



The Fats of Life

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Dawn L Thiselton, PhD produced this issue of *The Fats of Life*.



This is a particularly packed issue of *The Fats of Life* in that several topics are addressed.

These include 1) a review on hepatic lipase by our colleagues from Japan, 2) a fascinating editorial on the life and times of two leaders in our field, Ancel Keys and Salim Yusuf, and 3) a round table discussion on omega 3 fatty acids by the leading experts in this area. Our now Past, Past Chair, Dr. George Csako, also provides an excellent review of recent and pertinent literature to help keep you up to date.

Japanese investigators have recently developed methods to measure hepatic lipase activity in plasma after heparin administration. They are now using this method to help determine the role of hepatic lipase in health and disease. In the hepatic lipase article contained here the authors review studies conducted to date, designed to give us a better understanding of this important enzyme. Further understanding is necessary, and new tools have been developed which will help us design and perform studies to determine the clinical significance of hepatic lipase and how we might use it in clinical practice.

I highly recommend reading the editorial article on the biographical notes of Ancel Keys and Salim Kusuf. This is masterfully written and tells the life stories of two dedicated scientists who have made significant contributions to the science, particularly related to cardiovascular disease progression and prevention. The editorial describes the extreme passion of these men and the struggles they encountered as they persisted with their pursuits to conduct two extremely important studies: The Seven Countries Study and the INTERHEART Study. Reading this article you will see how passion and the love of science resulted in accumulation of knowledge that has helped the world to better understand atherosclerosis and disease prevention. It is both inspirational and educational. We are so pleased that the leadership and editors at the *Journal of Clinical Lipidology* have allowed us to print this and other articles in *The Fats of Life* and we sincerely thank them, on behalf of the membership of the

Lipoproteins and Vascular Disease Division of the AACC.

Also included in this issue is a round table discussion on the utility of omega-3 fatty acids in clinical practice. Dr. W. Virgil Brown is a master at leading such discussions and as usual, he gathered the leading experts in the field to discuss advantages and potential disadvantages of using omega-3 fatty acids in 1) the setting of hypertriglyceridemia, 2) in cardiovascular risk reduction, and 3) in past current and future research studies designed to test the usefulness of omega-3 fatty acids. You will find this to be a stimulating and information filled discussion.

On a last note, it is an extreme pleasure to have Dr. Sridevi Devaraj step up to the helm as Chair of the LVDD Division. She brings a wealth of knowledge and plans for new programs, new research and the forging of new collaborations. In the Chairs Corner she introduces an exciting plan to explore partnership with clinical colleagues in the National Lipid Association. The LVDD will certainly benefit from her leadership.

With best regards,
Joseph McConnell, Ph.D., DABCC



This is my first message to AACC LVDD Division members since assuming the Chair's position. I hope to continue the excellent efforts of my predecessors to keep our Division activities interesting and informative. I would like to take this opportunity to welcome the new Division officers that will assume responsibility in 2012.

I have assumed the responsibility of the Chair of the Division. In my professional life, I am the Medical Director of Chemistry and POCT at Texas Children's Hospital and Professor in the Department of Pathology and Immunology at Baylor College of Medicine, Houston, TX.

As the New Year unfolds, we look forward to the upcoming AACC Annual Meeting in Los Angeles. The President of the AACC is now a clinical chemist, Dr. Greg Miller, PhD, who is Professor of Pathology, Director of Clinical Chemistry, and Director of Pathology Information Systems at Virginia Commonwealth University in Richmond. The LVDD will hold its customary events at this meeting: i) The Executive Committee /Membership meeting on Sunday morning which is open to all members; ii) The Annual Mixer/Dinner meeting on Monday night and iii) the International Lipoprotein Standardization Meeting on Tuesday night. Since these are very educational, discussing state of the art topics and technologies, we encourage all members to purchase your tickets to these early, since they have been sold out in the last few years. As in the past, there will be several workshops that have been developed by LVDD members or in association with LVDD. LVDD will also select the best posters for awards that will be presented at the AACC National meetings. Please remember to submit abstracts for the meeting; the deadline is February 27th 2012.

This year, we also hope to actively participate and hold hands with our colleagues at the National Lipid Association and have made efforts to initiate a strong and mutual partnership. I also take this

opportunity to thank all of those volunteers for their generous donations and from the different companies that have supported us and made all these events possible. I also would like each and every one of you to actively participate as officers in the Division.

Happy 2012 and Look forward to seeing you all in Los Angeles.

Sridevi Devaraj
Chair, LVDD



Hepatic lipase: What is its clinical significance on plasma lipoprotein metabolism and atherosclerosis?

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Hepatic lipase (HL) plays a key enzyme catalyzing hydrolysis of triglycerides (TG) and phospholipids (PL) in several lipoproteins. It is generally recognized that HL is involved in the remodeling of remnant, LDL, HDL and production of small, dense low-density lipoproteins (sd-LDL). On the other hand, there is a controversy as to whether HL accelerates or retards atherosclerosis. In this review, we describe the clinical significance of HL on lipoprotein metabolism and its relation to atherosclerosis.

1) HL and lipoprotein metabolism.

It has been well recognized that remnant lipoproteins and sd-LDL are risk factors for cardiovascular disease. A large number of studies suggest that HL is mainly involved in the metabolism of those lipoproteins as well as HDL.

In 1990, authors from France showed that inhibition of HL activity using a specific goat antibody against rat HL impairs chylomicron remnant-removal in rats [1]. Authors in the same group suggested that HL may facilitate uptake of chylomicron remnant-like particles, not only as a lipolytic enzyme, but also as a ligand anchored to extracellular glycosaminoglycans in isolated rat hepatocytes [2]. Ji and colleagues proposed, using rat hepatoma cells transfected with a human HL cDNA, that HL contributes to the enhanced cell association of specific types of remnant lipoproteins by initiating their binding to cell-surface HSPG [3]. Another group from the USA reported that, in mice, anti-HL antibody caused a small but significant delay in remnant removal from plasma and a larger decrease in hepatic uptake, independent of lipolytic function of HL [4]. In a gene transfer study using recombinant adenovirus to express native and catalytically inactive HL (HL-145G) in apo E-deficient mice, NIH researchers suggested that HL may serve as a ligand that mediates the interaction between remnant lipoproteins and cell surface receptors and/or

proteoglycans [5]. In 2002, a human cohort study showed that, of 120 normolipidemic, nondiabetic, premenopausal women, those with more sd-LDL had higher HL activity and lower HDL₂ cholesterol [6]. These in vitro and in vivo reports support the notion that HL contributes to the metabolism of remnant lipoprotein mainly through its bridging action, and to the formation of sd-LDL and the metabolism of HDL₂ mainly through its enzyme activity.

Recently, we found that in middle-aged, obese or overweight American men or postmenopausal women, HL activity in post-heparin plasma (PHP) showed no association with remnant-like particle (RLP)-TG, RLP-TC or sd-LDL but was inversely associated with plasma HDL levels [7]. In that study, we had expected that HL activity would have been significantly associated with sd-LDL, RLP-TG and RLP-TC. Thus, we feel that aside from an effect of HL on HDL, the clinical significance of this enzyme on remnant and sd-LDL remain to be elucidated.

2) Relevance of HL deficiency to atherosclerosis.

One approach for understanding how this enzyme affects the development and progression of atherosclerosis is to clarify whether or not complete HL deficiency is an atherogenic disease. This issue does not appear to be resolved, in part because of the rarity of this disease. Canadian investigators reported that HL-deficient beta-VLDL in compound heterozygotes for HL mutations (Ser267-->Phe and Thr383-->Met) readily induces cholesteryl ester accumulation in J774 [8]. The same group suggested, in several other reports, that human HL deficiency in the context of a second factor causing hyperlipidemia could be strongly associated with premature coronary artery disease (CAD) [9,10].

In contrast to these human studies, Mezdoor and colleagues suggested, from studies of mice lacking both HL and apoE, that HL deficiency may increase plasma cholesterol but reduces



susceptibility to atherosclerosis [11]. Although homozygous or compound heterozygous deficiency of HL is rare, it is presumed that a considerable number of individuals with heterozygous HL deficiency may exist in the general population. One study reported that moderate elevation of total TG, IDL, LDL, HDL₂ and HDL₃-TG were observed in heterozygous HL deficiency with R186H or L334F in a Finnish pedigree [12]. Up to date, there is no clinical study on the association between heterozygous HL deficiency and the development of atherosclerosis.

We recently established a novel method for measuring HL activity in postheparin plasma (PHP) [13,14]. This method is easy, reliable and gives us important information on how HL activity associates with the series of lipoproteins (the kit for the assay of HL activity is going to be commercially available in the near future in Japan). Our method for measuring HL activity will enable us to detect individuals with low HL activity easily, leading to the identification of either heterozygous or homozygous HL deficiency. This will help to answer the question of whether HL deficiency is pro- or anti-atherogenic. There is some speculation as to whether genetic deficiency of another crucial lipolytic enzyme, lipoprotein lipase (LPL), is associated with atherosclerosis. Most of the studies suggest that it is unlikely that homozygous deficiency of LPL is associated with atherosclerosis. The findings in some reports, however, suggest that individuals who do not have LPL activity but have considerable inactive protein mass show evidence of the development and progression of atherosclerosis [15,16]. So what about HL deficiency with HL protein mass? To answer this question, it is essential to measure both activity and mass of HL and for this purpose an easy and reliable method for measuring HL activity is useful.

3) Polymorphism of the HL gene.

Studies have shown that the *LIPC* promoter -514C>T polymorphism is associated with a mild reduction in HL activity and elevation in HDL-C [17~21]. We previously reported that this polymorphism showed a weak effect on increasing HDL-C in the Japanese population [22] but no significant association with CAD in Japanese

familial hypercholesterolemia (FH) individuals [23]. There is a study showing that in men with CAD, HL activity in PHP was 15% to 20% lower in heterozygotes and 30% lower in homozygotes for the -514T allele [24]. Hokanson and colleagues [25] reported that the *LIPC* -480C>T polymorphism (the same as the -514T allele) was associated with subclinical coronary heart disease in type 1 diabetes. To the best of our knowledge, how this *LIPC* polymorphism affects the development of atherosclerosis has not yet been reported.

4) HL in various disease conditions.

In human studies, HL activity is increased in individuals with insulin resistance[26], type 2 diabetes[26], and high abdominal fat mass [27], all of which are closely related to the development of atherosclerotic disease. These aspects of HL appear to be quite opposite to LPL. Unlike HL, LPL activity or mass decreases in individuals with insulin resistance [28,29], type 2 diabetes [30,31], intra-abdominal visceral fat accumulation[32,33] or metabolic syndrome[34].

5) HL and fat accumulation in the liver.

Studies suggest that HL activity increases, or tends to increase, in individuals with fatty liver [35~37]. Whether or not HL is simply associated with these disorders is an interesting topic. A recent report by authors from the USA showed that unlike wild type mice, HL knockout mice showed no development of fat accumulation in the liver even after a high fat diet load [38]. This finding suggests that HL is not simply associated with the development of fatty liver but may have causal effect on the development of this disease. Combined with the fact that fat accumulation of the liver is responsible for the development of diabetes [39], this suggests that HL may indirectly contribute to the formation of this atherogenic disease.

6) HL and angiopoietin-like protein 3 (ANGPTL3).

Considerable numbers of studies using mice suggest that ANGPTL3 is involved in plasma TG metabolism by inhibiting LPL activities [40~42]. However, Shimamura et al [43] and Moon et al [44] found that, unlike in mice, plasma ANGPTL3 levels were not correlated with TG levels in human

plasma and were shown to be more strongly associated with HDL metabolism. We recently reported that in American overweight or obese subjects, ANGPTL3 concentration was inversely correlated with HL activities in PHP [7]. This data suggests that ANGPTL3 could be involved in HDL metabolism through inhibiting HL activity in humans.

In conclusion, despite multiple in vitro studies having been done regarding the function of HL on plasma lipoproteins, the clinical significance of this enzyme is not fully understood. To solve this, measuring HL activity in a large number of individuals in daily clinical practice is essential and our new method for measuring HL activity in PHP will provide a unique opportunity for this purpose.

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Conflicts of interest
Not declared

Clinical Lipidology Roundtable Discussion

Using omega-3 fatty acids in the practice of clinical lipidology[†]

W. Virgil Brown, MD*, Harold Bays, MD, William Harris, PhD, Michael Miller, MD

Editor-in-Chief, The Journal of Clinical Lipidology, Charles Howard Candler Professor of Internal Medicine Emeritus, Emory University School of Medicine, 1670 Clairmont Road, Atlanta, GA 30033, USA (Dr. Brown); Medical Director/President, L-MARC Research Center, Louisville, KY, USA (Dr. Bays); Senior Scientist, Health Diagnostic Laboratory, Inc., Richmond, VA, USA (Dr. Harris); and Professor, University of Maryland School of Medicine, Director, Center for Preventive Cardiology, University of Maryland Medical Center, Baltimore, MD, USA (Dr. Miller)

Acknowledgment

The *Journal* would like to recognize Megan Seery's work with the editorial issues for this Roundtable.

Disclosures

Dr. Bays has received consulting fees from Amarin Corporation, AstraZeneca, Daiichi Sankyo, GlaxoSmithKline, Merck & Co., and Zeomedex. Dr. Bays has received honoraria related to speaking from Amarin Corporation, AstraZeneca, Daiichi Sankyo, GlaxoSmithKline, Merck & Co., and Zeomedex. Dr. Bays has received grants from Amarin Corporation, Arena Pharmaceuticals, Boehringer Ingelheim, Bristol-Myers Squibb, California Raisin Board, Cargill Incorporated, Daiichi Sankyo, Eli Lilly and Co, Esperion, Essentialis, Forest Pharmaceuticals, Gilead, GlaxoSmithKline, Johnson & Johnson, Nicox, Novo Nordisk, Omthera Pharmaceuticals, Orexigen Therapeutics, Pfizer Inc., Pozen, Proctor & Gamble Inc., Schering Plough, Shionogi, Stratum Nutrition, Takeda Pharmaceuticals, Trygg Pharmaceuticals, Trygg Pharmaceuticals, and TWI Bio. Dr. Harris has received consulting fees from Acasti Pharmaceuticals and Omthera Pharmaceuticals. Dr. Harris has received honoraria related to speaking

from GlaxoSmithKline. Dr. Harris has received salary from Health Diagnostics Laboratory Inc. Dr. Harris has ownership interest in OmegaQuant LLC. Dr. Miller has received consulting fees from Amarin Corporation.



Dr. Brown

W. Virgil Brown, MD: Increasing the quantity of dietary omega-3 fatty acids appears to offer significant health benefits. The American Heart Association suggests an additional 0.5 to 1 g of omega-3 fats daily from fish and other dietary sources. The reduction of blood triglyceride concentrations with much larger doses has led to the use of these fats as pharmacologic agents. I have asked three clinical lipidologists, Drs. Harold Bays, William Harris and Michael Miller, each with considerable research experience, to join me in a Roundtable discussion on the use of these agents in the management of lipoprotein disorders.

Dr. Bays, will you please describe the current preparations of omega-3 fats that are available for prescription use?



Dr. Bays

Harold Bays, MD: Only one prescription omega-3 fatty acid is currently approved for clinical use. Years ago when we first began research on this agent, this prescription omega-3 fatty acid preparation was known as K85 and then subsequently known as Omacor[®] (as is still true in Europe). It is now known as Lovaza[®] in the United States. The reason it was initially termed K85 is presumably because it contained

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[†] The Roundtable was recorded in New York, NY in May 2011 at the time of the Annual Scientific Sessions of the National Lipid Association.

more than 85% omega-3 fatty acids. This agent is a true fish oil preparation in that it contains both eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) as well as other omega-3 fatty acid esters derived from fish such as anchovy, mackerel, herring, sardine, menhaden, smelt, salmon, tuna, and marlin.

This prescription omega-3 fatty acid therapeutic agent has a Food and Drug Administration (FDA)-indicated use as an adjunct to diet to reduce triglyceride levels in adult patients with severe (≥ 500 mg/dL) hypertriglyceridemia.

Brown: Dr. Harris, are there other preparations that are in development and that may soon be approved by the FDA?



Dr. Harris

William Harris, PhD: There are a few that I'm aware of that are being developed. One is a free fatty acid form, (not an ethyl ester like Lovaza) provided in an enteric-coated capsule. Presumably, this is going to be more readily absorbed because it doesn't need to be de-esterified the way ethyl esters do. That product is called Epanova and is being developed by Omthera.

Dr. Brown: Does that contain esters of EPA only, or is it a mix also?

Dr. Harris: It's a mix of EPA and DHA. The other product under development is from Amarin, and it's called AMR101. It is just pure EPA ethyl ester. It's virtually the same product as was used in the JELIS trial.

Dr. Brown: I believe that preparation has been in recent clinical trials in the United States?

Dr. Harris: True, yes.

Dr. Brown: Is it now being considered for approval as a prescription agent?

Dr. Harris: It will probably be submitted to the FDA in the early fall. It will seek approval both for very high triglycerides as well as for use in patients with triglycerides between 200 and 499 mg/dL who are taking statins.

Dr. Brown: Dr. Miller, what are the current indications that are approved for using these pharmacological preparations?



Dr. Miller

Michael Miller, MD: As Dr. Bays indicated, the present indication for the use of Lovaza is in subjects with very high triglycerides defined as a triglyceride level of at least 500 mg/dL. There might be a future indication in lower triglyceride range as Dr. Harris had suggested, if adequate clinical trial and clinical outcomes data are developed.

Dr. Brown: What can one expect in terms of triglyceride lowering with the use of these drugs? Dr. Harris, is it necessary to always use 4 grams in patients to lower triglycerides?

Dr. Harris: That's the indicated dose for the only approved product on the market. When these were being developed they tried 2 grams, 3 grams, and 4 grams. There was a clear dose-response relationship, even up to 6 grams. But the best compromise between effective triglyceride lowering and compliance was deemed to be at 4 grams.

Dr. Brown: Dr. Bays, were you going to say something about other agents?

Dr. Bays: As Dr. Harris mentioned, AMR101 is an EPA-only preparation being developed by Amarin Pharmaceuticals. According to data presented at the 2011 National Lipid Association Annual Meeting (and subsequently published), AMR101 significantly reduces triglycerides without increasing low-density lipoprotein (LDL) cholesterol in patients with very high triglyceride levels (≥ 500 mg/dL). In addition, in this challenging population with severe hypertriglyceridemia, AMR101 significantly reduced non-high-density lipoprotein (non-HDL) cholesterol, apolipoprotein B, lipoprotein-associated phospholipase A2, very low-density lipoprotein cholesterol, total cholesterol, and C-reactive protein.

As mentioned by Dr. Harris, another relevant therapeutic of interest is Epanova[®], which is an EPA/DHA combination agent developed by Omthera Pharmaceuticals. In contrast to ethyl ester omega-3 agents, Epanova[®] is a free fatty acid formulation that may facilitate gastrointestinal absorption. In studies presented at the 2011 National Lipid Association, when taken with low fat meals, Epanova[®] increased the gastrointestinal absorption of these fats compared with the omega-3 ethyl ester preparation (Lovaza[®]). Even with the consumption of a higher-fat diet, the omega-3 fatty acid preparation appeared to have significantly better gastrointestinal absorption in patients compared with the ethyl ester omega-3 fatty acid preparation.

Finally, according to the Internet, Trygg Pharma is a subsidiary of a company called Aker BioMarine, which develops products such as krill-derived pharmaceutical ingredients. ClinicalTrials.gov reports that Trygg Pharma is investigating the efficacy of AKR963 for lowering triglycerides in patients with severe hypertriglyceridemia.

Dr. Brown: So, there are at least three other preparations that are in development as potential agents for the purpose of lowering triglycerides.

Now let's talk more specifically about principles of management of high triglycerides. What is the first line therapy for a patient who has triglycerides of 2000?

Dr. Bays: Few lipid parameters respond better to appropriate nutrition and increased physical activity than elevated triglyceride levels. Clinically meaningful changes in LDL-C and HDL-C often require substantial change in nutritional and physical activity, as well as other lifestyle changes (which may include considerable weight loss). Even then, changes in these lipid parameters are often moderate. Conversely, patients with hypertriglyceridemia may experience substantial reductions in triglyceride levels

with nutritional interventions such as a low saturated fat, low-glycemic index diet and the avoidance of binge alcohol drinking. Increased physical activity is also often beneficial. In addition, hypertriglyceridemia may often be improved through corrections of metabolic disturbances as the result of adiposity/adiposopathy, poorly controlled blood sugars in patients with diabetes mellitus, untreated hypothyroidism, and nephrotic syndrome. Other potential secondary causes of hypertriglyceridemia include medications, such as estrogens, particularly high-dose estrogen, some antiretroviral therapies, certain antipsychotics, isoretinoin, glucocorticoids, as well as bile acid sequestrants, thiazazide diuretics, and nonselective beta blockers. Therefore, the best initial approach to the hypertriglyceridemic patient is a multifactorial one.

Dr. Brown: That is certainly good advice. Now, let me give you a specific scenario. A patient is referred for management with a blood plasma triglyceride concentration of 2000 mg/dL. He had already begun an exercise program, reduced his calories, and is now taking a fibrate. The original triglyceride value decreased from 3500 mg/dL in response. Would you use an omega-3 fatty acid preparation as an additional pharmacologic therapy? If so, would you start with the full 4-gram dose, or do you test for responsiveness with 2 grams? I realize that clinical trials indicate that 2 grams didn't do much in the patients who were tested. Are there people who are sensitive to these agents and who respond more dramatically?

Dr. Miller: Right. I would have them start off with two capsules just for 3 to 4 days to make sure that they have no issue with tolerability. Then I would increase to the 4-gram dose. At a triglyceride of that magnitude, we also recommend other lifestyle changes, including weight loss if overweight, a low-carbohydrate, low saturated-fat diet, complete abstinence from alcohol, and more intensive glycemic control in diabetic patients whose HbA1C exceeds 7%.

Dr. Brown: So fish oil would follow all of these earlier steps rather than simply starting it immediately? Why not start earlier?

Dr. Bays: Given this level of triglyceride elevation, my preference would be to start with two prescription omega-3 fatty acid capsules twice per day (for a total of 4 capsules per day), which is the recommended starting dose.

Dr. Harris: Were fibrates the appropriate first-line medication in this patient?

Dr. Bays: I believe this goes to more the art of medicine than the science of medicine. Depending upon the preparation recommended, fish oils are highly effective triglyceride-lowering agents and are extraordinarily safe. Fish oils may also have other potential health benefits, including other cardiovascular benefits, that I believe beneficial for patients.

Dr. Miller: Yes. I use omega-3 fatty acids as well as fibrates and niacin-based therapies. Both omega-3 fatty acids and fibrates are quite effective, especially in hypertriglyceridemic diabetic patients. Then niacin, might be a

third consideration, although I tend to be more cautious in the diabetic population.

Dr. Brown: Are there any other indicators in the risk profile, the LDL value, or the HDL value that would steer you to one or another of these triglyceride-lowering therapies? What would steer you most often to fish oil as the next step?

Dr. Bays: That is an evolving question. For patients with very high triglyceride levels treated with a fibrate or an EPA/DHA combination fish oil agent, the LDL cholesterol level may increase as much as 26% to almost 50%. However, as you correctly mentioned, in patients with very high triglyceride levels, the EPA only preparation may reduce triglyceride without significantly increasing LDL cholesterol levels. This EPA only agent (AMR101) may also reduce apoB, reduce C-reactive protein, as well as promote other favorable lipid effects. This may be especially important for lipidologists. So overall, I think the answer to the question is an evolving one, which must await further study results.

Dr. Brown: Would you consider the particle number as indicated by apoB (apolipoprotein B) concentration or as measured by nuclear magnetic spectral analysis? That seems to be an important indicator of risk. Do we see the same response with niacin, fibrates, and fish oil with apoB when you have such a severely hypertriglyceridemic patient?

Dr. Harris: I'm sure there's never been a 3-way head-to-head. I'm pretty sure there's never been a niacin trial in patients with triglycerides greater than 500 mg/dL, although Ann Goldberg has studied fenofibrate in this population. LDL increased similarly with both omega-3 and fenofibrate (apoB was not measured).

Dr. Bays: Dr. Harris makes an excellent point. It is interesting that the prescribing information for extended-release niacin cites an approved indication to reduce triglyceride levels in adult patients with severe hypertriglyceridemia. However, I cannot recall being an investigator in such a trial, nor can I find in the medical literature the confirmatory, supporting trials wherein extended-release niacin alone was studied exclusively in patients with very high triglyceride levels (≥ 500 mg/dL).

Regarding fibrates, clinicians sometimes forget that total cholesterol, LDL cholesterol, and apolipoprotein B are typically reduced when administered to patients with modest to no increase in baseline triglyceride levels.

It is mostly when baseline triglycerides are very high (and LDL levels are at the lower range) that fibrates increase LDL cholesterol levels. In potential contrast to EPA only agents, the effect of EPA and DHA omega-3 fatty acid therapy upon apoB is variable, with some studies suggesting a decrease, others showing a mild increase, whereas others basically demonstrating no significant effect.

Dr. Harris: Yeah, but apoB certainly can go up. Back in the 1990s we gave 6 grams of omega-3, 18 capsules a day

of MaxEPA in studies with Dr. William Connor and saw an increase in apoB.

Dr. Brown: It would appear that reduction of apoB cannot be expected to occur with this therapy. What about Lp(a)? If you had a patient with a plasma Lp(a) concentration of 100 mg/dL, would that affect your choice of drugs?

Dr. Harris: Fish oils don't lower Lp(a), and I presume that you would pick niacin in that case.

Dr. Brown: Do you agree with that, Dr. Miller?

Dr. Miller: I do, but with exception of subgroup analysis from FATS, we don't have a whole lot of clinical outcome data demonstrating that niacin induced Lp(a) reduction translates into improved outcomes.

Dr. Brown: I assume you would like to see triglycerides less than 500 as a target of your treatment?. What combinations make sense with fish oil? Is there a reason to prefer fibrates with fish oil or does niacin and fish oil work better in your experience in lowering severely elevated triglyceride levels?

Dr. Bays: As is true in the presence of other marked metabolic abnormalities, combination drug therapy is often required for patients with severe hypertriglyceridemia. However, patients with triglyceride levels ≥ 500 mg/dL may present unique challenges. In a study of prescription omega-3 fatty acids as an adjunct to fenofibrate therapy in severely hypertriglyceridemic patients, this combination of agents was not much greater than the use of fenofibrate alone. Certainly, little evidence supported an additive effect when both these agents were used together. My sense is that many of these patients with very high triglyceride levels may have a relative or absolute loss of function of lipoprotein lipase. This is important because one of the shared mechanisms of fibrates and omega-3 fatty acid therapy is enhancing lipoprotein lipase activity. If lipoprotein lipase isn't sufficiently available for enzyme activity alteration, and if fibrates and omega-3 fatty acids share this mechanism, then this may help explain why the combination of fibrates and omega-3 fatty acids may not produce additive results in severely hypertriglyceridemic patients. It also helps explain why drug therapy (such as fibrate therapy) may not always be effective for severe hypertriglyceridemia caused by absolute lipoprotein lipase deficiency, with aggressive nutritional intervention being most recommended.

Dr. Harris: But much of the fish oil effect is the result of reduced production of triglycerides, so one would expect that combining it with an agent that increased clearance would be effective. We've just finished a study in metabolic syndrome patients with Niaspan at 2 g/d and Lovaza at 4/g/d. We actually got better triglyceride lowering with the Niaspan than we got with the Lovaza, and their effects were additive.

Dr. Brown: Is there a place for statins in patients who have very high triglycerides but whose LDL cholesterol concentrations may be quite acceptable, in fact even low? Do statins help lower triglycerides, and would you use them in conjunction with fish oil?

Dr. Miller: Well, I would in proinflammatory hypertriglyceridemic conditions such as diabetes and the metabolic syndrome. So, I think in these patients, the combination of statins with omega-3 may be a reasonable consideration.

Dr. Bays: I agree with Dr. Miller that many patients benefit from the combined use of statins and omega-3 fatty acid therapies. Where this may really come into play is when omega-3 fatty acids increase lipoprotein lipase activity, and very-low-density lipoprotein particles are converted to LDL particles. This helps account for the increase in LDL cholesterol so often found with the use of EPA and DHA combination agents in patients with very high triglyceride levels. Given that non-HDL cholesterol typically decreases with omega-3 fatty acid therapy, then I would not characterize this as a known "toxic" effect, as is often perceived by many clinicians. Instead, I believe that this lipid effect is simply revealing the underlying atherogenicity of the patient's lipid profile. My point is that if a patient with marked hypertriglyceridemia is treated with a combination EPA/DHA therapeutic agent, and if LDL cholesterol increases above target goal, then a statin should either be added, or if a statin is already being prescribed, the statin dose should be increased. I believe this to be a more rational approach than stopping the omega-3 fatty acid therapy.

Dr. Brown: If you have a patient whose LDL cholesterol is perfectly acceptable, even low, and you have triglycerides that are very elevated, let's say 2000 mg/dL and they decrease to 1000 mg/dL with the addition of fish oil to his or her diet, what benefit might be expected if you add a statin to the regimen?

Dr. Miller: The statin effect on triglyceride tends to be more robust at higher baseline levels. So, when triglyceride levels are greater than 200 mg/dL, the additional triglyceride-lowering effect of statins may approach 30% or greater and is independent of omega-3 fatty acids or other triglyceride-lowering therapies.

Dr. Bays: Another interesting aspect regarding statins and omega-3 fatty acid therapy is that it appears the EPA only therapy has enhanced triglyceride-lowering effects among statin treated patients.

Dr. Brown: Well, I've certainly seen that in practice, that statins add quite a bit, 30% or 40% of further reduction when you add these drugs to the fibrate therapy. I've had less experience with fish oil as the initial intervention, followed by statins.

I would like to return a moment to the rationale for developing purely EPA containing preparation such as EPA ethyl ester. Why would it be useful to have EPA only as opposed to the more naturally occurring EPA/DHA mixture?

Dr. Harris: The short answer is that EPA ethyl esters are being developed in the United States because they reduced risk for cardiac events in a very large randomized clinical trial in more than 18,000 statin-treated patients in Japan. They did so without altering lipids to any meaningful extent, but the dose was only 1.8 g/d; not a dose you'd expect

to lower lipids, and this cohort in Japan was not hypertriglyceridemic. Nevertheless, with that kind of clinical efficacy, interest is great in developing such a product for the U.S. market. But here, the shortest (and cheapest) road to approval for an omega-3 product is for a triglyceride lowering-indication, not for event reduction. EPA alone, at higher doses than used in JELIS, does lower TGs in hypertriglyceridemic patients, and so Amarin is taking that approach. People have looked at the combined and separate effects of EPA and DHA on both TG-lowering and LDL-raising. There is a poster presented here at the National Lipid Association that is a review of literature on this topic that suggests that it actually is maybe the DHA component that contributes to the LDL-raising. Now, by the same token, in those same studies it looks as if DHA is a little bit better at triglyceride-lowering and HDL-raising than EPA. So, we have a lot to learn about the separate effects of the two, but if you can get, with EPA, decent TG lowering without raising LDL, that's a strong marketing story and likely to be a successful sell at the FDA.

Dr. Brown: Are there any significant adverse effects from the use of these high doses of EPA and DHA that have been documented either in community-based studies or in large clinical trials?

Dr. Harris: After the LDL increase in nonstatin-treated, severely hypertriglyceridemic patients, the next concern that always comes up is bleeding. Clinically significant bleeding however, has never been reported. I looked at 19 different trials that all involved coronary artery bypass grafting, percutaneous transluminal coronary angioplasty, or diagnostic cath—all bloody procedures—in a variety of different settings in which patients were given 4 to 7 grams a day of EPA and DHA on top of the standard anti-coagulants. Not one of them reported increased bleeding with the omega-3. In more recent studies authors have examined fish oils and plavix and found no increased risk for bleeding. So no, there are no significant adverse effects of EPA and DHA ... unless you call eructation with a fishy taste an adverse effect!

Dr. Miller: Correct, and this includes the recent study in patients with atrial fibrillation who were also receiving warfarin and where no excess risk of bleeding was reported.

Dr. Bays: One of the potential advantages of a prescription omega-3 fatty acid therapy is that it avoids potential toxicities that frighten patients and clinicians regarding fish oil therapy. Reports from the news or governmental agencies sometimes raise concerns of environmental toxins in fatty fish. However, given the rigorous purification processes required for regulatory approval, no evidence supports that prescription omega-3 fatty acids pose a clinical risk for exposure to environmental toxins. Patients and clinicians may also be wary of potential fatty acid oxidation over time, and the fishy taste with these agents. But again, because of the purity of their components, the fishy smell attributable to oxidation and the incidences of fishy eructations (burping) for prescription omega-3 fatty acids are negligible. Other potential safety matters include a potential

mild and transient increase in glucose levels with combination EPA and DHA agents. However, longer term measures of glucose control (such as hemoglobin A1C) are not typically affected. Liver enzyme elevation is sometimes described; however, no evidence exists for liver toxicity with omega-3 fatty acid therapy. These are very safe therapeutics.

Finally, patients and clinicians may be concerned about an increase in body weight when consuming capsules of fatty acids. Administration of four 1-gram capsules per day of fatty acids modestly increases daily calories, which one might suppose could promote an increase in body weight. Conversely, the literature suggests omega-3 fatty acids may increase fatty acid oxidation, which may promote fat loss. However, the clinical trial suggests that prescription omega-3 fatty acids really have no net effect on body weight.

Dr. Brown: With four grams you're only talking about 36 extra calories a day.

Dr. Harris: And you're not burning but storing those fatty acids in the membranes around the body.

Dr. Brown: I know there have been suggestions that the high hemorrhagic stroke rate in Japan was related to high fish consumption, implying high omega-3 fatty acid consumption. Is there any credible evidence for that?

Dr. Harris: Actually, that probably has more to do with the higher prevalence of hypertension in Japan than with the omega-3s. In the JELIS trial they looked at that question. Again, the JELIS trial included 18,600 patients who were all on statins, and its investigators tested the effects on several end points of 1.8 grams of EPA. They looked at stroke in a substudy and found that in those who had no history of a stroke going into the study, there was no difference in stroke risk, hemorrhagic or ischemic. In those who had a history of stroke, EPA actually reduced risk for recurrent stroke. So it's unlikely that the higher stroke prevalence is due to omega-3. Remember that the average life expectancy in Japan is 4 years longer than ours, so whether there is an age adjusted difference in stroke rates or not, I'm not sure.

Dr. Brown: Well, that's reassuring. There has also been a claim that there may be a risk of prostate cancer with high doses of omega-3 fatty acids?

Dr. Harris: That's a mixed bag. There have been several epidemiological studies reporting that greater omega-3 (or fish) intakes were associated with lower risk for prostate cancer. This recent one looked at plasma levels of omega-3 and future risk for stroke. It was not a supplementation study, but a prospective cohort study. They reported two very odd findings. One was that greater levels of omega-3 in the plasma were associated with increased risk for aggressive prostate cancer and that greater levels of trans fats were beneficial. Both findings in that study raise questions. We simply need to keep studying that question.

Dr. Brown: I would like now to turn to the issue of the use of these agents in cardiovascular disease prevention.

Where do we stand with that? Dr. Harris, I wonder if you would briefly describe the major studies in this area?

Dr. Harris: Two studies from the GISSI group have been done. The first one is called GISSI-Prevenzione, and that was using about 1 gram of Lovaza (also called Omacor). The second was the GISSI heart failure trial that just included patients with heart failure. The original GISSI-Prevenzione was in recent post-MI patients. Both used a single capsule of the prescription omega-3. Their intention was not to lower lipids because that dose does not affect lipids. Their intention was to see whether that dose would reduce risk for cardiac events, arrhythmias, and total mortality. In both the Prevenzione and Heart Failure trials, omega-3 reduced risk for death from any cause. The JELIS trial was as I mentioned a Japanese trial, 18,000 patients all with hypercholesterolemia and all treated with statin, 5 to 10 milligrams of pravastatin or simvastatin I believe. It was a very low dose, but as I understand in Japan that is enough to get the job done. That trial was not placebo-controlled, it was open label, but blindly adjudicated. The primary end point was major adverse cardiac events, a composite end point. There was a 19% reduction in the composite end point after 5 years, which is remarkable. I was surprised with that outcome. This trial, in Japan where they already eat much more omega-3 than we do, much more fish, and using a product that just had EPA and no DHA, provided an impressive beneficial effect.

Now, there have been a couple of recent neutral trials (I don't call them negative because the omega-3s were not harmful; just not shown to be helpful). One was called the OMEGA study. It was done in Germany with approximately 3600 patients, all of whom had recently experience a myocardial infarction. The authors were trying to replicate the GISSI-Prevenzione observation. They gave 1 gram of Lovaza and they did it for 1 year. They found no effect (no benefit and no harm) on sudden death or total mortality or any cardiac end point. They said in the paper that unfortunately the death rates were much lower than they expected, and so their power to detect even a 25% benefit was only about 20%. So this study simply failed to find an effect. It did not show that fish oil has no effect. It's a subtle difference, but an important one, characteristic of studies that are not properly designed or conducted.

Dr. Brown: Were patients in the OMEGA trial being treated with statins or other agents?

Dr. Harris: Yes. They were receiving the modern pharmacologic therapy. It's interesting if you go back and look at the total mortality. In GISSI, which was 10 years earlier in Italy, and compare it to OMEGA done in Germany, the death rates were essentially the same during the first year. So, whether pharmacologic "improvements" have actually improved anything or not is questionable. It wasn't clear that that was the reason why the OMEGA trial didn't work. I think it was just underpowered.

Dr. Miller: Then in terms of other studies that have been inconsistent or negative were the defibrillator-based studies

that did not demonstrate reduced arrhythmias and more recently, the failure to prevent recurrent symptomatic atrial fibrillation in patients with paroxysmal afib.

Dr. Harris: Yeah, generally negative or no effect. The other one I should mention is called the ALPHA OMEGA study, which came out last year in the *New England Journal of Medicine*. It was a comparison of EPA plus DHA versus alpha-linolenic acid (commonly found in flaxseed oil), alone or in combination, for about four and a half years in patients with coronary disease, performed in the Netherlands. The authors showed no effect in either class of omega-3 on cardiac events. Unfortunately, they administered the omega-3s as a mixture in margarines and asked the participants to use this margarine on their bread.

Dr. Brown: So, the actual dose taken is uncertain.

Dr. Harris: Well, the dose was uncertain, and it was also low. They report that the average EPA/DHA dose was about 370 milligrams. This is too low.

Dr. Brown: In these various trials with cardiovascular end points, were there any substantive changes in lipoprotein concentrations? Could any change in event rates be attributed to these lipoprotein modifications?

Dr. Harris: In neither JELIS nor the GISSI trials was there any effect on cholesterol or LDL; triglyceride were reduced by about 5% overall. So no, the effects observed were independent of meaningful lipid changes.

Dr. Brown: That seems to have left us with the concept that fish oil is doing something beneficial other than changing lipoprotein metabolism, right?

Dr. Bays: Yes.

Dr. Brown: Where are we with that concept? Is there any new data that suggest that the electrophysiology of the heart might be involved or that the clotting system might be altered to produce benefit?

Dr. Miller: Well Dr. Harris, you might want to mention the heart rate variability study?

Dr. Harris: Exactly. So, autonomic balance—which is typically assessed by measuring heart rate variability—seems to be improved by fish oils. That's not really a membrane effect per se, although it could be mediated through changes in nervous system membrane composition. We've also seen a reduction in heart rate, and this has been confirmed in a meta-analysis. Heart rate can drop two to five beats a minute with omega-3, and that in itself could reduce risk for sudden death or arrhythmic death. Exactly why that happens, nobody really knows. We have actually looked at this effect in patients who had transplanted hearts who didn't have any parasympathetic innervation to their hearts—and the omega-3s lowered heart rate in those people. So, it seems to be something intrinsic to the heart and does not have to be mediated by cardiac nerves.

Dr. Bays: I think that Dr. Brown brings up an important point for the clinician because it describes a phenomenon I call the "*time to efficacy paradox*." For most other lipid-altering drugs with clinical-trial supported cardiovascular disease (CVD) benefits (such as statins), the first demonstrable effects occur within days to weeks, and include

improved lipid levels, improved endothelial function, anti-inflammatory effects, antithrombotic effects, and increased nitric oxide bioavailability. This is often followed by reduced CVD after several months, with a subsequent potential (but more elusive) reduction in mortality after a longer period of years.

Conversely, although omega-3 fatty acids may have also had favorable lipid effects, improved endothelial function, anti-inflammatory effects, antithrombotic effects, and increased nitric oxide bioavailability within days to weeks, it is the demonstrable reduction in atherosclerotic progression occurring after years is more elusive, with the reduction in mortality often occurring in a shorter period of time (within months).

Thus, I believe it legitimate to ponder as to the real physiologic connection between omega-3 fatty acids' lipid effects, and their cardiovascular health effects. How much of a role do other cardiovascular effects of omega-3 fatty acids (such as potential anti-dysrhythmic effects) play in their clinical trial supported CVD benefits? What is going on from an antiatherogenic standpoint? What about antithrombotic effects? What's going on from an anti-inflammatory and endothelial protective effect standpoint? Many questions remain to be answered from this most interesting, and clinically useful class of agents.

Dr. Brown: The GISSI-Prevenzione study had a relatively high incidence and a significant reduction in sudden death, whereas JELIS didn't seem to show that.

Dr. Harris: JELIS did not. JELIS did not have a reduction in risk of sudden death, but that's because nobody in Japan is allowed to die suddenly! I mean, of 18,000 people over 5 years with hypercholesterolemia, there were a total of 35 sudden cardiac deaths. That's an extremely low rate, and due, I suspect, to the high omega-3 background diet.

Dr. Miller: But they were also statin treated though, right?

Dr. Harris: Yes. They were all statin treated, and if they are like other Japanese populations, their Omega-3 Index was very high—about 9.5%—compared with approximately 4% to 5% in the United States.

Dr. Brown: The baseline levels of omega-3 in the blood and tissues as well as coexisting statin treatment may have been playing a role here to make the Italians and the Japanese studies quite different in event rates.

Dr. Harris: Right.

Dr. Miller: Well, I think that's important because statin use is now commonplace as baseline therapy so it has become much more difficult to identify large differences between groups as compared to the traditional placebo-controlled studies.

Dr. Brown: A victim of our own success. At this point in time, where are the promising areas of research in this area? Are there genetic studies that suggest susceptibility? Are there any other physiologic measures or biomarkers that point to benefit and point to a better understanding of the mechanism of omega-3 fatty acids in risk reduction?

Dr. Harris: I think Dr. Bays has listed some of them as he reviewed some of the other systems. Certainly anti-inflammatory effects have been seen in studies of patients who were pre-treated with omega-3 before endarterectomy. When the plaques were examined after removal, they found fewer inflammatory cells and thicker caps. So there may be a plaque stabilizing effect, but there are only two such studies and the pre-treatment periods were very short.

Dr. Brown: Similar things have been observed with statins. Statin therapy preceding an acute vascular event seems to predict fewer complications and better survival.

Dr. Harris: Right. Endothelial function is to some extent improved by omega-3 fat supplementation. It depends on the way you measure it, whether it's the flow-mediated dilation, digital tonometry or pulse wave velocity.

Dr. Brown: Vascular compliance?

Dr. Harris: Vascular compliance.

Dr. Bays: It is certainly interesting to speculate on the cellular and tissue effects of omega-3 fatty acids. But as a clinical trialist, I believe what is most important is that we are way past due in conducting the first clinical trial specifically designed to assess the clinical benefits of lowering triglycerides in hypertriglyceridemic patients. Given the decades of widespread use of triglyceride-lowering therapies, prescribed predominantly to reduce CVD risk, patients might reasonably expect us to point to several clinical trials wherein specifically lowering triglycerides was a primary end point, and cite such trials as conclusively proving that a reduction in triglycerides concentrations reduces cardiovascular events. Unfortunately, we are still waiting for the first such clinical trial. It is my hope, if not expectation, that some of the manufacturers of these newer omega-3 fatty acid investigational agents will finally have the courage, and make the substantial investment to conduct these overdue clinical outcomes trials.

In the interim, in this age of almost daily reports of adverse findings in both investigational and established drugs, it is reassuring to know that fish oil, or omega-3 fatty acid therapy, has a favorable efficacy and safety profile. Herein lies a potential benefit to clinicians. Because after prescribing an omega-3 fatty acid treatment, many patients might reasonably go to the Internet and make their own assessment. Here they may find clinical trial evidence (although not always) that fish oil therapy does indeed have antidysrhythmic, antiatherogenic, antithrombotic, and anti-inflammatory effects, as well as potentially favorable effects upon endothelial function and blood pressure. In addition, they may find that fish oil therapy has other benefits as well, including orthopedic/rheumatologic, neurologic, neuropsychic, ophthalmologic, oncologic, and other favorable effects, including benefits regarding women's health. Although not all of these potential benefits are proven, they are widely described on the Internet, which may instill confidence, and thus potentially improve compliance among patients.

Dr. Harris: I think that you're right. There are just so many claims for benefits here. I remember a study

suggesting that asthma was reduced, that colitis was reduced, and these inflammatory disorders that we haven't been able to really understand very well.

Dr. Brown: We have discussed two trials that show CVD reduction. There are other smaller studies that have failed to confirm this but these had much less power to detect a difference. They were also done outside the United States. If a company making an omega-3 containing product wishes to have this approved by our FDA as a prescription agent, what would need to be done to achieve an indication for cardiovascular disease prevention? Is there any current effort to achieve such an indication?

Dr. Miller: I think it would be important to do an outcomes study in high-risk cardiovascular patients who are hypertriglyceridemic. If it can be established that the addition of omega-3 supplementation reduces cardiovascular events, it would behoove the FDA to consider such an indication.

Dr. Brown: Are you aware of anyone actually calculating a sample size estimate for such a trial? Would it require 20,000 or 50,000? How many patients do you think would be required?

Dr. Harris: Perhaps Dr. Bays or Dr. Miller has been part of such planning?

Dr. Bays: As one involved in the development of these kinds of outcomes-based protocols, you are exactly correct that given the recent challenges in CVD outcomes trials in achieving differences of clinical significance, tens of thousands of patients are now often the standard sample size. This is an important point clinicians should understand. The design and conduct of CVD outcomes studies are more challenging in today's environment, largely because most such studies require that study participants adhere to "standards of care." As a tribute to the extraordinary success in establishing interventions that reduce CVD risk, a controlled clinical trial wherein all study participants are to adhere to "standards of care" necessitates clinical trial inclusion of large numbers of high CVD risk patients to provide any chance that a drug can indeed demonstrate CVD benefits.

Having said all this, I want to go back to something Dr. Miller mentioned earlier. I've been told of marketing data suggesting that as much as 70% of prescription omega-3 fatty acid therapy is used in patients with triglyceride levels between 200 and 500 mg/dL. Yet, its labeled indication is to treat triglycerides greater or equal to 500 milligrams per deciliter. So, some might say that most prescription omega-3 fatty acid therapy is being used "off label," with the suggestion that clinicians are engaged in inappropriate prescribing. I feel this is an unfair characterization of clinicians who are trying to do what is in the best interest of their patients.

First, the meaning of the labeling of "indicated" means that the therapeutic has undergone sufficient studies to warrant a specific indicated use, as defined by regulatory agencies. However, the lack of an indicated use is not the

same as a contraindicated use. The label of the currently available prescription omega-3 fatty acid gives no suggestion that it is contraindicated in patients with triglycerides between 200 and 500 mg/dL.

Second, within the prescription omega-3 fatty acid label is a description of the COMBOS trial, which demonstrated lipid efficacy of Lovaza[®] in patients with triglycerides between 200 and 500 mg/dL. I find it difficult to make the case that prescription omega-3 fatty acids are being used "off label" when the use is exactly as described in the FDA approved label.

Finally, it is not solely the opinion of clinicians, but rather the recommendations of international organizations and established guidelines that prompt such use. The National Cholesterol Education Program, Adult Treatment Panel III recommends that once LDL is at treatment goal, if triglycerides are between 200 and 500 mg/dL, then the secondary treatment target is non-HDL cholesterol. Multiple clinical trials support omega-3 fatty acids as reducing non-HDL cholesterol in this patient population. So if a clinician uses prescription omega-3 fatty acid therapy in a way consistent with established guidelines, in a patient population identified in the label of the therapeutic agent, and in a way that the clinician feels is in the best interest of patients, then again, it is difficult for me to characterize this as somehow inappropriate.

One might therefore argue that the contradiction is not how clinicians are using omega-3 fatty acids, but rather how the regulatory agencies worded their indicated use. My sense is that the regulatory agencies are challenged by a preference to go ahead and approve prescription omega-3 fatty acids for patients with triglycerides between 200 and 500 mg/dL, as long as they had some clinical trial data to support such use. Hence, as Dr. Miller mentioned, one might imagine a scenario wherein the FDA might agree that once an omega-3 fatty acid CVD outcomes trial was substantially enrolled, it would grant an indicated use for patients with triglyceride levels between 200 and 500 mg/dL. Such a change in labeling indication would not necessarily require having the outcome results; rather, the study would have to be sufficiently ongoing to obtain such an indicated use. Maybe that is exactly the kind of incentive that is required to finally get the kind of CVD outcome study regulatory agencies want to see, and the kind of CVD outcome study that would be in the best interest of patients and their treating clinicians.

Dr. Brown: I believe you are suggesting that an appropriate indication would actually be a triglyceride lowering indication in that range, not prevention of an acute coronary syndrome.

Dr. Bays: Well, if at the end of 5 to 7 years it showed CVD benefit, then you could get that.

Dr. Brown: Right, but in the interim the indication for triglyceride lowering in this lower range might be approved?

Dr. Bays: That's right.

Dr. Harris: But you'd still have the problem because you could never pin the benefit directly on the triglyceride lowering.

Dr. Brown: There you are.

Dr. Harris: I don't know if we're ever going to be able to do that with any agent we've got.

Dr. Brown: I have been told that the most commonly prescribed dose of omega-3 fatty acids today is one capsule a day. My assumption is that these are physicians who are aware of the GISSI study and believe that it has meaning in their practice. What are the problems with doing that? Should anything be done? Should you feel that this is within the purview of medicine without adequate science?

Dr. Harris: Well, if you look at risk and benefit, you see some evidence for benefit and virtually zero risk.

Dr. Brown: The American Heart has said it would be good for us all to be consuming at least a half gram of omega-3 fatty acids every day. So, perhaps the clinician using this 1-gram dose is assuring that the patient meets that guideline.

Dr. Harris: I think they're well within the purview. The average Japanese intake is about a gram of omega-3 a day.

Dr. Brown: So, there's no harm.

Dr. Harris: There's no harm.

Dr. Bays: Let's be clear. It would represent hubris to suggest that the United States corners the market on what is in the best interest of patients. It may therefore be relevant that at least the last time I reviewed it, in Europe, 1 gram of Lovaza[®] is approved for secondary prevention of postmyocardial infarction, and two to four capsules per day of Lovaza[®] are approved to lower triglyceride levels. So just because prescription omega-3 fatty acids do not have an approved indication in the United States at lower doses does not mean that other regulatory agencies looking at the same data have come to the same conclusion.

Dr. Brown: I think we can say that even though there isn't an FDA approved indication for it, that a low dose may be good medical practice and we don't expect the FDA to express a warning about such dosing.

Dr. Harris: Agreed. It would be hard to justify warnings about giving a lower-than-approved dose of a drug.

Dr. Brown: I think if a clinician simply tells his patient the truth, we don't have the data that are adequate for the FDA to allow the claim of vascular disease prevention but there's a lot of evidence that a gram or so might be beneficial for you. After all, we are talking about a component of a normal diet in many cultures with less vascular disease.

Dr. Harris: Now, whether your payor is going to pay for a prescription that's one-fourth the indicated dose is another question.

Dr. Brown: Maybe we should discuss that issue. In Georgia I haven't found a payor yet who actually reimburses you for omega-3 fatty acids. With some third-party payors, this may have changed recently for the specific treatment of hypertriglyceridemia with the 4-gram dose. Without that indication, they were not covering costs for

the 1-gram dose in the patients with normal triglyceride concentrations.

Dr. Harris: In that case, you might as well just recommend going to Sam's and buying fish oil.

Dr. Brown: Is that the same thing? Is the fish oil that is on the market today safe in terms of heavy metal or other toxins?

Dr. Miller: About 5 years ago, *Consumer Reports* examined data on a number of OTC preparations of fish oil and did not find any appreciable degree of toxins. That may in part reflect again that salmon, as a primary component of these products, has a very low mercury content.

Dr. Miller: I don't think there are any appreciable toxins in these capsules.

Dr. Bays: From a practical standpoint, I do think clinicians should educate their patients on blatantly misleading supplement labels. Sometimes the front label of a fish oil preparation will state "1000 milligrams" right above the words "omega-3 fatty acids." Patients may then logically assume that each supplement fish oil capsule will contain 1000 mg of omega-3 fatty acids. That is not always true. If the bottle is turned around, the ingredients will often list 180 of EPA and a 120 of DHA with a total of 300 milligrams of omega-3 fatty acids. In this case, the "1000 mg" just means it has 1000 mg of fish oils of all types and not 1000 mg of omega-3 fatty acids.

This has cost and tolerability considerations. If a patient desires to substitute over-the-counter omega-3 fatty acids for prescription omega-3 fatty acids, then both the patient and the clinician should ensure that everyone is clear on the amount of consumed omega-3 fatty acids with a given capsule. If the supplement preparation has a total of 300 mg of EPA and DHA, then the patient would need to take 11 or more capsules of the supplement to match the same amount of omega-3 fatty acids in 4 capsules of prescription omega-3 fatty acids. Again, this may have cost and tolerability considerations.

The good news is that from a safety standpoint, fish oil or omega-2 fatty acid supplements are likely to be reasonably safe, particularly if the clinician is willing to sit down with the patient and ensure that the fish oil supplement brand has a United States Pharmacopeia (USP) stamp. Although this is often wrongfully marketed as meaning that the supplement is "pharmaceutical grade," it does provide some reassurance that the supplement has undergone oversight regarding the manufacturing process.

Dr. Brown: There are preparations now that are sold over the counter with a very high percentage of omega-3 fatty acids.

Dr. Harris: There are some preparations that have more omega-3 per capsule than Lovaza. It's a bigger capsule.

Dr. Miller: One of the tricks of the trade that I use is unless they're taking Lovaza is I have them freeze or in some cases put in a refrigerator. That tends to at least attenuate some of the eructation symptoms, and also if there is any concern about oxidation, it may lessen it. So, that's my recommendation.

Dr. Harris: Taking fish oil capsules with food or taking them at bedtime are other ways to reduce the burping.

Dr. Brown: This can be a problem. I have had a number of patients who will not tolerate that taste.

Dr. Harris: I agree.

Dr. Bays: But let's be clear. The prescription omega-3 fatty acids are so purified and without the kinds of other things that the rate of eructation is negligible if not absent. Again, especially if they have undergone the rigorous testing necessary to achieve a prescription status, omega-3 fatty acids are efficacious, safe, and generally well tolerated.

Dr. Brown: Are there any other suggestions for the practical use of these that should mention to physicians?

Dr. Harris: I think we covered a lot of it.

Dr. Brown: This has been a very interesting session for me. I sincerely appreciate your answering my questions. I know that many clinical lipidologists find the data surrounding omega-3 fats to be confusing. I believe your discussion will clarify major points in the science behind recommending these and will also provide helpful guidance for their successful use in their patients.

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Editorial Article

Biographical notes on Ancel Keys and Salim Yusuf: Origins and significance of the Seven Countries Study and the INTERHEART Study

KEYWORDS:

Ancel Keys;
Salim Yusuf;
Cholesterol;
Risk factors;
Coronary artery disease;
Prevention;
Lifestyle

Abstract: Ancel Keys and Salim Yusuf are both pioneers in preventive cardiology. Each overcame significant obstacles to demonstrate, through large international studies, how culture and environment influence cardiovascular disease. This paper will explore the origins and outcomes of their landmark studies: the Seven Countries Study, a prospective cohort model, and the INTERHEART Study, a case-control model. Each study advanced our understanding of the interplay between lifestyle, culture, and heart disease.

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Paul Dudley White, cardiologist to President Dwight D. Eisenhower, who suffered a heart attack after 27 holes of golf on September 23, 1955, once said “A heart attack after age eighty is the work of God, before age eighty, a medical failure.”¹ According to the American Heart Association,² over 50% of the heart attacks in America occur before age 75 (for men, almost 70% are before age 74, whereas for women, the figure is 40%). Our current understanding of how modifiable risk factors can promote or prevent heart disease owes much to two men who lead original and ambitious international field studies with limited resources, motivated by the desire to prevent heart attacks.

The first man, Ancel Keys, came to be known as “Mr. Cholesterol.” His major study tracked over 12,000 men in seven countries for 10 years.³ Published in 1970, it concluded that saturated fat in the diet and blood cholesterol levels were the principle risk factors for heart disease. The American Heart Association endorsed these findings and through effective public campaigns beginning in the 1970s, succeeded in significantly reducing saturated fat and cholesterol in the American diet.

The second man, Salim Yusuf, conceived and organized a study across 52 countries with 29,972 subjects. Instead of observing his subjects over time, like Keys, he and his team enrolled 15,152 at their presentation to the hospital with a first heart attack, and 14,820 as random age and gender-matched subjects categorized by not having any evidence of heart disease. Those with heart attacks were “cases,” and those without heart disease were “controls.” Yusuf’s study,

called INTERHEART,⁴ published in 2004, showed that the risk for heart attacks was due to nine modifiable risk factors, which, if effectively controlled, would prevent over 90% of first heart attacks around the world.

Ancel Keys

Keys was born in Colorado Springs in 1904. His parents were teenagers with no formal education.⁵ In search of a better life, they moved to California and settled in Berkeley. Ancel’s early interest in science became evident when, at his eighth birthday party, he tried to chloroform a fly and inhaled enough fumes to lose consciousness himself.

After finishing college in 3 years with honors, Keys worked briefly at Woolworths, then returned to Berkeley and earned an MS in Biology, followed by a PhD from the Scripps Institute of Oceanography. In 1930 he traveled to Copenhagen to work with Nobel laureate August Krogh, studying the ability of eels to survive in both fresh and salt water environments. He then went to Cambridge and earned a second PhD in animal physiology from King’s College.

When Keys returned to the United States in 1933, he joined the Fatigue Laboratory at Harvard, renowned for its research in exercise physiology. His first project was a study on the effects of altitude on blood oxygenation,⁶ which he believed might have practical benefits for Chilean copper miners. He took a small group down to the Chilean Andes and gradually acclimatized to an altitude of 22,000

feet, collecting blood samples on himself and others along the way. Reminiscing in 1979 for the University of Minnesota newsletter, he said “We had a little snow shelter—put up a few poles and blankets over them—and crawled in there to get out of the wind and cold. At night the temperature dropped to 50 below. We didn’t do much cooking, of course. Through it all I lost a little weight but wasn’t sick in any important sense, though of four others who came up from time to time two were very sick. One of them was John Talbott, who later was editor of the Journal of the American Medical Association.... We had an awful time getting him down. He was not blue but black with gas pain and retching. We thought he was going to die.”

Keys’ work eventually came to the attention of the United States War Department. In 1939 he was asked to develop and test a food ration for parachute troops. By this time, he had established the Laboratory of Physiologic Hygiene at the University of Minnesota. It was from this location, under the bleachers of the football stadium, that he would conduct studies on human nutrition over the next several decades. His first project for the War Department would produce K-rations (“K” for Keys). The little meals, eventually assembled by the Cracker Jack Company, achieved iconic status as America’s World War II soldiers’ food. Each waterproof box of 3200 daily calories contained meal units for breakfast, lunch, and dinner. A tin could contain meat or cheese, biscuits, a chocolate bar and hard candy, coffee, lemon or soup powder, chewing gum, toilet paper, and cigarettes.

As millions of people across the world suffered the consequences of disrupted food supplies, Keys began a research project called the Minnesota Starvation Experiment.⁷ He wanted to identify the effects of semistarvation on the human body, and then define nutritional techniques for restoring health in the aftermath of starvation. His subjects were chosen from conscientious objectors at the Civilian Public Service work camps. The brochure used to recruit potential participants was entitled “Will you starve that they be better fed?”

The final results of the experiment filled 1385 pages in a two-volume tome entitled, “The Biology of Human Starvation,” published in 1950. The book became a classic in the field of human starvation. It was the first study to show how starvation can profoundly alter personality, inducing depression, apathy, and obsessive behavior. It was the first study to document the effects of food deprivation on physiologic parameters such as blood pressure, basal metabolism, serum cholesterol levels, and resting heart rate. The rehabilitation phase of the study showed that up to 4000 calories a day for several months would be needed to build up tissues that had wasted away. Perhaps most importantly, Keys came away from the study with a new understanding of the complex interactions between nutrition and human health. Ironically, his great future contributions would be focused on the effects of diets with too much fat and cholesterol, as he now turned his attention to the epidemic of heart disease in affluent middle-aged American males.

Keys’ work on starvation gave him access to data on health and disease in post-war Europe. A finding that intrigued him was the dramatic drop in heart attacks that occurred in countries where the populations had been deprived of their typical high fat, high calorie diets. This trend quickly reversed as the countries recovered during the post-war period. He was also puzzled by the new epidemic of heart attacks in affluent middle-aged businessmen. He suspected that dietary factors, particularly saturated fat, might play a key role in clogging the coronary arteries. To establish the connection between saturated fat and blood cholesterol levels, he conducted a series of carefully designed experiments where subjects were fed diets with varying amounts of saturated fat, polyunsaturated fat and cholesterol. He was able to derive an equation, called the Keys Equation:

$$\Delta\text{chol} \left(\frac{\text{mg}}{\text{dl}} \right) = \left(2.16\Delta\% \text{sat. fat kcal.} - 1.3\Delta\% \text{polyunsat. fat kcal.} + \sqrt{1.5 \text{ dietary chol. (mg)}} \right) \div \frac{1000 \text{ kcal}}{\text{day}}$$

The equation predicted a 2.7 mg/dl rise in cholesterol for every 1% of calories derived from saturated fat. The equation also showed that polyunsaturated fat lowered serum cholesterol, and dietary cholesterol raised serum cholesterol, but to a lesser extent than saturated fat. This equation remains one of the most important in nutritional science.

Keys then proceeded to design a new kind of trial for teasing out the causes of coronary heart disease. The concept of risk factors did not yet exist. In 1947 he proposed to enlist 283 businessmen and professionals from Minnesota, between the ages of 45 and 55. Keys explained the rationale for the study as follows: “The aim of this study was to find individual characteristics in apparently healthy middle-aged men related to the future tendency to develop coronary heart disease, an aim based on the conviction that the physico-chemical characteristics of the individual should have predictive value”.

The Minnesota men were followed for 40 years, when only 54 remained alive. The most frequent cause of death was heart disease. Keys’ results showed that men with entry cholesterol levels greater than 260 were four times more likely to suffer a heart attack compared to men with cholesterol levels below 200.⁸

In 1951 Keys took his family to Oxford for a 1-year sabbatical. While there, an Italian colleague bragged that heart disease was almost nonexistent in Italy. Keys was skeptical. He took his wife, Margaret, a biochemist who often worked at his side, and set up a portable laboratory in Naples. He soon verified the low incidence of heart disease, and also found that Neapolitans had very low serum cholesterol levels. Keys and his wife then visited several European and African countries to measure cholesterol levels and

conduct diet surveys. Gradually a pattern emerged, suggesting that diets rich in saturated fats, by raising serum cholesterol, were a principle cause of coronary heart disease.

In the 1950s, the notion of diet causing heart disease was controversial. Keys presented his ideas for the first time in an international setting at the World Health Organization in 1955. His ideas were met with skepticism. Sir George Pickering, the Oxford physician who was one of the world's experts on hypertension, challenged him: "Yes, and Professor Keys would you be kind enough to cite for us the principle piece of evidence that you think supports this diet-heart theory of yours?"³ He was unable to defend his position against Pickering's skepticism. He came away from the meeting humiliated, but all the more motivated to prove his theory. He would spend the next 15 years designing, implementing, and analyzing the Seven Countries Study.

Between 1955 and 1958 Keys began to organize teams of clinicians and scientists in several countries. The study would require fieldwork that took researchers deep into the lifestyles of predominantly rural populations. It required the transportation of bulky medical equipment, such as electrocardiograph machines that were much larger and more primitive than those in use today. For each area chosen, the entire male population between the ages of 40 and 59 had to be convinced to participate. Once the data was collected, it was sent to back to "Gate 27," the nickname for Keys laboratory under the bleachers at the University of Minnesota stadium. The techniques that were developed eventually formed the basis for a manual published by the World Health Organization on cardiovascular disease survey methods. From the thousands of electrocardiograms that were collected and sent to Gate 27, the team developed a precise system for coding electrocardiographic abnormalities. It came to be known as the Minnesota Code, and is still one of the most widely used coding systems for population studies and clinical trials.

Alessandro Menotti gave one example of the challenges the field workers faced with their primitive electrocardiograms when he reminisced at the 35th Anniversary Conference of the Seven Countries Study in Fukuoka, Japan, in 1993: "In the mornings between 9:30 and 10:30 the electrocardiograms were terrible because of AC interference. After three days of this, we discovered that in the basement of our building was a kitchen. At that moment each day, the cook started an enormous potato-peeling machine, and the electrical fields created were disturbing the ECG recording. We had to call back the men who had been seen during those hours, because their records weren't readable at all by the Minnesota Code!"⁹

The countries included in the study were Yugoslavia, Italy, Greece, Finland, the Netherlands, the United States, and Japan. Why were these particular countries chosen? Yugoslavia offered coastal and inland populations with vegetable fat versus animal fat diets. Italy represented a prototypical Mediterranean lifestyle, with a diet full of grains, pasta, legumes, fruits and vegetables, olive oil, bread, and wine. Greece provided a setting with very high

dietary fat intake, principally in the form of olive oil, but very little saturated fat. Finland had an exceptionally fit population, but high rates of heart disease, and a diet extremely high in saturated fat. The Netherlands represented a European population with an intermediate dietary pattern, with meat, butter, and tuberous vegetables. The United States component consisted of railroad workers, originally chosen to study the effects of different activity levels on heart disease, but then incorporated into the Seven Countries Study because the participants tended to remain in one place over time, making follow-up relatively simple. Finally, Japan was chosen as representing a lifestyle with minimal dietary fat intake.

The chief operations officer for the study was a young doctor named Henry Blackburn. He had joined Keys' laboratory in 1956, just after completing his medical fellowship. During the many months of field work, he noted his impressions in a personal journal.¹⁰ These writings provide an invaluable picture of the trials and tribulations encountered by the teams of clinicians and technicians, as they worked in primitive conditions with limited resources.

The Seven Countries Study formally began September 28, 1958 at the Hotel Jadran in Makarska, on the Adriatic coast. Two regions had been chosen in Croatia: coastal Dalmatia, and Slovenia, further inland. Keys was interested in seeing how disease rates would be affected by the primarily vegetable (low saturated) fat diet on the coast compared to the inland animal (high saturated) fat diet.

For 3 weeks the little band of scientists moved down the Adriatic coast, setting up their station in each village between Makarska and Dubrovnik. They encountered the wind known as the Bura, which would sweep down from the mountains to the sea, taking anything in its path that was not firmly anchored. When they hung cholesterol specimens on filter paper to dry, they found that the microscopic smudges left by flies contained enough cholesterol to throw off their results.

Today an electrocardiogram (ECG) can be done in 2 or 3 minutes. But in 1958, the ECG had to be done using brine-soaked bits of cotton, while the technicians awaited the arrival of electrode paste from America. Each of the 12 leads that make up a standard electrocardiogram was traced onto separate strips, which were then cut and stapled onto cardboard. Because of problems with local electricity, a U.S. Army surplus gasoline generator was frequently used to run the electrocardiographs and centrifuge machines.

Once they had finished with all males between the ages of 40 and 59 in one village, the team would break camp, hop a ferry, and head for the next location. Gradually they made their way from Tucepi down to Gradac. When the last of the coastal villages was finished in mid-October, the team moved inland.

Slovenia was described by Blackburn as a grim land, with poor villages surrounded by fertile plains and abundant livestock. Wealthy farmers were distrustful of government and men of science. They feared having their taxes increased if word got out of their rich diet. Peasants were suspicious and

resentful. A constant rain fell. Blackburn had to take the electrocardiograph to Paris for repairs. The testing center was damp from incessant fog and was surrounded by geese and pigs. But by November 3, the work was done and all eligible men had been enrolled except for one, who was away at sea.

The experience in Croatia provided a solid foundation for the rest of the Seven Countries Study. Challenging conditions required ingenious solutions, and the manual of procedures evolved into a roadmap that would become a template for similar studies. The teams returned to Croatia again in 1963 and in 1968, for the 5- and 10-year follow-ups.

From 1958 to the early 1970s, the Seven Countries team built their database, translating cultural differences into a set of equations that could predict heart disease.¹¹ Five years and 10 years after the initial visits the teams returned for follow-up visits with the participants, identifying those who had experienced a heart attack. The lowest rates were found in Crete, the highest in East Finland. The second highest rates were in the American cohort, the railroad workers. After Crete, the lowest rates were in Japan, followed by Corfu. Comparing East Finland to Crete, there was an almost 100-fold increase in the incidence of heart attacks. On average, 3.2% of the participants experienced heart attacks over the 10-year study period. In Crete, the rate was 0.1%, whereas in East Finland it was 9.5%. The U.S. railroad workers had a rate of 5.7%, whereas Japanese fishermen had a rate slightly under 1%.

The variables that were measured were smoking, overweight status, physical activity, resting pulse rate, lung capacity, blood cholesterol level, blood pressure, and diet. Keys and his team used multivariate logistic analyses to build mathematical models that combined all the measured variables and then determined which ones were related to heart attack rates.

Seven Countries was the first study ever done providing prevalence rates of heart attack and stroke in contrasting cultures. Even at the beginning, during enrollment, striking differences were found in rates of disease. In Japan, only 0.3% had evidence of heart disease at entry, whereas in the United States, 4.6% did. The study also was the first to describe population differences in risk factors. For example, the bell-shaped curves describing serum cholesterol levels in Eastern Finland and in Japan were so far apart there was almost no overlap. In Finland, 77% of the population had cholesterol levels over 200, whereas in Japan only 3% were in this range. Diets between countries varied between 3% and 22% of calories from saturated fat, and between 9% and 40% for total fat calories.

Saturated fat as a percentage of calories was the most powerful lifestyle predictor of heart disease. Blood cholesterol was the most important physiologic variable, explaining 40% of the variation in heart attack rates. Keys' equation had shown that cholesterol level could be predicted by saturated fat intake. The second most significant risk factor for heart attacks was high blood pressure. Blood cholesterol and high blood pressure together accounted for 60% of heart disease risk.

Keys was the first medical scientist to understand the importance of the Mediterranean diet. Together with his wife he wrote "Eat Well and Stay Well,"¹² and "How to Eat Well and Stay Well the Mediterranean Way." He lived for many years in Italy, following his own advice. He died at the age of 100. His obituary appeared in the New York Times on November 23, 2004,¹³ and was written by Jane Brody, the well-known medical journalist.

Henry Blackburn, in a tribute to Keys, wrote: "He has had a major influence on the public, its food choices and eating patterns. We physicians and fellow scientists learned from him that if common diseases are the result of mass behaviors there is a social responsibility for us to address these larger issues in practice and in our communities.... He showed us that there is a personal responsibility to model behavior so as to provide an example of healthful living to our families and our patients. This is the large legacy of Minnesota's senior scientist, Ancel Keys."¹⁴

Salim Yusuf

Dr. Salim Yusuf is a soft-spoken yet intense cardiologist based at McMaster's University in Hamilton, Ontario.* In 2004 he published the results of the INTERHEART Study, which concluded that 90% of heart attacks were due to nine modifiable risk factors. Dr. Yusuf grew up in India, and in 1976 received a Rhodes Scholarship to Oxford University. His joy at receiving this honor was cut short by the premature death of his father at age 56 from coronary heart disease. He acknowledges that on an unconscious level this loss played a role in the evolution of the INTERHEART Study. In 1984 he moved from Oxford to the National Institutes of Health, where he worked in the clinical trials branch of the National Heart Lung and Blood Institute. In 1992 he came to McMaster to set up the new Division of Cardiology. He served as its Chief of Cardiology for over a decade, and currently occupies the Heart and Stroke Foundation Chair in Cardiology and is Director of the Population Health Research Institute.

Around the time that he moved to Canada, Dr. Yusuf began to collaborate with his former colleague Dr. Prem Pais, now the Dean of St John's Medical School in Bangalore. Although he did not realize it at the time, the relatively small study they conducted together would become a pilot for the INTERHEART Study. Dr. Yusuf wanted to explore the causes of premature heart disease in India. With little money available, he could not conduct a trial like Seven Countries, known as a prospective cohort study. Large prospective cohort studies became the dominant model for studying the epidemiology of heart disease as a result of the Framingham Heart Study.

* Dr. Wright interviewed Dr. Yusuf, who kindly provided many personal biographical insights as well as updated information on the INTERHEART study.

Dr. Yusuf, however, did not have the funds for this kind of study. He needed a method that would provide meaningful results with several hundred participants, not several thousand, enrolled over 2 to 3 years, and not followed for 2 to 3 decades. He decided to use a method called a case-control study. The concept is simple: subjects are enrolled when they present to the hospital with a first heart attack. Then someone of the same age and gender is identified who is free of heart disease. The former is the "case" and the later is the "control." Baseline data is collected on the cases and controls. Unlike a cohort study, where only a small percentage of the total participants have a cardiac event, half the participants in a case-control study by definition have the disease, making it easy to see significant differences between the cases and the controls. In Seven Countries, by contrast, the rate of heart attacks over 10 years was 3.2%, meaning only 408 of 12,763 subjects had a definite coronary event, whereas 96.8% had no event. The small number of events can make it difficult to identify weaker risk factors.

A major challenge for a case-control study, according to Dr. Yusuf, is the selection of the control. Extreme care must be taken to assure that the controls have no heart disease. Often the controls were chosen from patients hospitalized at the same time as the case. Controls could not have emphysema, for example, because emphysema is usually caused by cigarette smoking, which increases the likelihood of heart disease. Even someone admitted for a hernia repair would be disqualified, because hernias are more common in smokers, who cough more. An acceptable control would be someone admitted for acute trauma, or for an elective procedure such as ear surgery. Another challenge for Dr. Yusuf was a psychological one, known as recall bias. People who have just had a heart attack tend to distort their memories about diet and exercise, and their perceptions of stress levels before the event. Field workers have developed techniques for minimizing recall bias.

The small Indian case-control study was a success. It was finished in 3 years. The results were published in the British medical journal the *Lancet* on August 10, 1996.¹⁵ With only 400 subjects, the study clearly demonstrated that the strongest predictor of a first heart attack in Bangalore was cigarette smoking. High blood pressure, blood sugar level, and abdominal obesity were also associated with higher risk. Cholesterol levels were not a risk factor. Vegetarianism and higher socioeconomic status were protective.

During the 1990s, cardiovascular disease was becoming a global epidemic. Dr. Yusuf believed that the case-control model he used in Bangalore might well be standardized for use across many countries. A global prospective cohort study, like Seven Countries or Framingham, would be impossible to do, as it would require hundreds of thousands of subjects followed for 5–10 years. Therefore, the case-control method seemed to be a reasonable alternative. He believed the study would identify different risk factors at work in different countries. He also believed, along with many experts, that risk factors would only be able to

explain about half the risk, with unknown genetic factors accounting for the rest.

INTERHEART began with a \$25,000 grant from Merck Pharmaceuticals. With this seed money, Dr. Yusuf convened a meeting of former collaborators from around the world, and described the INTERHEART concept: the largest study ever done to map out the global causes of cardiovascular disease. From this humble beginning, the concept slowly built momentum. Little by little he added countries and medical centers. Recruitment remained low until he gave an impassioned plea at a large international conference nearly a year after the Merck-funded meeting. Dr. Yusuf used his considerable persuasive skills and international reputation to galvanize the crowd of medical scientists representing dozens of countries. From then on, INTERHEART became a movement of committed researchers, willing to work almost for free. Final funding permitted payment of around \$50 per subject enrolled, but many researchers participated for only \$15 per patient. People signed on because they believed in Dr. Yusuf's vision. Was there a chance to really understand the causes of heart disease at work in different parts of the world? Could the global burden of heart disease be reduced by finding these causes? In the end, 252 centers in 52 countries participated. Funding ultimately came from 41 separate sources, and totaled \$2.5 million, a paltry sum for such a large and demographically complex study. But the true cost of the study will never be known, because all the participants donated so much of their time for free. Dr. Yusuf estimates that he visited about half of the 52 countries, but only went to a participating center if it was for another study, and then stayed an extra day or two to work on INTERHEART. Through years of conducting international clinical trials, Yusuf had built up a network of clinical researchers who were friends and collaborators, and who now supported and promoted his cause. In some cases, they would only ask for a few dollars to rent freezer space for blood samples, and do all the rest for free, or seek a local funding source. Yusuf described the funding process as "quilt work built up from the trenches."

Dr. Yusuf's grant proposal for the study was based on the hypothesis that different risk factors would be important in different parts of the world. He believed that there would be differences between developing and developed countries, or between Eastern and Western or Northern and Southern countries. His second hypothesis was that only about 50% of the risk would come from known and modifiable risk factors. He hoped to discover a number of novel risk factors, especially in non-Europeans/non-Americans. The final results, quoting Yusuf directly, "blew our minds." He never expected that the risk factors would be virtually the same everywhere, nor that over 90% of the risk would be modifiable, meaning preventable.

INTERHEART involved the collection of data from 52 countries, including people from China, South and Southeast Asia, Africa, Europe, North and South America, the Mideast, and Australia. To keep costs down, data

collection was limited to a simple, eight-page questionnaire, blood samples, and anthropomorphic measurements. The amount of data collected was so great that papers will continue to be published for at least another decade.

To examine the population effect of a risk factor, the study, in addition to calculating odds ratios, also looked at “population attributable risk” or PAR. PAR represents the impact of a risk factor on the entire population’s risk. If the risk factor were not present at all in the population, by how much would one lower the total population’s risk for a first heart attack?

Overall, the average age for presentation with a first heart attack was only 58 for men, and 65 for women. North Americans were close to this average, with men presenting at age 59 and women at 64. The youngest victims were in the Middle East, with men presenting at 51 and women at 57. The oldest were in China and Western Europe, where men were 63 and women were 67 and 68, respectively. Why these age differences? Are there genetic reasons, or can all the variability be explained by known risk factors? INTERHEART demonstrates that these age differences are entirely due to the prevalence of the nine modifiable risk factors.

An individual with all nine risk factors has 129 times the risk for a first heart attack compared to someone with none of the risk factors. If all nine risk factors were removed from a population, there would be a 90% reduction in the number of first heart attacks (based on the PAR calculations).

INTERHEART showed that the most important risk factor is the ApoB/ApoA1 ratio. ApoB is the structural protein in the spherical particles circulating in our blood that can carry cholesterol into the wall of an artery. Measuring the quantity of ApoB protein in the blood is more predictive of risk than measuring LDL cholesterol.¹⁶ Conversely, ApoA1 is the structural protein in HDL (high density lipoprotein). ApoA1 is a better measure of protection than HDL cholesterol. The ratio of ApoB/ApoA1 is about twice as accurate in risk prediction as the ratio of LDL cholesterol to HDL cholesterol. In INTERHEART, those with the worst ApoB/ApoA1 ratio had 3.87 times the risk of those with the best ratio. If everyone in a given population had the best ratio, population risk would be reduced by 54%.

The second strongest risk factor identified was diabetes. Those with diabetes had three times the risk of those without diabetes. Because the number of people with diabetes is much less than the number of people with abnormal ApoB/ApoA1 ratios, eliminating diabetes would only lower population risk by 12.3%.

Cigarette smoking was the next strongest risk factor. A current smoker had 2.95 times the risk of someone who never smoked, whereas a former smoker had 2.27 times the risk. If no one smoked in the entire studied population, heart attack rates would have been reduced by 36.4%. Yusuf was able to calculate risk based on the number of cigarettes smoked a day. The relationship between number of cigarettes and risk is linear. Each cigarette confers a little more risk. Someone who smoked seven to eight cigarettes a day

doubled his risk, whereas someone who smoked 21 or more cigarettes a day experienced a six-fold increase in risk.

The next risk factor surprised many experts. Stress as a cardiac risk factor is difficult to measure and therefore to prove. Based on a large literature suggesting its importance, Yusuf decided to include a psychosocial stress assessment tool in the questionnaire. He graded responses to questions about stress in the home or workplace, general stress, financial stress, stressful life events, feelings of depression, and the degree to which one felt in control of one’s life. Those with the most stress had 2.5 times the risk of those with the least, and if stress were not present in a population (certainly an unrealistic assumption!), the incidence of heart attacks would drop by 28.8%.¹⁷

Hypertension increased risk by 2.48%, and its removal lowered heart attack incidence in a population by 23.4%. The final risk factor was abdominal obesity. Yusuf determined that the ratio of waist circumference to hip circumference was a much better predictor of risk than the traditionally used body mass index (BMI). BMI is used to classify people as normal weight, overweight or obese, but does not distinguish between muscle and fat, or between different frame sizes. BMI category was a very poor predictor of risk. Waist/hip ratio was a very good predictor. Those with the highest ratio had 2.24 times the risk of those with the lowest ratio, and removing this risk factor reduced heart attack rates by 33.7%. Not only were smaller waists protective, but bigger hips also conferred protection. Dr. Yusuf was unable to provide a scientific explanation for the so-called “hip factor.”

Besides the above-described six modifiable risk factors, there were three protective factors: eating vegetables and fruits daily, exercising, and having alcohol in moderation. Each reduced risk by 20% to 30%, and in combination reduced heart attack rates by a bit over 50%.

The conclusion of the study, as published in the *Lancet*, on September 3, 2004, states: “Our study has shown that nine easily modifiable risk factors are associated with more than 90% of the risk of an acute myocardial infarction in this large global case-control study. These results are consistent across all geographical regions and ethnic groups of the world, men and women, and young and old. Although priorities can differ between geographical regions because of variations in prevalence of risk factors and disease and economic circumstances, our results suggest that approaches to prevention of coronary artery disease can be based on similar principles throughout the world. Therefore modification of currently known risk factors has the potential to prevent most premature cases of myocardial infarction worldwide.”²

The results of INTERHEART confirm Paul Dudley White’s earlier quoted words describing a heart attack before age 80 as a medical failure. According to Dr. Yusuf, a close look at all the known genetic factors for heart disease shows their influence on total risk to be modest. The well-known difference in risk levels for men and women can also be explained based on the prevalence of the nine modifiable risk factors. The risks between different ethnic

groups are entirely related to these same risk factors. Further analyses of the data have improved the population attributable risk to the 95% range, by refining the diabetes score, the diet risk score, and the quantification of sedentary versus active lifestyles. This means that all but 5% of heart attacks are potentially preventable.

Dr. Yusuf continues to work passionately on the goal of preventing cardiovascular disease. His team published in the *Lancet* on July 10, 2010 another global study called INTERSTROKE, a case-control study in 22 countries with 6000 subjects, half presenting with a first stroke and half serving as the controls.¹⁸ This time, 10 modifiable risk factors were found to account for 90% of the risk for stroke. He is also working on ways to translate his findings into public policy initiatives around the world that would reduce the prevalence of these modifiable risk factors. He has drilled down into his substantial database to look at how owning a car and a television affect risk (each confers about a 47% increase in risk). He has analyzed leisure time physical activity and found that those in the highest category have 45% lower risk. His dietary risk score has been further refined to identify three dietary patterns around the world: *Oriental*, which is high in tofu, soy, and other sauces, *Prudent*, with higher intake of fruits and vegetables, and *Western*, with higher intakes of fried foods, salty snacks, eggs, and meat. The prudent diet is associated with a 30% lower population risk, whereas the Western diet raises population risk by 35%. The Oriental pattern is neutral, probably because the higher intake of fruits and vegetables is offset by other factors, such as high salt intake.

Conclusion

Ancel Keys' and Salim Yusuf's landmark studies across many cultures and ethnic groups confirmed the universality of the major cardiovascular risk factors. Both scientists overcame significant obstacles to accomplish objectives they believed in passionately. Their results advanced our understanding of the complex interactions between lifestyle and heart disease. Keys carried out the first international study of cardiac risk factors. He extended basic research in lipid nutrition to large scale field studies that corroborated the relationships between lipid intake, cholesterol levels and heart disease. Yusuf used the case-control field study method on a global scale and demonstrated the same risk factors at work around the world in many different ethnic groups. His use of population attributable risk showed that

interventions to lower risk could prevent 90% or more of heart attacks. As we try to intervene to reduce the worldwide epidemic of cardiovascular disease, the work of these two scientific pioneers gives hope that prevention is a realistic and achievable goal.

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By Gyorgy Csako, M.D.

Title: Rosuvastatin does not affect human apolipoprotein A-I expression in genetically modified mice: a clue to the disputed effect of statins on HDL.

Authors: Marchesi M, Parolini C, Caligari S, Gilio D, Manzini S, Busnelli M, Cinquanta P, Camera M, Brambilla M, Sirtori CR, Chiesa G.

Journal: Br J Pharmacol. 2011 Nov;164(5):1460-8.

Comment: In addition to significantly reducing low-density lipoprotein (LDL) cholesterol, statins tend to moderately increase high-density lipoprotein (HDL) levels. *In vitro* studies have suggested that this effect may be the result of an increased expression of apolipoprotein (apo)A-I, the main protein component of HDL. The aim of the present study was to investigate *in vivo* the effect of rosuvastatin on apoA-I expression and secretion in a transgenic mouse model for human apoA-I. Human apoA-I transgenic mice were treated for 28 days with 5, 10 or 20 mg/kg/day of rosuvastatin, the most effective statin in raising HDL levels. Possible changes of apoA-I expression by treatment were investigated using quantitative real-time RT-PCR on RNA extracted from mouse livers. The human apoA-I secretion rate was determined in primary hepatocytes isolated from transgenic mice from each group after treatment. Rosuvastatin treatment with 5 and 10 mg/kg/day did not affect apoA-I plasma levels, whereas a significant decrease was observed in mice treated with 20 mg/kg/day of rosuvastatin (-16%, $P < 0.01$). Neither relative hepatic mRNA concentrations of apoA-I nor apoA-I secretion rates from primary hepatocytes were influenced by rosuvastatin treatment at each tested dose. Thus, in human apoA-I transgenic mice, rosuvastatin treatment does not increase either apoA-I transcription and hepatic secretion, or apoA-I plasma levels. These results suggest that mechanisms other than the increased expression of apoA-I may account for the observed HDL increase induced by statin therapy in humans.

Title: Behaviour of human erythrocyte aggregation in presence of autologous lipoproteins.

Authors: Saldanha C, Loureiro J, Moreira C, Silva JM.

Journal: Biochem Res Int. 2012;2012:261736.

Comment: Several clinical studies evidenced associations between complex lipid macromolecules; for example, high LDL-C concentrations and blood

rheological behavior, like blood hyperviscosity, that are both referred to as cardiovascular risk factors. Blood viscosity is dependent on macro-(hematocrit and plasma viscosity) and micro-(erythrocyte deformability and aggregation) hemorheological parameters. Disturbances in blood rheological behavior, such as high blood and plasma viscosity and increased erythrocyte aggregation tendency, have been described in patients with ischemic heart diseases. Red blood cells (RBCs) participate in acute coronary occlusion, mainly under conditions of lower shear rate, for example, within the microcirculation in the peri-infarct domain of myocardium. Under *in vitro* stasis conditions, RBCs in normal human blood form loose aggregates with a characteristic morphology, similar to a stack of coins, that is described as rouleaux formation. After prolonged stasis, individual rouleaux can cluster, thereby forming 3-dimensional structures. In the circulation, the attractive forces involved are relatively weak, and the aggregates can be dispersed during flow by the shear rate. Increasing RBC aggregation at low shear rate affects blood viscosity and microvascular flow dynamics and both are markedly enhanced in certain clinical conditions. Factors influencing RBC aggregation can be (i) *extrinsic factors* such as levels of plasma proteins (fibrinogen, lipoproteins, macroglobulins, or immunoglobulins), hematocrit, and shear rate, and (ii) *intrinsic factors* such as RBC shape, deformability and membrane surface properties. RBC membrane surface properties and structure, such as surface charge and the ability of macromolecules to penetrate the membrane glycocalyx, greatly affect aggregation of cells suspended in a defined medium. Different studies have shown that hyperlipoproteinemia is associated with RBC hyperaggregation. The inverse correlation of erythrocyte aggregation with HDL₂-C subfraction was reported in a hypercholesterolemic middle-aged male population without apparent symptoms of cardiovascular disease. It was evidenced *in vitro* that LDL-C enhances the RBC aggregation induced by fibrinogen in aggregation models. The aim of this work was to evaluate the effect of autologous plasma lipoprotein subfractions on the aggregation of RBCs *in vitro*. Aliquots of anticoagulated whole blood samples obtained from 10 healthy fasting adult male volunteers were enriched with their own HDL-C, LDL-C, or



VLDL-C fractions obtained from the same batch by density gradient ultracentrifugation. Plasma osmolality and erythrocyte aggregation index (EAI) were determined. Blood aliquots enriched with LDL-C and HDL-C showed significantly higher EAI than untreated aliquots, whereas enrichment with VLDL-C did not induce significant EAI changes. This may be explained, at least in part, by differing binding abilities of these lipoprotein particles to the RBC membranes. For the same range of lipoprotein concentrations (under the same number of particles), expressed as percentage of osmolality variation, the EAI was higher in the presence of HDL-C than LDL-C ($P < 0.01$) and EAI generally increased with increasing particle number. It was concluded that particle size, up to LDL diameter values, promotes the tendency of RBCs for aggregation at the same plasma osmolality (particle number) range of values. Additional studies are needed to establish whether the cross-bridging or the depletion layer model fits the effects of lipoproteins in RBC aggregation.

Title Establishment of chemiluminescence enzyme immunoassay for apolipoprotein B-48 and its clinical applications for evaluation of impaired chylomicron remnant metabolism.

Authors: Hanada H, Mugi S, Okubo M, Maeda I, Kuwayama K, Hidaka Y, Kitazume-Taneike R, Yamashita T, Kawase R, Nakaoka H, Inagaki M, Yuasa-Kawase M, Nakatani K, Tsubakio-Yamamoto K, Masuda D, Ohama T, Matsuyama A, Ishigami M, Nishida M, Komuro I, Yamashita S.

Journal: Clin Chim Acta. 2012 Jan 18;413(1-2):160-5.

Comment: Apolipoprotein B-48 (apoB-48) is a constituent of chylomicron remnants synthesized in the small intestines. The serum concentration of apoB-48 at fasting has been reported to be a marker of postprandial hyperlipidemia, a presumed risk factor for atherosclerosis. The authors evaluated the analytical performance of a recently developed chemiluminescent enzyme immunoassay (CLEIA). Using multiple regression analysis, they also examined the influence of other serum lipid values, age, sex, smoking, drinking status and BMI on serum apoB-48 values in 273 clinical samples. Regarding the performance of the CLEIA, within-run and between-run precisions were 1.7-2.7% and 1.2-7.3%, respectively. Limit of detection was 12.5 ug/dL with

linearity up to 3,000 ug/dL. The recovery of apoB-48 ranged from 96.3 to 103.5% in 1:9 diluted sera after adding recombinant apoB-48 at concentrations of ~1,510 ug/dL and ~3,990 ug/dL. The CLEIA highly correlated with a previously validated in-house ELISA ($r=0.953$, $y=1.02 \times -1.59$). Serum apoB-48 concentrations were higher in males than in females, and were correlated with smoking status as well as with remnant-like particle-cholesterol (RLP-C) concentrations. Patients with the metabolic syndrome showed higher values of serum apoB-48 compared with control subjects. The authors concluded that serum apoB-48 measurement by CLEIA is satisfactory for clinical use to assess abnormalities in the chylomicron remnant metabolism.

Title: Fasting Serum Apolipoprotein B-48 Can be a Marker of Postprandial Hyperlipidemia.

Authors: Masuda D, Sakai N, Sugimoto T, Kitazume-Taneike R, Yamashita T, Kawase R, Nakaoka H, Inagaki M, Nakatani K, Yuasa-Kawase M, Tsubakio-Yamamoto K, Ohama T, Nakagawa-Toyama Y, Nishida M, Ishigami M, Masuda Y, Matsuyama A, Komuro I, Yamashita S.

Journal: J Atheroscler Thromb. 2011 Dec 22;18(12):1062-70.

Comment: Several epidemiologic studies have demonstrated that both fasting and non-fasting hypertriglyceridemia are closely related to the development of atherosclerosis. Non-fasting hypertriglyceridemia is partially associated with postprandial hyperlipidemia (PH) in patients with dyslipidemia which is characterized by the postprandial accumulation of excess triglyceride (TG)-rich lipoproteins (TRLs) and their partially hydrolyzed product, remnants (R) or remnant lipoprotein particles. In the postprandial state, serum TG levels increase rapidly around 3-4 hours after the meal because of the prompt production of TRLs. TRLs and their remnants are heterogeneous and originate from two different organs, that is, the small intestines (chylomicrons [CM] and CM remnants) and liver (VLDL and VLDL remnants), respectively. However, it is unclear whether the increase in TRLs is mainly due to the increase in CM or VLDL in the postprandial state and whether the postprandial increase in remnant lipoprotein particles is due to the increase in CM-R or VLDL-R. It is difficult to

estimate the presence of PH without performing a time-consuming oral fat loading (OFL) test, so postprandial lipoprotein metabolism was analyzed by measuring the postprandial levels of apolipoprotein (apo)B-48 and apoB-100, and the correlation between postprandial TG increase and fasting apoB-48 levels was assessed to establish a good marker of PH without performing an OFL test. Ten male normolipidemic subjects were loaded with a high-fat (HF, 1045 kcal) or standard (ST, 566 kcal) meal, and the lipids, apolipoproteins and lipoprotein profiles were analyzed after each meal. TG, apoB-48, remnant-like particle (RLP)-cholesterol and RLP-TG levels were increased and their levels were significantly higher after intake of the HF meal than the ST meal; however, there was no postprandial increase in apoB-100 and LDL-C levels. Postprandial increases in TG levels of CM, VLDL, LDL and HDL were significantly higher after intake of the HF meal than the ST meal. Fasting apo B-48 levels were strongly correlated with the incremental area under the curve of TG after intake of the HF meal, but not the ST meal. In conclusion, postprandial TG increase was mainly due to increased CM and CM-R, but not VLDL. Measurement of fasting serum apoB-48 may be a simple and useful method for assessing the existence of PH.

Title: Serum apolipoprotein B-48 levels are correlated with carotid intima-media thickness in subjects with normal serum triglyceride levels.

Authors: Nakatani K, Sugimoto T, Masuda D, Okano R, Oya T, Monden Y, Yamashita T, Kawase R, Nakaoka H, Inagaki M, Yuasa-Kawase M, Tsubakio-Yamamoto K, Ohama T, Nishida M, Ishigami M, Komuro I, Yamashita S.

Journal: *Atherosclerosis*. 2011 Sep;218(1):226-32.

Comment: Postprandial hyperlipidemia (PPHL), based on the accumulation of chylomicrons (CM) and CM remnants containing apolipoprotein B-48 (apoB-48), is an independent risk factor for coronary heart disease (CHD). Since atherosclerotic cardiovascular diseases are frequently observed even in subjects with normal serum triglyceride (TG) level, the correlation between fasting apoB-48 containing lipoproteins and carotid intima-media thickness (IMT) was analyzed in subjects with normal TG levels. For this study, 164 male subjects were selected from a total of 245 males who took their annual health check at the Osaka

Police Hospital. Exclusion criteria included systolic blood pressure ≥ 140 mmHg, intake of antihypertensive or antihyperlipidemic drugs, or age >65 years. The association between biochemical markers and IMT was analyzed and independent predictors of max-IMT were determined by multiple regression analysis in all subjects and in groups N-1 (TG <100 mg/dl, n=58), N-2 ($100 \leq$ TG <150 mg/dl, n=53) and H ($150 \leq$ TG mg/dl, n=53), respectively. Fasting total cholesterol, LDL-cholesterol, HDL-cholesterol, apoB-100 and ln[RemL-C] (remnant lipoprotein-cholesterol) levels were not correlated with max-IMT, but ln[TG] and ln[apoB-48] were significantly correlated with max-IMT in all subjects. Ln[apoB-48] and apoB-48/TG ratio were significantly correlated with max-IMT in group N-2. By multiple regression analysis, age and ln[apoB-48] were independent variables associated with max-IMT in group N-2. According to these results, serum apoB-48 level might be a good marker for the detection of early atherosclerosis in middle-aged males with normal-range levels of blood pressure and TG.

Title: Combined use of high-sensitivity C-reactive protein and apolipoprotein B/apolipoprotein A-1 ratio prior to elective coronary angiography and oral glucose tolerance tests.

Authors: Wen ZZ, Geng DF, Luo JG, Wang JF.

Journal: *Clin Biochem*. 2011 Nov;44(16):1284-91.

Comment: Coronary angiography (CAG) is considered as the gold standard for the diagnosis of coronary artery disease (CAD), and echocardiography is also identified as a useful tool to assess cardiac structure and function in cardiology patients. To date, however, both angiographic and echocardiographic techniques are still costly for many patients. Thus, doing CAG in all patients with suspected CAD and doing echocardiography in all cardiology patients are not cost-effective. Additionally, CAG is a traumatic method and has potential risk, which may bring about physiological and psychological pressure for some patients. Similarly, the oral glucose tolerance test (OGTT) is considered the gold standard for the diagnosis of diabetes mellitus (DM), and is a reliable measurement of the glucometabolic state, even for patients with acute myocardial infarction (MI) before hospital discharge. However, the consistency of OGTT in patients with acute coronary syndrome

measured at admittance and 1 month later was poor, and the overall reproducibility of the OGTT was reported to be only 65.6%. Consequently, in order to do these examinations in cardiology individuals cost-effectively, the best approach to one-time accurate diagnosis may be to target people selectively. This study aimed to investigate the predictive value of the combination of high-sensitivity C-reactive protein (hs-CRP) and apolipoprotein B (apoB)/apoA-1 ratio for the outcomes of coronary angiography, echocardiography and oral glucose tolerance tests. Hs-CRP, apoB, apoA-1, and the profiles of CAG, echocardiography and OGTTs as well as traditional risk factors were measured in 1,757 cardiology patients. Hs-CRP or apoB/apoA-1 ratio was significantly correlated with the presence and severity of angiographic profiles, the levels of left ventricular (LV) ejection fraction, LV mass and LV mass index, and the presence of abnormal glucose metabolism. The combination of hs-CRP and apoB/apoA-1 ratio had greater correlation with abnormal glucose metabolism than its individual components in patients with normal fasting glucose, and was an independent predictor for CAD. Accordingly, the combination of hs-CRP and apoB/apoA-1 ratio may be a strong predictor for CAD and abnormal glucose metabolism.

Title: Apolipoprotein B assessment for evaluating lipid goals.

Authors: Tekiner E, Oguz A, Uzunlulu M, Tekiner F.

Journal: Acta Cardiol. 2011 Aug;66(4):433-8.

Comment: It has been suggested that the apolipoprotein (Apo) B levels are more valuable than LDL cholesterol (LDL-C) levels in assessing cardiovascular risk associated with hyperlipidaemia. However, although non-HDL cholesterol (non-HDL-C) levels are accepted as a secondary objective after achieving LDL-C levels in the guidelines, Apo B has not been widely accepted as a therapeutic goal yet. The objective of this study was to determine how many of the patients who achieved the LDL-C and non-HDL-C targets recommended by the guidelines with a statin therapy achieved the Apo B target. The study included a total of 182 consecutive hypercholesterolaemic (119 male, 63 female) patients who were over 18 years of age (mean age: 54.96 +/- 9.27 y) and on statin therapy within the past 3 months with periodic assessment of lipid levels. Apo B and non-HDL-C levels were

determined for all patients irrespective of achieving the LDL-C target according to the cardiovascular risk categories as defined in the Adult Treatment Panel-III report. Serum Apo B levels were assessed using an immunonephelometric method. According to the results, the prevalence of patients who achieved the LDL-C, non-HDL-C and Apo B target was 63.2%, 79.7% and 72.5%, respectively. All patients who achieved the LDL-C target also achieved the non-HDL-C target. However, 6 of 115 patients (5.2%) who achieved the LDL-C and non-HDL-C target failed to achieve the Apo B target, and 23 of 132 patients (17.4%) who achieved the Apo B target failed to achieve the LDL-C target. Because 95% of patients who received a statin therapy and achieved LDL-C and non-HDL-C targets also achieved the Apo B target, the authors concluded that determination of Apo B does not contribute to the assessment of treatment efficacy. This conclusion is in conflict with numerous earlier reports such as the MERCURY II study (Ballantyne *et al.* J Am Coll Cardiol 2008;52:626-32). Relatively small number of patients in the present study and differences in treatment goals, presence of hypertriglyceridemia and other confounding factors may explain the conflicting results.

Title: Investigational CETP antagonists for hyperlipidemia and atherosclerosis prevention.

Author: Sirtori CR.

Journal: Expert Opin Investig Drugs. 2011 Nov;20(11):1543-54.

Comment: This is an excellent review on the current status of cholesteryl ester transfer protein (CETP) antagonists for hyperlipidemia and atherosclerosis prevention. Reverse cholesterol transport (RCT) is a function of high-density lipoproteins (HDL) in humans and higher species. It is enabled by the CETP, a high molecular weight protein exchanging cholesteryl esters in HDL for triglycerides in very low-density lipoproteins (VLDL). Inhibition of CETP may provide a useful strategy to raise HDL, the protective lipoprotein fraction in plasma. The review evaluates the clinical and experimental findings with the three drugs developed or is in advanced development for CETP inhibition. As an expert opinion, inhibition of CETP, both inherited and drug induced, at times leads to dramatic elevations of HDL-cholesterol (HDL-C) levels. Epidemiological data presently available do not,

however, provide convincing evidence that reduced CETP levels or activity due to genetic factors and associated with HDL-C elevations reduce cardiovascular risk. Indeed, the opposite may be true in some instances. All the three CETP inhibitors were the object of experimental and clinical evaluation. Large clinical trials with torcetrapib led to disappointing results with raised cardiovascular morbidity and mortality in addition to raised risk of cancer and sepsis. Off-target effects of the drug, such as aldosterone retention and raised blood pressure, were believed to provide an explanation for these negative findings. The two newer agents, dalcetrapib and anacetrapib, do not exert off-target effects. The two drugs differ because anacetrapib has a more dramatic effect on HDL cholesterolemia (+139%) than dalcetrapib (+20-30%). Anacetrapib, however, may impair formation of pre- β HDL which is the primary particle in the process of cholesterol removal. The initial large trial with anacetrapib (DEFINE study) in coronary patients on statin treatment, appeared to confirm a remarkable HDL raising property, together with some reduction in vascular end points, in particular coronary procedures. The issue of other potentially harmful effects of CETP inhibition (sepsis and others) has yet to be clarified. Large clinical end-point trials, however, will be necessary to provide convincing evidence that, in addition to raising HDL-C, CETP inhibitors provide a valid additional treatment, for example, to statins in patients with coronary heart disease (CHD) or at high risk of CHD.

Title: High prevalence of mutations in LCAT in patients with low HDL cholesterol levels in the Netherlands: Identification and characterization of eight novel mutations.

Authors: Holleboom AG, Kuivenhoven JA, Peelman F, Schimmel AW, Peter J, Defesche JC, Kastelein JJ, Hovingh GK, Stroes ES, Motzack MM.

Journal: Hum Mutat. 2011 Nov;32(11):1290-8.

Comment: Lecithin:cholesterol acyltransferase (LCAT) is a plasma enzyme that is synthesized and secreted by the liver and small intestine, and is a member of the lipase superfamily which includes lipoprotein lipase, endothelial lipase, and hepatic lipase. In plasma, LCAT primarily associates with HDL where it esterifies free cholesterol molecules with acyl groups derived from phosphatidylcholine

(lecithin). This leads to the maturation of HDL from a nascent discoidal particle, consisting of apo A-I, phospholipids, and free cholesterol, to mature spherical HDL with a core of cholesterol esters. Twin studies have shown that HDL-cholesterol (HDL-C) levels are, to a large extent, determined by genetic variation [heritability of 50%]. Deleterious mutations in three specific genes have thus far been shown to cause Mendelian forms of HDL deficiency. Homozygosity for LCAT mutations underlies rare disorders characterized by HDL-C deficiency while heterozygotes have half normal HDL-C levels. The authors studied the prevalence of LCAT mutations in referred patients with low HDL-C to better understand the molecular basis of low HDL-C in these patients. LCAT was sequenced in 98 patients referred for HDL-C <5th percentile and in four patients referred for low HDL-C and corneal opacities. LCAT mutations were highly prevalent: in 28 of the 98 participants (29%), heterozygosity for non-synonymous mutations was identified while 18 patients carried the same mutation (p.T147I). The four patients with corneal opacity were compound heterozygotes. All previously identified mutations are documented to cause loss of catalytic activity. Nine novel mutations—c.402G>T (p.E134D), c.403T>A (p.Y135N), c.964C>T (p.R322C), c.296G>C (p.W99S), c.736G>T (p.V246F), c.802C>T (p.R268C), c.945G>A (p.W315X), c.1012C>T (p.L338F), and c.1039C>T (p.R347C)—were shown to be functional through *in vitro* characterization. The effect of several mutations on the core protein structure was studied by a three-dimensional (3D) model. Unlike previous reports, functional mutations in LCAT were found in 29% of patients with low HDL-C, thus constituting a common cause of low HDL-C in referred patients in The Netherlands.

Title: Serum total and HDL cholesterol and risk of prostate cancer.

Authors: Mondul AM, Weinstein SJ, Virtamo J, Albanes D.

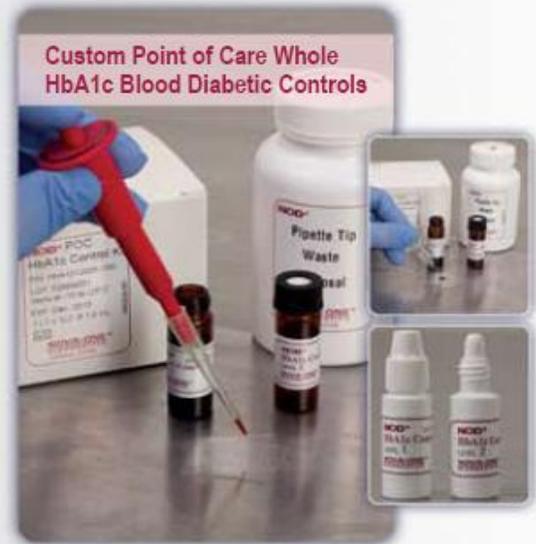
Journal: Cancer Causes Control. 2011 Nov;22(11):1545-52.

Comment: Growing evidence supports the hypothesis that low cholesterol levels may protect against aggressive prostate cancer. Recent prospective studies have shown a decreased risk of high-grade prostate



cancer in men with lower circulating total cholesterol. In addition, several investigations found that statins, a class of drugs commonly prescribed to lower cholesterol, may protect against high stage or high-grade prostate cancer. Earlier prospective and case-control studies examined the association between circulating cholesterol and total incident or fatal cancer and reported site-specific findings, including prostate cancer, with mixed results including positive associations, inverse associations, and null associations reported. Most of these studies, however, included relatively few prostate cancer cases, and none reported the association for advanced or high-grade prostate cancer, although the case distribution was likely shifted toward more advanced cases in studies conducted prior to the widespread use of PSA screening. Furthermore, the few prospective studies that examined high-density lipoprotein (HDL)-cholesterol found no association or that higher HDL-C was associated with a lower risk of prostate cancer. Only one of these studies examined advanced prostate cancer separately. Recently, a study from the Alpha-Tocopherol, Beta-Carotene Cancer Prevention (ATBC) Study cohort examined total and HDL-C in relation to risk of cancer overall and for specific sites, including total prostate cancer, and found inverse associations between both total and HDL-C and prostate cancer that were attenuated when the first 10 years of follow-up were excluded. Given differing etiological associations observed for overall and advanced or high-grade prostate cancer and that the association for HDL-C is understudied, the authors conducted a more detailed analysis of total and HDL-C and risk of prostate cancer in the ATBC Study cohort (n = 29,093) with additional years of observation. Cox proportional hazards models were used to estimate the relative risk of total (n = 2,041), non-aggressive (n = 829), aggressive (n = 461), advanced (n = 412), and high-grade (n = 231) prostate cancer by categories of total and HDL cholesterol. After excluding the first 10 years of follow-up, men with higher serum total cholesterol were at increased risk of overall (≥ 240 vs. < 200 mg/dl: HR = 1.22, 95% CI 1.03-1.44, p-trend = 0.01) and advanced (≥ 240 vs. < 200 mg/dl: HR = 1.85, 95% CI 1.13-3.03, p-trend = 0.05) prostate cancer. Higher HDL-C was suggestively associated with a decreased risk of prostate cancer regardless of stage or grade. In this population of

smokers, high serum total cholesterol was associated with higher risk of advanced prostate cancer, and high HDL-C suggestively reduced the risk of prostate cancer overall. These results support the findings of previous studies and, indirectly, support the hypothesis that statins may reduce the risk of advanced prostate cancer by lowering cholesterol.



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