way to reduce CHD. Although cholesterol concentrations in the U.S. have dropped, ~50% of adults still have total cholesterol (TC) concentrations ≥5.18 mmol/L, putting them at "borderline-high risk" for heart disease. Whether the decline has continued into the 21st century was not known. The authors assessed 20-year trends in cholesterol, hypercholesterolemia, lipid-lowering drug use, and cholesterol awareness, treatment, and control from Minnesota Heart Survey (MHS) data. They assessed TC rather than LDL-C because the differential risk levels associated with various lipoprotein cholesterol fractions were not as firmly established when MHS was begun, as they are today, and validated methods for cholesterol fractions were not developed until recently. The authors conducted five independent, cross-sectional, population-based surveys of 2500 to 5000 adults in the Minneapolis-St. Paul, MN area from 1980 to 2002. Mean (nonfasting) TC concentrations have continued a 20-year decline, punctuated by an intervening lull. Age-adjusted mean TC concentrations in 2000 to 2002 were 5.16 and 5.09 mmol/L for men and women, respectively (in 1980 to 1982, 5.49 and 5.38 mmol/L for men and women, respectively). However, the decline has not been uniform across all age groups. Middle-aged to older people have shown substantial decreases, but younger people have shown little overall change and recently had increased TC values. The mean prevalence of hypercholesterolemia in 2000 to 2002 was 54.9% for men and 46.5% for women and has decreased significantly for both during the study. Age-adjusted mean HDL-C concentrations in 2000 to 2002 were 1.09 and 1.40 mmol/L for men and women, respectively, and were not different from the prior survey. Lipid-lowering drug use rose significantly for both sexes aged 35 to 74 years. Awareness, treatment, and control of hypercholesterolemia have increased; however, more than half of those at the NCEP level of borderline-high risk remain unaware of their condition. Although hypercholesterolemia prevalence continued to fall, significant population segments still have TC concentrations near or at the level of increased risk. In summary, this report from the MHS on lipid trends from 1980–1982 to 2000–2002 indicates both positive and troubling data. The downward trends in TC concentrations reported nationally since 1960 are continuing to the present and reflect a decrease in age-adjusted TC levels and hypercholesterolemia prevalence and an increase in lipid-lowering medication. However, a matter of concern is that younger adults (25 to 34 years) have recently shown an increase in TC levels. In addition, more than half of those with hypercholesterolemia remain unaware of their condition. Thus, the study underscores the need for continued vigilance to increase screening as well as lifestyle and pharmacological management of lipid disorders.

We've all heard the line that nothing is free. However, I'd like to tell you about two great pieces of software that I've been using free of charge (and legally!). Dr Bill Sadler (Christchurch, New Zealand) has written some vary useful code known as the *Variance Function Program* (VFP), which estimates the relationship between the mean and the variance of groups of data. The second piece of software is *OpenOffice.org* (a.k.a., *OOo*), a free program that is nearly identical to Microsoft's suite of *Office* programs. In addition to being free, one of the things that makes these two programs unique is that they are not tied to some annoying advertisement, they don't expire, and they don't install with limited capabilities and nagging enticements to try and get you to pay for the "full" version.

Although there are several aspects to the utility of the VFP, the introduction of the accompanying documentation suggests "the single most important use is probably the construction of precision profile plots." If you have the need to determine the points of functional sensitivity for immunoassays such as cardiac troponin (cTnI, cTnT) or TSH, the VFP is all you need. If you are evaluating two vendor's assays for the same analyte, this program offers an objective means to compare the low-end precision. It's also a good way to generate data for feedback to manufacturers. Do it—the more feedback they get, the more they are inclined to listen!

So as not to trigger a block in email systems with size limitations on incoming mail, Dr. Sadler has cleverly packaged the program in such a way that it can be sent in six or seven emails, each with ~1.4 MB attachments. Upon installation, everything gets compiled into the full program; the current version (VFP 6.1) installs in ~11 MB, including 1.7 MB for the PDF documentation. The program is intuitive and easy to use. For those who want to learn more about the statistics and validation supporting this software, the documentation includes a bibliography with references that describe the details of such. If you think you have need of this program, the easiest way to obtain a copy is to simply send a request by email to Bill at bill.sadler@cdhb.govt.nz.

The second piece of software, OOo (version 2.0.1), is a behemoth compared to the VFP. Just to download requires 76 MB and, once installed, the size increases to a little over 200 MB. But wait, don't let the size scare you; with a high-speed connection, it takes less than two minutes to download and the installation only takes another minute and a half. The application is a multiplatform (Windows, Mac, Linux, etc.) and multilingual (over 45 supported languages) office suite that's compatible with other major office programs (e.g., Office, Word Perfect, Lotus), and it's free to download, use, and distribute. Since I own a recent version of Office, I have not made the transition, but I have installed OOo and spent a good deal of time "playing" with it. While I've had it "lock up" during use, I doubt there's been a program that I've spent any significant amount of time with that has not. It seems to have few bugs, is easy to use, and very complete. If you have problems or questions, there is a tremendous amount of online help available.

There are six modules in the application—*Writer*, a word processor; *Calc*, a spreadsheet; *Impress*, a multimedia presentation module (similar to *PowerPoint*); *Draw*, a simple graphics program; *Base*, a relational database; and *Math*, a straightforward mathematical formula editor. If you're familiar with one of the commercial competitive products, there's no need to even review any "how to get started" documents; it's intuitive and can be used interchangeably with competitor's files. The roots of this Sun Microsystems product go back 20 years and the software has been extensively tested and revised during that time.

This newsletter is created with MS *Word* and the final document is converted to PDF with Adobe *Acrobat*. An added feature of OOo's *Writer* is that it has the ability to export documents directly to PDF (no need to use *Acrobat*)—just one of many nice capabilities of this applications suite. If you've wanted to upgrade from your old version of *Office* or *Word Perfect*, but didn't want to pay the exorbitant fee for the latest, check out OOo at www.openoffice.org.

Some might argue that *free* implies *shoddy*, leading to wasted time using a substandard product, inciting the adage that *time is money*; and, therefore, it's *not* free. Clearly, that is not the case with these two software packages. Kudos to the individuals who have made these excellent programs freely available!!!

Dan Hoefner, LVDD Information Officer