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The Fats of Life

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

The recent AACC Annual Meeting in Chicago was an opportunity to reacquaint with old friends and catch up on new developments in our area of interest. I have been attending AACC meetings for over 30 years and have been involved with the Lipoproteins and Vascular Diseases Division from its inception. Most interesting to me this year was to experience a rekindling of the enthusiasm and energy present at the founding of the Division.

The Division was organized in the mid-1980s in step with the transition of cholesterol and lipoprotein measurements into the "mainstream". The NIH-funded Lipid Research Clinics Study had for the first time demonstrated unequivocally that lowering cholesterol could decrease the risk of developing CVD. Even though the implications were highly controversial at the time, national consensus conferences had endorsed the value of aggressive intervention. The National Cholesterol Education Program was organized to develop expert guidelines, implement professional programs and focus public attention on "Know Your Number," identifying and treating elevated cholesterol. Because CVD was then and remains today the number one cause of morbidity and mortality, the stakes were high, generating much energy and enthusiasm.

The newly organized Lipids and Lipoproteins Division played an integral role in developing lab guidelines, driving various publications and educating lab professionals in responding appropriately. We were all "young" back then and many of us focused our careers on the intersection of lipoproteins with CVD. We forged ahead with considerable enthusiasm, driving programs like the Manufacturer's Symposium, held every year during the AACC Annual Meeting, which brought diagnostic manufacturers and laboratories in step with the recommendations. We also sponsored the "Frontiers Symposium," which provided an opportunity for experts and various practitioners to communicate.

Over the ensuing two decades as programs have been implemented and become integral, the LRC generation has aged gently and many have even gone on to retirement. Most heartening to me this year is to see the "second" and even "third" generation Division members picking up the ball and carrying on the effort. And we seem to be on the verge of a new revolution in practice. In development of new lipoprotein technologies, much of the focus has shifted to scientists and diagnostic firms in Japan. The newly organized association with our colleagues in Japan, mentioned by our current Chair, Dr. McConnell, will provide a new focus in driving improvements in the measurement technology. Presentations at the Annual meeting on next generation methods; lipoprotein subclasses, markers of inflammation, and all the "omics" suggest that the focus on the lipid panel over the past two decades will likely be supplemented by more definitive and "personalized" indicators.

Pushing the practice to a more definitive and individualized level will require new research, careful planning, thorough organization and considerable energy to drive changes to a new paradigm. The Lipoproteins and Vascular Diseases Division members have another opportunity now to step up, get involved and assist in creating the framework for the future. I am very optimistic that our current Division leadership and the core group of members are up to the challenge.

Russ Warnick, Editor
The Fats of Life

Welcome readers to the Summer issue of the *Fats of Life*. We are all just getting back form a very successful AACC meeting. There was record attendance this year, and the LVDD sponsored events were all well attended. We had nearly 150 people at the LVDD mixer and dinner, where we were entertained with excellent presentations from Drs. Michael Tsai and Alan Wu. We thank them for their time and efforts and for providing an excellent program for us. Dinner after the meeting was very nice and I noticed much interaction, and hopefully productive networking amongst the division members. By this time, if you are like me, you are addressing the many new ideas and collaborations that have been put in place as a result of our exchange with colleagues that always come about when we all get together.

At the request of AACC President-Elect, Dr. Gary Myers, the AACC-LVDD management group met with the Japanese Lipid standardization group. An important new relationship was developed as a result of this meeting. For the last three years, members from our division have met during the annual AACC meeting to discuss lipid/lipoprotein standardization efforts in Japan. Since many of the reagent systems now used in the US for lipid measurements are produced by Japanese manufacturers, we felt it a very worthwhile effort. In the past, these meetings have not officially been a part of the AACC National meeting agenda. We are proposing the development of a subdivision of the AACC-LVDD, yet to be named, but focusing on standardization efforts for lipid measurements in Japan and the US. AACC leadership supports this effort and, in 2007, we will incorporate a program for Japan/USA lipid/lipoprotein standardization efforts into the official AACC program. Our knowledge regarding the importance and intricacies of HDL and LDL measurements is increasing and the program that we are planning for AACC in 2007 will be worth attending, so please watch for it and attend if possible.

As was discussed in my last Chair's Corner, we are continuing a plan to assemble experts to discuss/assess alternative measures to LDL cholesterol like apolipoprotein B or LDL particle number with the ultimate goal of providing a position statement from AACC-LVDD. We also plan to submit a program (short course or symposium) on this topic to be presented at the 2007 AACC annual meeting. Slots for the meeting are competitive, but we are hopeful this will be supported.

I'll leave you with one additional topic that I feel is of importance to us as laboratorians focused on measurement of analytes to assess lipoproteins and cardiovascular disease. I hope you all had a chance to read the recent publications in the July 10th issue of the *Archives of Internal Medicine* regarding incremental risk prediction using novel cardiovascular risk markers. This includes a manuscript (lead author Aaron Folsom) and an editorial by Donald Lloyd-Jones and Lu Tian. The authors present findings from the Atherosclerosis Risk in Communities (ARIC) study examining 19 novel cardiovascular risk markers and their clinical utility above and beyond traditional risk measurements. Change under the area of a receiver operating characteristic curve was used to assess incremental contribution of each novel risk marker over that of traditional risk factors. Of the 19 markers tested, only lipoprotein-associated phospholipase A_2 increased the area under the curve (AUC) significantly, with a modest increment of 0.006, from 0.774 to 0.780, suggesting minimal clinical improvement in risk discrimination. None of the other markers tested significantly increased the AUC. The fact that the AUC is only 0.780 suggests in and of itself that we have much more work to do.

However, on review of documents such as this, one could lose focus and interest in these important new risk markers that deserve further study and assessment of their potential clinical utility. Although our traditional cardiovascular risk prediction models are very good and useful in current clinical practice, it is widely recognized that they are imperfect. The analyses performed in this study could be flipped on its head, simply by establishing an area under the curve using the

novel risk marker and determining if traditional measures, like lipids, add significantly to that curve. Is it possible that these new markers may ultimately replace traditional markers providing improved risk prediction? It is an interesting question, and admittedly, none of the novel risk markers tested in the study have been evaluated sufficiently to suggest that might occur, but we need to not lose focus on these important markers. Indeed, some of the markers currently have clinical utility in certain populations, such as in individuals at intermediate risk for cardiovascular disease, where abnormal values suggest a need for more aggressive therapy. Many questions are yet to be answered as is nicely pointed out in the editorial accompanying the article. We as clinical chemists can play an important role in answering these questions by collaborating with our clinical colleagues to develop protocols to assess measurement of novel risk markers and specific treatment options based on laboratory measures. However, to do that well, we must control our enthusiasm for our favorite analytes and the allure such analytes might provide in the market place and maintain our commitment to science and data. It is indeed a challenging area, but one I believe will be rewarding to those who become involved.

Best regards,

Joseph P. McConnell
Chair, LVDD

Enzyme Cycling Methods and Their Application in Clinical Diagnostics

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Enzymes catalyze a variety of biochemical reactions under physiological conditions and are characterized by their high efficiency and high substrate specificity. Due to these unique properties, enzymes have been widely used as clinical diagnostic reagents for quantitative determination of various clinical indicators (analytes) in body fluids (1). For example, blood levels of glucose, cholesterol, triglycerides, uric acid, and many other analytes have been routinely tested by enzymatic methods in clinical laboratories. Though the enzymatic assays have advantages in their simplicity of detection (colorimetric), cost effectiveness, and ease of use (homogeneous, 1-2 liquid reagents), conventional enzymatic assays have limitations in their detection sensitivities. Most of the conventional enzymatic assays require µmol/L or mmol/L concentrations of analytes in test samples. Analytes of lower concentrations ranging from nmol/L to pmol/L are mainly determined by immunoassays in clinical laboratories using instruments equipped with special detection systems such as fluorescence polarization, chemiluminescence, and electrochemiluminescence. Though immunoassays have much higher detection sensitivity than that of conventional enzymatic assays, there are drawbacks for immunoassays. These include the complexity of heterogeneous immunoassays, involving incubation, separation and wash steps, and the requirement of special instruments and detection systems, which are often expensive and sensitive to interference from substances within samples. There is a constant need to develop better assays with the simplicity of the enzymatic assay and the sensitivity of the immunoassay. The enzyme cycling method has recently emerged as the method of promise.

What is the Enzyme Cycling Assay?

As indicated by the name, the enzyme cycling assay is a method that utilizes one or more coupled enzymatic reactions to convert one of the enzyme reaction products back to the enzyme substrate so as to form a substrate-product-substrate (S-P-S) conversion cycle with a concomitant formation of a reaction by-product that is detected at *n* times higher sensitivity at the end of *n* times the S-P-S cycle. As shown in Figure 1, the test substance A is converted to product B by the enzyme 1 (Enz-1), and the product B is converted back to the test substance A by the enzyme 2 (Enz-2) with a concomitant accumulation of the by-product P2 that can be detected colorimetrically. S1 and S2 are co-substrates for Enz-1 and Enz-2, respectively. As long as enough co-substrates S1 and S2 are present in the reaction, the enzyme cycling reaction from A to B to A (A→B→A) or the S-P-S cycle continues with accumulation of both P1 and P2. When this cyclic reaction repeats n times, both P1 and P2 are accumulated by n times the test substance A. Therefore, at the end of the cycling reaction, the test substance A can be measured at n times higher sensitivity through measuring the amount of P1 or P2 formed during the cycling reaction.

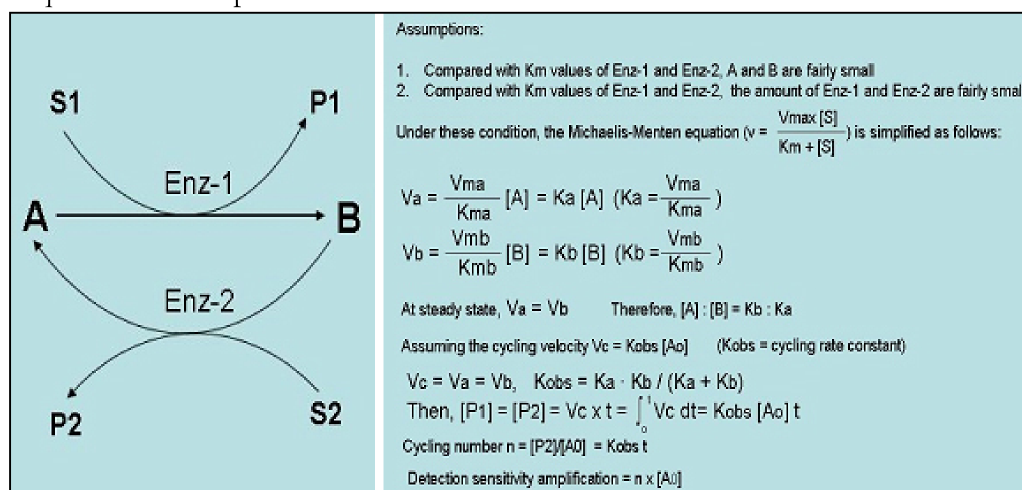

Figure 1. Enzyme cycling principle.

Sensitivity of The Enzyme Cycling Assay

As indicated in Figure 1, the sensitivity of the enzyme cycling assay is dependent on the cycling rate constant K_{obs} and reaction time *t*. The magnitude of detection sensitivity enhancement is equal to the cycling number *n* times initial test substance concentration $[A_0]$ or it can be expressed as $[P_2] = K_{obs} [A_0] t$, if $[B] = 0$ at t_0. The cycling rate constant K_{obs} is determined by the amounts of Enz-1 and Enz-2, which in turn are determined by their K_m values and their specific activity values under the cycling assay conditions (pH, temperature, and buffer system). As the detection sensitivity is K_{obs}-dependent, achieving the maxima K_{obs} can lead to the highest sensitivity. The theoretic maxima K_{obs} is ½ of K_a or K_b when K_a is equal to K_b. The sensitivity is also dependent on the reaction time; therefore, increases in the reaction time can enhance the detection sensitivity. Well-designed enzyme cycling assays can have a rate of 1000 cycles/min, and can reach a detection sensitivity of low nmol/L or even high pmol/L ranges.

For example, if the K_m value of Enz-1 is 10 µM, the specific activity is 5.0 IU/mg, and the test substance A concentration is 50 nM in the sample, total reaction volume is 200 µL, reaction time is 10 min, and the sample dilution factor is 10, then the reaction rate of Enz-1 is five of ten thousand (5/10,000) of its V_{max}, or 2.5 mU/mg which is equivalent to 2.5 nmol/min/mg. If 20 µg of Enz-1 is used per reaction, then the rate of the enzyme reaction will be 50 pmol/min. Since there is only 1 pmol of test substance A in the reaction system after 10 times of dilution, the cycling rate is 50 cycles per min (50 pmol/min divided by 1 pmol). After a 10 minute reaction, the total number of cycles is 50 x 10 = 500 and the final amount of P_2 or P_1 in the reaction system will be 500 pmol or 2.5 µM. If P_2 in Figure 1 is the co-enzyme NADH with an extinction coefficient of 6.25 x 10^3, the $\Delta A_{340\ nm}$ = 6.25 x 10^3 x 2.5 x 10^{-6} = 0.0156. The ΔA/min is 0.00156, which is within the detection sensitivities of most modern chemistry analyzers. With some enzymes that have higher specific activities, such as alcohol dehydrogenase and diaphorase, the cycling rates can easily reach more than 1000 cycles per minute.

Types of Enzyme Cycling Reactions

Various cycling methods can be designed based on the nature of the analytes and type of enzymes used. Enzyme cycling methods cannot only be used to measure analytes that are enzyme substrates or co-enzymes, but also can be used to measure enzymes themselves. There are three types of commonly used enzyme cycling methods: they are Type I, Type II and Type III. The Type I reaction cycles between the test substance A and reaction product B, or A→B→A cycle, using one or two enzymes. Type II reactions cycle between product B and secondary product C or B→C→B using one or two enzymes. On the other hand, the Type III reaction cycles between reduced and

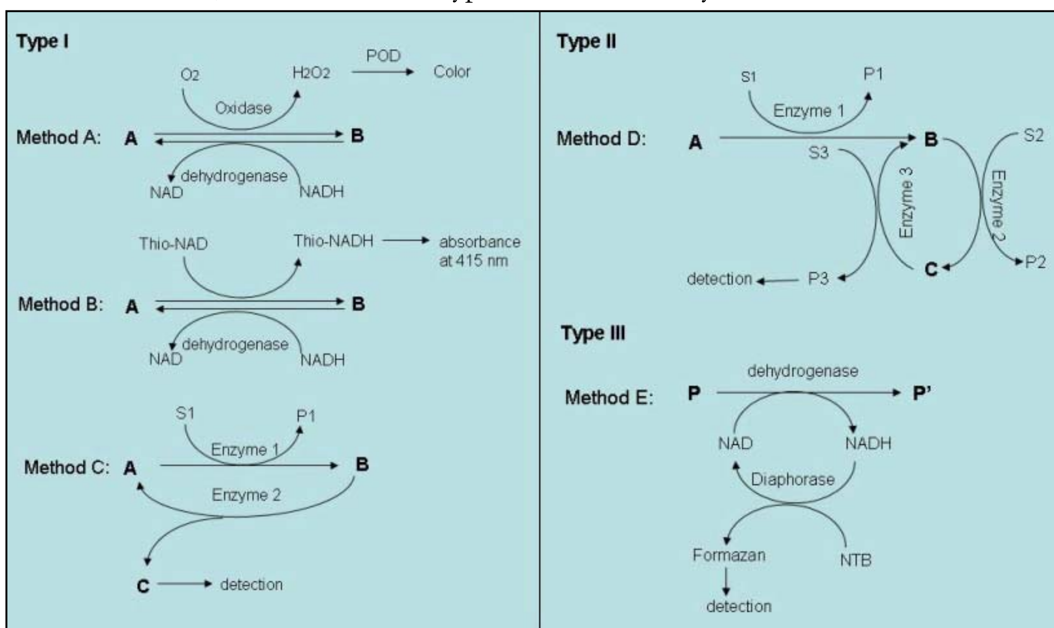

Figure 2. Types of enzyme cycling reactions.

oxidized forms of co-enzymes using oxidase and dehydrogenase enzymes. Figure 2 shows the representative cycling schemes of the three types. The Type III cycling reaction can be further coupled with Type I and Type II cycling to form double cycling reactions to further increase the detection sensitivity of assays.

Methods A, B and C cycle substrates, while method D cycles product. In contrast, method E cycles coenzymes. An example of method A is the measurement of L-glycerol-3-phosphate (G3P) or dihydroxyacetone phosphate (DHAP) using glycerophosphate oxidase and glycerophosphate dehydrogenase. An example of method B is the measurement of total bile acids using 3-α-hydroxysteroid dehydrogenase (3-α-HSD) and thio-NAD^+. An example of method C is the measurement of homocysteine using homocysteine:S-adenosylmethionine methyltransferase and S-adenosylhomocyseteine hydrolase. An example of method D is the measurement of α-methylacyl-CoA racemase (AMACR) using both acyl-CoA oxidase and reductase. An example of method E is the measurement of alcohol using alcohol dehydrogenase and coupled reactions in EIA assays.

Illustration of Enzyme Cycling Assays

To illustrate the principle of enzyme cycling assays, total bile acids (TBA) will be discussed as a representative for method B assays and the assay for total homocysteine (tHcy) will be used for method C assays (see Figure 2). Both of these products are manufactured by Diazyme Laboratories and have been cleared by the FDA for clinical diagnostic use.

Total Bile Acids (TBA) assay (method B)

Reagents:
R1: Thio-NAD
R2: 3-α-HSD and NADH
Assay principle: enzyme cycling, kinetic
Detection wavelength: 405 nm
Linear range: 1 – 180 μmol/L

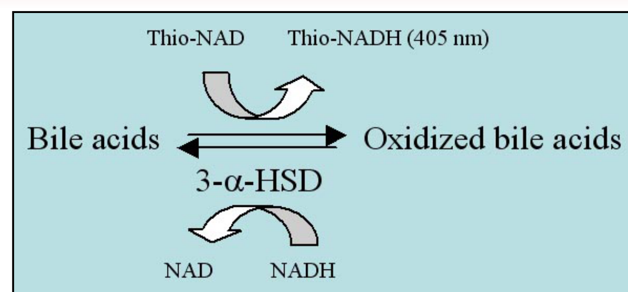

Figure 3. Assay principle for Total Bile Acids.

The enzyme cycling-based TBA assay utilizes the property of the enzyme 3-α-hydroxysteroid dehydrogenase (HSD) that catalyzes reversible reactions converting bile acids to oxidized bile acids and vice versa (Figure 3). The enzyme reactions are driven by the coenzyme NAD and NADH. In the forward direction, the enzyme uses thio-NAD, which is in large excess, to produce oxidized bile acids and reduced coenzyme thio-NADH that has a unique absorbance wavelength at 405 nm. In the reverse direction, the enzyme uses NADH, which is in large excess, to produce bile acids and oxidized co-enzyme NAD^+. As the enzyme catalyzes the reactions back and forth, the reaction product, thio-NADH, is accumulated at each reaction cycle. The assay takes less than 10 min with 3 – 5 μL of serum sample. A typical reaction scheme of the assay is shown in Figure 4.

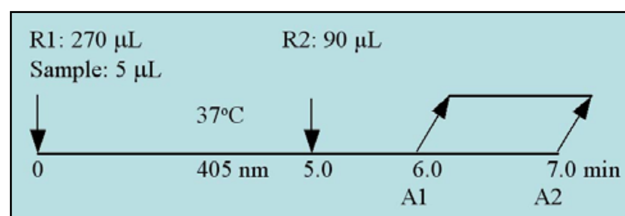

Figure 4. Total Bile Acids assay procedure.

The major advantages of this enzymatic cycling-based TBA assay are: a) no interference from hemolytic and lipemic samples; b) both reagents are liquid stable and suitable for any chemistry analyzer; and c) the assay has a high sensitivity and dynamic range (1 – 180 μmol/L) with intra- and inter-assay CV values of less than 3%. The assay is being widely used in clinical and veterinary laboratories world wide, especially in Asia where it is routinely used to aid in the diagnosis of liver diseases. In Europe, the assay is used to test

cholestatic conditions in pregnant women. The frequency of obstetric cholestasis has been shown to be 1 in 100 pregnant European women and 1 in 10 pregnant South American women (2).

Total homocysteine (tHcy) assay (method C)

Reagents:
R1: NADH
R2: homocysteine S-methyltransferase (HMTase) and glutamate dehydrogenase (GLDH)
R3: S-adenosylhomocysteine hydrolase (SAHase) and adenosine deaminase (ADA)
Assay principle: enzyme cycling, kinetic
Detection wavelength: 340 nm
Linear range: 2 – 50 µmol/L

In the enzyme cycling-based total homocysteine (tHcy) assay, oxidized homocysteine (Hcy) in the sample is first reduced to free Hcy using a reducing agent, TCEP. The reduced Hcy is then reacted with a co-substrate, S-adenosylmethionine (SAM), catalyzed by a Hcy conversion enzyme, Hcy S-methyltransferase (HMTase), to irreversibly form methionine (the Hcy conversion product of Hcy) and S-adenosylhomocysteine (SAH, the co-substrate conversion product that does not contain any structural element from the sample homocysteine). SAH is hydrolyzed into adenosine (Ado) and Hcy by SAH hydrolase. The formed Hcy that originated from the co-substrate, SAM, is cycled back to the Hcy conversion reaction by HMTase, producing an enzymatic cycling system with substantial amplification of detection signals. The reaction scheme of the enzymatic cycling-based Hcy assay is depicted in Figure 5. The formed Ado

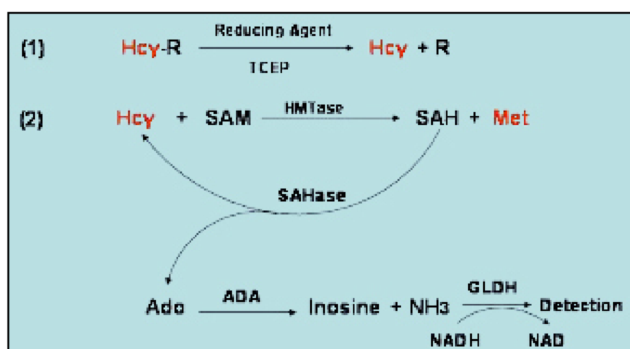

Figure 5. The principle of enzyme cycling-based total homocysteine assay.

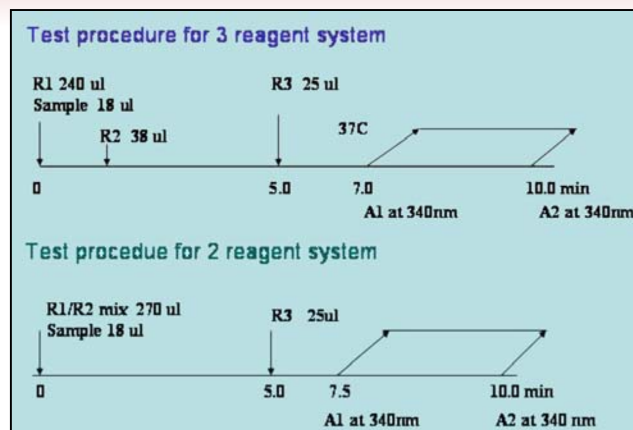

Figure 6. Total homocysteine assay procedures.

is hydrolyzed into inosine and ammonia by adenosine deaminase, and ammonia is reacted with glutamate dehydrogenase with concomitant conversion of NADH to NAD^+. The concentration of Hcy in the sample is proportional to the amount of NADH converted to NAD^+ ($\Delta A_{340\ nm}$) (3). The assay takes about 10 min with 15 – 20 µL of serum or plasma sample. The enzymatic tHcy assay uses liquid stable reagents and can be used in all types of clinical chemistry analyzers that can handle 2 or 3 reagents, as shown in Figure 6. A typical reaction curve is shown in Figure 7 where a sample containing 28.5 µmol/L tHcy was tested on a Hitachi 917 chemistry analyzer.

The major advantage of this enzyme cycling-based tHcy assay is its ability against interferences from other thio-containing substances such as cysteine

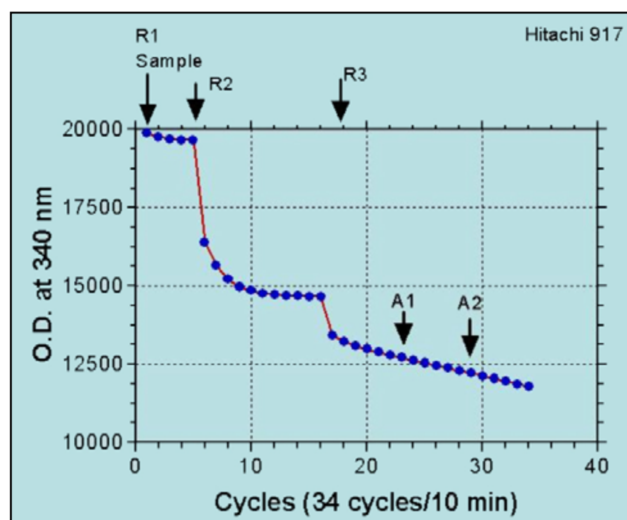

Figure 7. The reaction curve of the enzyme cycling-based tHcy assay on Hitachi 917.

and cystathionine. This is particularly important for accurately determining tHcy levels in renal failure patients whose plasma levels of cystathionine are often significantly elevated. The assay interference by endogenous cystathionine in samples from dialysis patients can be significant, ranging from 20% to 300%, depending on the tHcy assay method used. As shown in Figure 8, the enzyme cycling method described above completely eliminated cystathionine interference as compared with other tHcy assay methods.

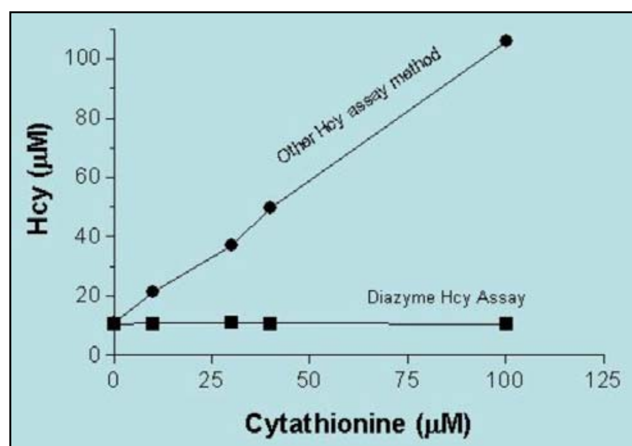

Figure 8. Comparison of cystathionine interference on tHcy assays.

Other advantages for the enzymatic tHcy assay include the use of fewer numbers of reagents and shorter time needed per assay when compared to both immunoassay and HPLC-based methods.

Elevated levels of plasma tHcy have been recognized as a new independent risk factor for the development of cardiovascular disease (4). Meta-analysis of clinical studies has established that a 5 µmol/L increase in tHcy is equivalent to approximately a 20 mg/dL increase in total cholesterol levels (5). Elevated levels of tHcy are also reported to be associated with increased risks of diabetes and Alzheimer disease (6). In connection with cardiovascular disease, the Nutrition Committee of the American Heart Association issued a statement in 1999 regarding tHcy testing that states: "a reasonable approach is to determine levels of fasting homocysteine in 'high-risk patients,' i.e., in those with strong family history for premature atherosclerosis or with arterial occlusive diseases, particularly in the absence of other risk factors, as well as in members of their families. Other conditions that may be associated with high homocysteine are advanced age, hypothyroidism, impaired kidney function, systemic lupus erythematosus, and certain medications, e.g., nicotinic acid, nitrous oxide exposure, theophylline, methotrexate, and L-dopa."

Summary

As described above, enzyme cycling methods have the capacity and potential to measure low levels of analytes that are currently measured by immunoassays. The enzyme cycling method can be divided into 3 types of cycling reactions (Type I, Type II, and Type III). Two examples of Type I cycling were described for the determinations of plasma levels of total bile acids and total homocysteine. Enzyme cycling methods are superior to the conventional enzymatic assays with higher detection sensitivities and less interferences from endogenous substances. Differing from traditional enzymatic assays, the enzyme cycling method amplifies the amount of detection product over time by repeatedly reacting with the target analyte. This enzymatic amplification technique makes it possible for enzymatic measurement of various clinical analytes that can currently be measured only by immunoassays with specially equipped detection systems.

The main benefits of an enzyme cycling assay are: (1) assay sensitivity can be increased by the increase of reaction times; (2) the cycling rate can be increased by increasing the amount of enzymes; (3) an enzyme cycling assay is homogenous and the reagents can be all liquid stable and ready to use; and (4) enzyme cycling assays have less interferences from endogenous substances, and reagents are free of hazards and environmentally friendly. Since enzyme cycling methods have the sensitivity to detect nmol/L levels of analytes, it is likely that they will be developed to measure steroid hormones that are currently measured by heterogeneous immunoassays. The enzyme cycling methods may have even greater application potential if coupled with fluorescence or lumi-

nescence detection methods to measure analytes in the concentrations of pmol/L range. As more and more enzymes become available, owing to the progress in genomic and proteomic projects coupled with advances in recombinant protein technology, enzyme cycling methods will likely play a major role in the development of the next generation of clinical diagnostic reagents.

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Laboratory Determination of Omega 3 Fatty Acids – An Emerging New Risk Factor for CAD-and CAD-Death Prevention? Nutritional Genomic Implications

Michael Y. Tsai, PhD

Objective of Presentation: To discuss:

1. The beneficial effects of the active ingredients of marine fish oil – eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA).
2. The potential role of the clinical laboratories in monitoring the intake of EPA and DHA.
3. Nutritional genomic implication of fish oil as a dietary supplement.

The beneficial effects of consumption of marine fish have been extensively studied. Among the anti-atherogenic effects, the most pronounced is the effect of EPA and DHA in lowering serum triglycerides (TG). In fact, a meta-analysis of 36 controlled crossover studies showed that 2-4 g of EPA+DHA lowers TG by 24% in normolipidemic subjects and 34% in hypertriglyceridemic patients (Harris WS 1997). The mechanism of EPA and DHA in lowering TG is thought to be mediated through reductions of very low-density lipoprotein (VLDL) secretion as well as increased catabolism (Park Y 2003). The former is through enhanced beta-oxidation of fatty acids by the liver, while the latter is through binding of DHA to peroxisome proliferation activated receptor (PPAR) alpha, and the subsequent increased expression of lipoprotein lipase in various tissues. (Varghese Z 2006).

Other proven benefits of fish oil include a moderate lowering of both systolic and diastolic blood pressure (Geleijnse J M 2002) and a moderate lowering of insulin-resistance in non-diabetic individuals (Delarue J 1996).

More recently, several large epidemiological studies have demonstrated that consumption of marine fish or supplements containing EPA and DHA reduce sudden cardiac deaths. For example, the Physicians Health Study demonstrated that individuals who consume one or more servings of fish per week had a 52% lower risk of sudden cardiac death compared to individuals who consume less than one serving per month (Albert CM 1998). In the GISSI-Prevention Trial involving more than 11,000 post-MI patients of both sexes, individuals were given either 850 mg of EPA+DHA or no supplements. The individuals who received supplements had a 45% reduction of cardiac death (Stone NJ 2000). The reduction of sudden cardiac death is thought to be mediated through direct protection on the heart by EPA and DHA in reducing malignant ventricular arrhythmia and improving heart rate variability (Sellmayer A 1995).

The overwhelming evidence of the benefits of EPA and DHA led to the recommendation by the American Heart Association (AHA) of eating a variety of fish twice a week. Also, for patients with documented CHD, the consumption of 1 g per day of EPA +DHA either from fatty fish or from supplements was recommended. In addition, 2-4 g of EPA+DHA provided as capsules was recommended for the lowering of serum triglycerides (AHA Nutrition Committee 2003). In turn, this led Harris and Von Schacky to propose the so-called "Omega-3 Index," which measures the sum of EPA+DHA in the membrane of erythrocytes as the percent of all fatty acids. These authors also determined that an ideal level of Omega-3-Index is when membrane EPA+DHA exceeds 8% (Harris WS, Von Schacky C 2004).

A recent study in our laboratory demonstrated that supplementation with 2g of EPA+DHA for 8 weeks raised the mean Omega-3 Index of our participants from 4.3 % to 7.8%. We calculated that there was a mean increase of 1.4% per gram of EPA supplementation and 1.9% per gram of DHA supplementation. Moreover, the rates of incorporation were fairly constant among our participants. These data are in good agreement with previous studies. Thus, it is theoretically possible to determine the dose necessary to reach the ideal level of EPA+DHA based on an initial determination of the baseline level of an individual.

While there are many proven benefits of EPA and DHA, there are also areas where the findings are controversial. This is particularly true for the effect

of fish oil on inflammation. Several observational studies have reported that fish consumption is associated with lowered inflammation as measured by one or more markers of inflammation. For example, in the Nurse Health Study I, it was found that, comparing individuals in the top quintile versus the bottom quintile of omega-3 fatty acid intake, those who consumed more fish had 29% lower C-reactive protein (CRP) and 23% lower interleukin (IL)-6 (Lopez Garcia E 2002). In the Nurses Health Study II, it was reported that EPA and DHA intake was inversely associated with tumor necrosis factor-receptor (TNF-R) 1 and TNF-R2 (Pischon T 2003). However, interventional studies using relatively high doses of omega-3 fatty acid supplement of 2-4 g per day failed to demonstrate a lowering of CRP levels (Balk EM 2006). The discrepancy between observational studies and interventional studies could be due to unidentified covariates in observational studies or to the influence of inter-individual genotype differences in their responsiveness to diet in general and, in this case, to fish oil intake.

Nutritional genetic and nutritional genomic studies have been gaining increased attention in recent years and have been featured on the cover pages of many national magazines such as *Newsweek* and *Time*. With regard to long chain omega-3 fatty acids, there are many studies in the literature on the nutritional genetic influences on individuals' responsiveness. For example, while fish oil supplementation is effective in lowering triglycerides levels, the responsiveness of individuals depends in part on their apolipoprotein E genotypes. This is particularly true for postprandial triglycerides levels, which are more responsive to fish oil intake in individuals who carry the apo E2 allele than those who carry only the apo E3 or E4 alleles (Minihane AM 2000). Other studies demonstrated that omega-3 intake helped lower intimal-medial wall-thickness only in those individuals who carry the variant alleles of the 5-lipoxygenase (LOX) gene (Dwyer JH 2004). Other examples of nutritional genetic influences have also been published on apoCIII, and PPAR-gamma 2 genes (Olivieri O 2005, Lindi V 2003).

In conclusion:

- Supplementation of omega-3 fatty acids is now recommended by AHA because of the numerous beneficial cardioprotective effects, and is particularly useful in secondary prevention and prevention of sudden cardiac death.
- The measurement of RBC membrane content of EPA and DHA, known as the Omega-3 Index, has been advocated as a new risk factor.
- Controversies exist on some of the beneficial effects of fish oil, such as its effect on lowering inflammation.
- Variations in individual responses may be responsible for the differences obtained in different studies.
- Future studies on nutritional genetics and genomics may explain at least in part the discrepancies in the findings of different studies.

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The origins of the discovery of HDL began over 50 years ago when Richard Havel at the National Institutes of Health developed the density gradient ultracentrifugation method for isolating and separating the major lipoprotein fractions (1). Even before that time, cholesterol in a plasma fraction containing alpha-lipoproteins produced by the Cohn precipitation method was found by Howard Eder and colleagues in 1951 to be inversely related to the development of coronary heart disease (CHD). This early observation and the overall importance of HDL in the pathogenesis of CHD was not fully appreciated until 1975 when two brothers, Norman and George Miller, wrote a provocative review article summarizing the atheroprotective role of HDL based on early experimental and epidemiologic data (3). Great progress, however, was made during these early years of lipoprotein research on the cholesterol biosynthetic pathway and on LDL metabolism, such as the discovery of the LDL receptor by Michael Brown and Joseph Goldstein (4). This work provided the foundation for the development of statin drugs, which are clearly the most valuable drugs we now have for preventing CHD.

Although statins have been a great therapeutic success, there is growing realization that they are not fully adequate at preventing CHD. Most well controlled clinical trials of statins for the primary prevention of CHD disease have shown only about a 30% reduction in clinical events (5), hence, accounting for the current interest in further lowering LDL-C treatment goals. Until recently, developing drugs for raising HDL to compliment statin drugs have been difficult because of our lack of knowledge of HDL metabolism. The recent finding of the gene defect in Tangier Disease, an autosomal recessive disorder associated with low HDL, was a major breakthrough that eventually led to the identification of the role of a whole new class of proteins involved in lipoprotein metabolism, namely the ABC transporters (6). The discovery of the SR-B1 receptor and its multifunctional role in lipid transport was also a major step in our understanding of HDL metabolism (7). These and many other recent discoveries have stimulated the search for new drugs that raise HDL.

Currently, the best drug for raising HDL is niacin, which is, in fact, one of the first lipid lowering drugs ever used (8). Although it can raise HDL-C by as much as 40% and can also lower LDL-C, triglycerides, and is the only effective drug for lowering Lp(a), patients not infrequently develop uncomfortable side effects when first starting niacin, such as facial flushing, which has limited its use. Fibrates and statins can also raise HDL but only modestly in the range of 5-20%. The few clinical trials that have used both an agent for lowering LDL-C and one specifically for raising HDL-C, such as niacin, have shown almost a doubling in the reduction of CHD events compared to just treatment with statins (9). The implication from these studies is that new drugs to raise HDL may be a valuable addition to our current LDL lowering treatment strategy.

A diagram of our current understanding of HDL metabolism and the targets for some of the new HDL raising drugs are shown in Figure 1, which illustrates the Reverse Cholesterol Transport (RCT) Pathway. The RCT pathway mediates the transfer of excess cellular cholesterol from

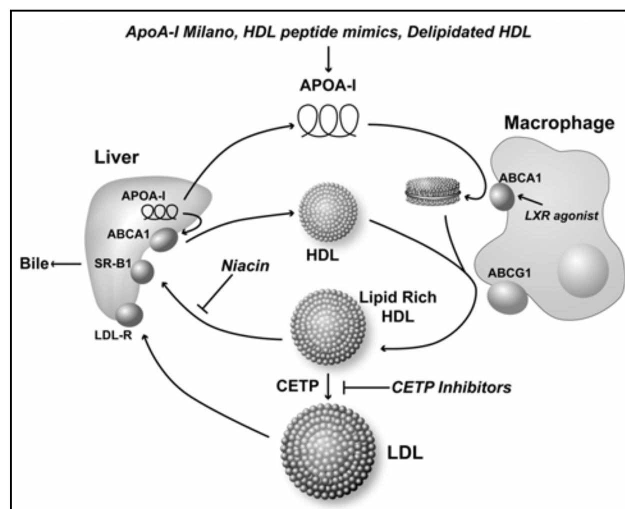

Figure 1. The reverse cholesterol transport pathway.

peripheral cells, such as macrophages in plaques, to the liver for excretion. HDL, however, has many other potential beneficial effects, such as anti-inflammatory, anti-oxidant, and anti-thrombotic properties, which are increasingly being recognized as also being important in the athero-protective role of HDL (10).

ApoA-I, the main protein component of HDL, is produced by the liver and intestine and is thought to be secreted with only a relatively small amount of bound lipid. The ABCA1 transporter, the defective gene in Tangier disease (6), mediates in the liver and the intestine the transfer of phospholipids and cholesterol to secreted apoA-I to form nascent pre-beta HDL. Based on several transgenic animal models that selectively express the ABCA1 transporter in various tissues, it appears that the liver accounts for about 70% of plasma HDL-C and the intestine accounts for most of the remainder (11). Once produced, nascent HDL then interacts with ABCA1 in peripheral cells, resulting in the transfer of excess cellular cholesterol to HDL. This additional cholesterol is esterified by Lecithin cholesterol acyl-transferase (LCAT), which then traps the cholesterol as a cholesteryl ester in the hydrophobic core of HDL until it is removed by the liver. As a result of the LCAT reaction, the discoidal-shaped pre-beta HDL is transformed into the spherical lipid-rich HDL, the predominant form of HDL in plasma. Recently, it was discovered the lipid-rich HDL can also remove even more cholesterol from peripheral cells by another ABC transporter, namely ABCG1 (12). In addition, SR-B1 and a passive aqueous diffusion process promotes the exchange of cholesterol between cells and extra-cellular lipoproteins based on the cholesterol concentration gradient (7). The only cells in the body that have a significant capacity for catabolizing cholesterol are hepatocytes, which can convert cholesterol to bile salts. The liver can also directly secrete any excess cholesterol into the bile. HDL delivers its cholesterol to the liver by at least two mechanisms (13). The majority of cholesteryl esters are returned to the liver by hepatic LDL receptors, after first being transferred to LDL from HDL by the Cholesteryl ester transfer protein (CETP). Cholesterol is also returned to the liver by hepatic SR-B1 receptors, which selectively extracts cholesteryl esters from HDL. In addition, there are likely other pathways for the delivery of free cholesterol to the liver that are currently not well characterized.

New drugs that are being developed for raising HDL can be classified based on their duration of use as either chronic or acute drugs. Chronic drugs, as the name implies, are similar to statins in that they are to be used for protracted periods, usually on a daily basis for lowering CHD risk. Two new potential HDL drugs in this category are a niacin combination drug and CETP inhibitors. Although niacin has been used since 1950s, the receptor that mediates its effect was only discovered in the last few years. Niacin binds to HM74A, a G-coupled receptor in the liver and in other cells (14). Stimulation of this receptor inhibits intracellular lipolysis and this alters lipoprotein metabolism by delaying the catabolism of HDL, thus raising HDL-C. Stimulation of this receptor in dermal macrophages leads to prostaglandin production, which triggers the facial flushing side effect of niacin. This problem has already been partly solved by formulations of niacin that slow its rate of absorption. A new niacin combination drug that contains a prostaglandin synthesis inhibitor is currently in late phase clinical trials, and the expectation is that this will eliminate most of the side effects of niacin, thus improving the compliance problem with this drug and increase its overall use.

CETP inhibitors raise HDL-C, because they block the transfer of cholesteryl esters from HDL to LDL, which results in the formation of a large lipid-rich species of HDL (Fig. 1). This change in HDL metabolism was initially postulated to reduce CHD risk largely based on the initial observation that patients with CETP deficiency have less CHD events and appear to show longevity (15). Subsequent studies have not always confirmed this relationship and there is still not a consensus on the effect of CETP deficiency on the risk of CHD. There is also a concern that the inhibition of CETP would potentially interfere with at least one

of the two major pathways for the delivery of cholesterol to the liver (Fig. 1), thus potentially inhibiting the RCT pathway. CETP inhibitors, however, have been shown to inhibit atherosclerosis in rabbits (16), and the changes in lipoprotein profile that it induces in man appear to be favorable (17). Two different CETP inhibitor drugs are currently being examined in late phase clinical trials, which will hopefully establish their value for preventing CHD.

One obvious approach to raising HDL is to increase apoA-I production, but an arduous search by most of the major drug companies for many years have failed to find a drug that can effectively do this. The discovery of the role of the ABCA1 transporter in the biogenesis of HDL have now prompted a search for drugs that would instead increase the expression of this transporter. In cells, ABCA1 is rapidly induced when they become loaded with excess cholesterol and this has been discovered to be mediated by the LXR transcription factor (18). LXR binds to its agonist, oxysterols, which are produced in cholesterol-loaded cells, and it then heterodimerizes with another transcription factor called RXR. This transcription factor complex then activates the expression of ABCA1 by binding to specific DNA element in the ABCA1 promoter. In addition, LXR also activates several other genes that have been predicted to be beneficial for enhancing the RCT pathway (18). Not all of the genes that LXR induces, however, are beneficial and some lead to hypertriglyceridemia and a fatty liver. The current strategy for developing LXR-based drugs is to develop drug agonists that would only selectively activate ABCA1, similar to the approach used for developing selective estrogen receptor modulator drugs. Drug agonists that would only activate ABCA1 in the intestine, thus potentially avoiding liver steatosis and hypertriglyceridemia, are also being developed.

Recently, a new concept has emerged for treating atherosclerosis called acute HDL therapy (19). It is largely based on one intriguing clinical trial, involving the intravenous infusion of a recombinant form of HDL (20), which recently has generated great interest in HDL research. Numerous animal studies have convincingly shown that HDL can have rapid effects on vascular function and can rapidly reduce plaque size (21). This is in contrast to statin therapy in which it has been difficult to demonstrate plaque reduction, even after several year use of the drug (22). The concept of acute HDL therapy is that patients with a recent myocardial infarction or with acute coronary syndrome, who are at great risk for developing future events, would be candidates for this therapy. The goal of acute HDL therapy is to try to rapidly stabilize patients. It is envisioned that following acute HDL therapy, patients would then be given statins or other conventional drugs for lowering LDL-C, as well as a drug for chronically raising HDL-C.

Only one clinical trial, however, has been published to date on acute HDL therapy (20). In this trial, patients with acute coronary syndrome were infused intravenously with an apoA-I variant called apoA-I Milano complexed with phospholipid once a week for 5 weeks. ApoA-I Milano has an Arg to Cys substitution at position 173 and may have some superior anti-atherogenic properties compared to normal apoA-I and is associated with longevity. The plaque volume in coronary vessels of patients were monitored by intravascular ultrasound and compared to their baseline values and to a placebo-treated group. The apoA-I Milano-phospholipid complex was found to significantly reduce plaque size at the end of the study. The mechanism, however, for the rapid plaque reduction is not fully understood, and the first trial was relatively small, so more clinical and basic research is needed to establish the usefulness of this approach.

A major hurdle with acute HDL therapy is the production of sufficient amounts of apoA-I that is free of any endotoxin. Doses as much as 1 g per treatment were used in the initial clinical trial, which may be cost prohibitive to produce in such large quantities. Because of this concern, several alternative approaches are also being investigated. Short synthetic peptide mimics of apoA-I, which would be less costly to produce, have been shown to mediate some of the beneficial effects of apoA-

I, such as the cholesterol efflux (23) and antioxidant (24) properties. In several animal models of atherosclerosis, these peptides have been shown to markedly reduce atherosclerosis (25). One of the peptides most studied, D-4F, is produced with D-amino acids and appears to be orally available and can perhaps also be used as a chronic agent. Several peptide mimics of apoA-I are currently in early stage clinical trials.

Another approach inspired by the apoA-I Milano trial, is called selective delipidization and is envisioned to be used in a manner similar to plasmapheresis (26). In this procedure, plasma removed from the patient is treated with various solvents that selectively extract cholesterol from HDL, producing pre-beta-like HDL. Plasma treated in this way has been shown to be better for mediating cholesterol efflux from cells. The main advantage of this approach is that the patient's own endogenous HDL is used for the therapy, thus avoiding the need for making and infusing recombinant HDL. A stage I clinical trial of selective delipidization in patients with acute coronary syndrome has recently started.

In summary, much progress has recently been made in understanding HDL metabolism since its discovery over 50 years ago, which has stimulated the search for new drugs for raising HDL. Results from several clinical trials on these new drugs should be available in the next few years. If they are shown to be safe and effective, these new HDL raising drugs may represent the next major advance in our treatment for CHD.

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By Gyorgy Csako, M.D.

Title: Applications of nanotechnology to atherosclerosis, thrombosis, and vascular biology.

Authors: Wickline SA, Neubauer AM, Winter P, Caruthers S, Lanza G.

Journal: Arterioscler Thromb Vasc Biol. 2006 Mar;26(3):435-41. Epub 2005 Dec 22.

Comment: This is a concise review about the applications of nanotechnologic techniques to molecular and cellular imaging of atherosclerosis, thrombosis and vascular biology. While the techniques described are not performed in diagnostic laboratories, they comprise an essential part of future laboratory studies in these areas. Advanced imaging techniques with new, targeted nanoparticle contrast agents allow for early characterization of atherosclerotic and cardiovascular pathology at the cellular and molecular levels. Thus, the evaluation of laboratory markers in the future will require using these molecular and cellular imaging techniques for assessing both diagnostic performance and therapeutic benefits. By precisely engineering atoms and molecules to yield new molecular assemblies on the scale of individual cells, organelles, or even smaller components (generally in the range of 5 to 500 nm), nanotechnology produces materials that possess unique chemical and biological properties on the basis of interactions that occur at their surfaces. Synthesis of such materials may occur from a "top-down" approach by miniaturizing existing microscopic materials, or from a "bottom-up" approach involving "self-assembly" of molecules into reproducible and well-defined nanoscale constructs. Examples of nanoparticle classes used in molecular and cellular imaging include liposomes (50-700 nm uni- or multilamellar vesicles), emulsions of perfluorocarbons (200-400 nm), modified micellar particles such as high-density lipoprotein (HDL) particles, polymers (20-200 nm), metallic particles such as iron oxide nanoparticles (15-60 nm) and gold shell nanoparticles (~120 nm), carbon nanotubes and fullerenes (4 nm), and quantum dots (2-8 nm) constructed from semiconductor materials (e.g., cadmium selenide. There are a variety of examples in which nanotechnology methods were combined with cardiovascular imaging (nuclear, optical, ultrasound, and magnetic resonance imaging, MRI). For instance, early detection of disrupted atherosclerotic plaques was achieved by nanoparticle-targeted fibrin imaging as early as 1996. In this case, a ligand comprised of an antibody fragment highly specific for certain cross-linked fibrin peptide domains was complexed to nanoparticles either through avidin-biotin linkages or covalent binding to the functionalized nanoparticle. Similar technique was used for tissue factor imaging. Echogenic liposomes with specificity for intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), fibrin, fibrinogen, and tissue factor were used to target thrombi and various vascular signatures associated with atheroma development in injured vessels. The utility of $\alpha_v\beta_3$-integrin-targeted nanoparticles was demonstrated for the detection and characterization of angiogenesis associated with growth factor expression, tumor growth and atherosclerosis. Macrophage imaging was shown to be possible with the use of non-targeted USPIOs. Recombinant HDL-like nanoparticles were able to enhance detection of atherosclerotic regions. Even stem cell imaging is possible with nanotechnology. Stem cells ingest nanoparticle contrast agents through endocytosis by various strategies, including coating the particles with dendrimers, transfection agents, or antibodies/peptides. Intracellular accumulation of these nanoparticles appears to be well tolerated by the cells over the long term and allows for their tracking. The future of cardiovascular diagnosis already is being impacted by nanosystems that can both diagnose pathology and treat it with targeted delivery systems. It is, however, important to recognize that, despite promising observations, the ultimate role of these technical advances in imaging must be established in clinical trials. Most of the current evidence comes from animal experiments and no solid proof of efficacy has been yet provided with respect to altering courses of therapy or patient outcomes.

Title: One third of the variability in HDL-cholesterol level in a large dyslipidaemic population is predicted by age, sex and triglyceridaemia: The Paris La Pitie Study.

Authors: Hansel B, Kontush A, Giral P, Bonnefont-Rousselot D, Chapman MJ, Bruckert E.

Journal: Curr Med Res Opin. 2006 Jun;22(6): 1149-60.

Comment: The objective of this study was to identify key determinants of high-density lipoprotein-cholesterol (HDL-C) level, including subclinical inflammation and insulin resistance, and to determine the prevalence of a low HDL-C phenotype in dyslipidemic patients at high cardiovascular risk. In a cross-sectional study, the prevalence of low HDL-C phenotypes was assessed in 14,667 dyslipidemic patients attending a specialized lipid clinic and the potential relationships between HDL-C level and 16 clinical and biological parameters was also evaluated. In univariate analysis, women exhibited higher plasma concentrations of HDL-C as compared with men. Levels of triglycerides (TG), fasting blood glucose, uric acid, waist circumference, body mass index, high sensitivity C-reactive protein (hs-CRP), insulin resistance (as HOMA-IR index) and smoking were all negatively correlated with HDL-C, whereas age was positively correlated with HDL-C levels. Moderate drinkers (10-30 g alcohol/day) displayed higher HDL-C concentrations than abstinent subjects. In contrast, consumption of more than 30 g/day was associated with a further non-significant elevation of HDL-C levels as compared to moderate drinkers. Multivariate analysis identified eight independent correlates of HDL-C. Age, sex and TG accounted for 37% of variability in HDL-C; modifiable factors including waist circumference, alcohol consumption and smoking, in addition to HOMA-IR and hs-CRP, accounted for an additional 5% of the variability in HDL-C. Using a cut-off of 40 mg/dL (1.03 mmol/L) for men and 50 mg/dL (1.29 mmol/L) for women, 33% and 28% of men and women displayed low levels of HDL-C. The high prevalence of low HDL-C phenotypes in dyslipidemic patients at elevated cardiovascular risk emphasizes the need for both lifestyle and pharmacological strategies of intervention to raise HDL-C.

Title: Genetic variability at the leptin receptor (LEPR) locus is a determinant of plasma fibrinogen and C-reactive protein levels.

Authors: Zhang YY, Gottardo L, Mlynarski W, Frazier W, Nolan D, Duffy J, Marescotti MC, Gervino EV, Johnstone MT, Mantzoros CS, Avogaro A, Doria A.

Journal: Atherosclerosis. 2006 Mar 30; [Epub ahead of print]

Comment: The mechanisms linking increased adipose tissue mass to increased risk of atherosclerosis are not completely understood, but recent evidence points to molecules secreted by the adipose tissue as mediators. Leptin is now considered as such a mediator. Cellular and animal studies suggest that leptin has proinflammatory and prothrombotic effects that could link increased adipose mass directly to atherogenesis. To investigate this hypothesis, the authors of the present work examined the effect of genetic variability at the leptin receptor (LEPR) locus on the plasma levels of fibrinogen and CRP—two markers of inflammation and susceptibility to atherosclerosis. Linkage disequilibrium analysis of 71 single-nucleotide polymorphisms (SNPs) spanning the LEPR locus revealed four haplotype blocks that could be tagged by 11 SNPs. In 630 healthy Caucasian individuals, variability in block #4 was significantly associated with plasma fibrinogen (P=0.005), accounting for 3% of its variance (r^2=0.030). The same block was also associated with CRP levels (P=0.049, r^2=0.022). The effect was strongest for two of the SNPs in this block. At rs3790432, fibrinogen was 10% higher in minor allele homozygotes than in major allele homozygotes and intermediate in heterozygotes (P=0.015). At rs1805096, it was 5% higher (P=0.007) and CRP 32% higher (P=0.011) in major allele homozygotes than in minor allele carriers. This pattern of association was also evident in the haplotype analysis. How leptin may modulate CRP and fibrinogen levels is not clear at this time. One possibility is an action on IL-6, IL-1, or TNF-α production by macrophages/monocytes. Alternatively, leptin may have a direct effect on the synthesis of CRP and fibrinogen in the liver. Whatever the mechanism, association of leptin receptor variability with inflammatory traits

supports the hypothesis that leptin may play a role in atherogenesis.

Title: Leptin and atherosclerosis.
Author: Beltowski J.
Journal: Atherosclerosis. 2006 Mar 30; [Epub ahead of print]
Comment: Obesity (body mass index, BMI >30 kg/m^2) and overweight (BMI 25-30 kg/m^2) are among the most important health problems in industrialized countries and also an increasing problem in developing countries. As many as one billion people may be overweight and ~300 million may be obese, worldwide. Obesity is now recognized as one of the components of the metabolic syndrome and most components of the metabolic syndrome are independent risk factors of atherosclerosis. Therefore, it is not surprising that the prevalence of cardiovascular diseases is markedly increased in obese individuals. Accumulating evidence indicates that adipose tissue hormones ("adipokines") are involved in the proatherogenic effect of obesity. The first adipose tissue hormone, leptin, was identified in 1994. It is a 167-amino acid peptide produced almost exclusively by white adipose tissue and is primarily involved in the regulation of food intake and energy expenditure. Leptin receptors are expressed in many tissues including the cardiovascular system. Plasma leptin concentration is proportional to body adiposity and is markedly increased in obese individuals. Recent studies suggest that hyperleptinemia may play an important role in obesity-associated cardiovascular diseases including atherosclerosis. Leptin exerts many potentially atherogenic effects such as induction of endothelial dysfunction, stimulation of inflammatory reaction, oxidative stress, decrease in paraoxonase activity, platelet aggregation, migration, hypertrophy and proliferation of vascular smooth muscle cells. Leptin-deficient and leptin receptor-deficient mice are protected from arterial thrombosis and neointimal hyperplasia in response to arterial wall injury. Several clinical studies have demonstrated that high leptin level predicts acute cardiovascular events, restenosis after coronary angioplasty, and cerebral stroke independently of traditional risk factors. In addition, plasma leptin correlates with markers of subclinical atherosclerosis such as carotid artery intima-media thickness and coronary artery calcifications. Inhibition of leptin signaling may be a promising strategy to slow the progression of atherosclerosis in hyperleptinemic obese subjects.

Title: Coronary atherosclerosis and alcohol consumption: angiographic and mortality data.
Authors: Femia R, Natali A, L'abbate A, Ferrannini E.
Journal: Arterioscler Thromb Vasc Biol. 2006 Jul;26(7):1607-12. Epub 2006 Apr 20.
Comment: A large number of epidemiological studies have documented that moderate alcohol use is protective against atherosclerotic cardiovascular diseases (CVD) such as acute myocardial infarction (AMI). A number of mechanisms have been postulated to explain this association, including increases in serum HDL and apolipoprotein A-I levels, anticoagulant effects (either direct platelet inhibition or inhibition of the fibrinolytic system), a reduction of inflammation, and an enhancement of insulin sensitivity. Whether the protective effect is based on a reduction in coronary atherosclerosis has not yet been established. The authors of the present work studied 1676 men and 465 women consecutively undergoing coronary angiography. A score (ATS) was calculated by summing the percent lumen narrowing of all main vessels; alcohol consumption was quantified by questionnaire. In univariate analysis, ATS was significantly ($P \leq 0.001$) associated with male sex, age, familial CVD, smoking, diabetes, hypertension, and serum cholesterol levels; alcohol consumption was associated with less frequent diabetes ($P<0.001$) and lower ATS ($P=0.02$). By multivariate analysis, alcohol intake was associated with lower ATS ($P<0.01$) independently of the other risk factors; the estimated effect size was comparable to that associated with a 1 mmol decrement in serum cholesterol. Over a median follow-up of 93 months, 37 women and 194 men died from a cardiac cause. By Cox analysis, positive predictors for cardiac mortality were male sex (hazard ratio [HR], 1.7; 95% confidence interval [CI], 1.1 to 2.6]), age (HR, 2.1; 95% CI, 1.8 to 2.5 per decade) and diabetes (HR, 1.7; 95% CI, 1.2 to 2.4), whereas alcohol consumption was the only negative

predictor (HR, 0.84; 95% CI, 0.71 to 1.00). Thus, in a selected high-risk population, moderate alcohol consumption was independently associated with less coronary atherosclerosis and lower risk for cardiac mortality.

Title: Genome-wide expression studies of atherosclerosis: critical issues in methodology, analysis, and interpretation of transcriptomics data.
Authors: Bijnens AP, Lutgens E, Ayoubi T, Kuiper J, Horrevoets AJ, Daemen MJ.
Journal: Arterioscler Thromb Vasc Biol. 2006 Jun;26(6):1226-35. Epub 2006 Mar 30
Comment: Genome-wide approaches allow for surveying the expression level of thousands of genes simultaneously. During the past six years, gene expression profiling of atherosclerosis has been used to identify genes and pathways relevant in vascular (patho)physiology. This review discusses some critical issues in the methodology, analysis, and interpretation of the data of gene expression studies that have made use of vascular specimens from animal models and humans. Analysis of gene expression studies has evolved toward the genome-wide expression profiling of large series of individual samples of well-characterized donors. Despite the advances in statistical and bioinformatical analysis of expression data sets, studies have not yet fully exploited the potential of gene expression data sets to obtain novel insights into the molecular mechanisms underlying atherosclerosis. To assess the potential of published expression reports, the data of a CC chemokine gene cluster between 18 murine and human gene expression profiling articles were compared. The analysis revealed that an adequate comparison is mainly hindered by the incompleteness of available data sets. The challenge for future vascular genomic profiling studies will be to further improve the experimental design, statistical, and bioinformatical analysis and to make data sets freely accessible.

Title: Combining serum biomarkers: the association of C-reactive protein, insulin sensitivity, and homocysteine with cardiovascular disease history in the general US population.
Authors: Cummings DM, King DE, Mainous AG, Geesey ME.
Journal: Eur J Cardiovasc Prev Rehabil. 2006 Apr;13(2):180-5.
Comment: Most studies evaluate cardiovascular (CVD) risk markers singly, adjusting for other known CVD risk factors. Elevated levels of C-reactive protein (CRP) and homocysteine have been independently associated with cardiovascular risk. However, the prevalence of concurrent elevations of these biomarkers in the general population is unknown, as is their association with cardiovascular disease (CVD). One strategy for improving the predictive power of various markers is to combine them. In this study, data from adults (n=4900) in the National Health and Nutrition Examination Survey were used to investigate the relationship between combinations of serum biomarkers of inflammation (CRP), atherosclerosis (homocysteine), and insulin sensitivity [homeostatic model assessment (HOMA), fasting insulin] and CVD. Using SUDAAN (a specialized statistical program), logistic regression models were constructed to examine the relationships between elevated serum biomarkers (CRP, homocysteine, HOMA, or insulin), singly or in combination, and having a history of heart failure, myocardial infarction (MI), stroke, or any CVD, while controlling for age, race, sex, obesity, smoking, cholesterol level, diabetes history, hypertension history, exercise level, and dietary fiber intake. After adjustment for covariates, there was a significant relationship between concomitant elevations of CRP plus homocysteine and a history of MI [odds ratio (OR) 2.21], heart failure (OR 2.14), and any CVD (OR 1.87) that was stronger than the relationship between individual biomarkers alone and a history of CVD. In addition, combinations of elevated CRP plus HOMA and CRP plus insulin, remained significantly related to having a history of any CVD. These findings support the possibility for improving cardiovascular risk stratification through the concurrent evaluation of multiple biomarkers. In particular, the combination of CRP and homocysteine should be considered prospectively as a predictor of CVD.