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Background on dietary fat and blood lipids

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□ In the consideration of dietary fat and heart disease several questions arise. □ First, how much fat is "healthy", both in terms of the total dietary amount (in grams) and as a percent of your total daily calorie intake (abbreviated as %en), for example, 20%en, 30%en, or 40%en? □ Second, once these fat intake parameters are ascertained, what is the proper balance of fatty acids, ie. the ratio of saturated fatty acids (abbreviated as SFA or S) to monounsaturated (MUFA or M) to polyunsaturated (PUFA or P)]? □ And within each of these categories, which specific SFA and PUFA are most healthy? Third, how can one measure a "healthy response" to changes in these dietary fat components? □ Fourth, what influence does dietary cholesterol have in this scenario? □ Finally, how important is the underlying lipoprotein (LP) profile of an individual when considering their response to fat?

□ The answer to these questions is complex, but it is generally agreed by most in the field that an individual's response to dietary fat can be best evaluated by measurements of total blood cholesterol (TC), and its two main lipoprotein subcomponents, LDL and HDL, with the lowest TC and LDL/HDL ratio being considered ideal.

Also to be considered is how fat affects the total blood triglycerides (TG), or fat pool, and the size of the LDL particle, because small, dense LDL are associated with increased coronary heart disease (CHD).

□ How much fat? The current National Cholesterol Education Program (NCEP) and American Heart Association (AHA) Dietary Guidelines encompass the best and most relevant guide for fat and cholesterol intake. □ These heart-healthy programs recommend limiting fat to 30-40%en, with prudence favoring the low end since 40%en from fat, which is common in the American diet, tends to have the undesirable consequence of raising TC and LDL. □ Decreasing fat intake to 20%en also can be troublesome because even though TC and LDL may decline, HDL may also fall, while triglycerides tend to rise. This combination typically leads to more-dense, atherogenic LDL particles that induce CHD. □ The reason for this adverse LP profile can reflect the fatty acid imbalance between S:M:P, which is often distorted at 20%en. □ That is, PUFA can easily become limited, thereby distorting LP metabolism and the LP profile.

□ FATTY ACID BALANCE. □ Each fat molecule includes 3 fatty acids (FA) that are responsible for the consistency of the fat. For example, is it an oil containing mostly PUFA, or a solid fat, containing mostly SFA? The original AHA Step I Diet fat recommendation was perceptive because it recognized the significance of the FA balance contributed from all our diet fats, suggesting it would be best at approximately 1:1:1 for S:M:P. Careful review of numerous reports in the literature has revealed the importance of this balance for generating the best LDL/HDL ratio. Furthermore, it would appear that the S:M:P balance is critical at any level of fat intake if one wishes to avoid adversely affecting the LP profile. Currently the AHA recommends slightly less SFA and PUFA than MUFA in the balance.

SATURATED and TRANS FATTY ACIDS. Within the concept of a "balance" among classes of SATs, MONOs and POLYs (ie. the whole fats) is the issue of which specific fatty acids, among the SFA or PUFA, are best. For example, there are typically four SFA that contribute the major pool of fatty acids attached to a SATURATED FAT molecule (remember that each fat molecule contains three fatty acids). Similarly, several specific PUFA of the omega 3 (n-3) or omega 6 (n-6) families are the major contributors to the pool of fatty acids in a POLYUNSATURATED fat. Many studies have clearly shown that SFA, in general, raise TC, LDL, and HDL while PUFA lower these values. But certain SFA (as consumed in our daily diet) are less problematic in terms of their impact on the LDL/HDL ratio than others. Fats rich in lauric and myristic acids (12:0+14:0, in milkfat, coconut oil, palm kernel oil) are the worst for raising LDL. Stearic acid (18:0) is not very prevalent in saturated fats, but it is neutral in its effect on blood cholesterol when consumed in natural fats. The most common SFA is palmitic acid (16:0), (so named because it represents the major SFA in palm oil, the fat). The 16:0 SFA is present to some degree in essentially all fats and is by far the most prevalent SFA in our diets. Our liver also makes 16:0 from extra carbohydrate molecules we consume. Considering the influence on TC and the LP profile, 16:0 is intermediate among the SFA, ie. it can be neutral when representing one of the 3 fatty acids in a triglyceride (fat) molecule in conjunction with MUFA, PUFA or 18:0, or it can help raise blood cholesterol if placed in a fat molecule along with 12:0+14:0, ie. such that all 3 fatty acids in the fat molecule are SFA. In high amounts dietary 16:0 can raise TC and LDL when substituted for 18:0, MUFA, or PUFA in people who already have elevated TC or who eat large amounts of cholesterol. Accordingly, the general advice has been to remove as much SFA from the diet as possible. But this is not practical in the extreme because the manufacture of many food products requires SFA (or some facsimile thereof, such as trans fatty acids) for technical reasons; and extreme restriction of dietary SFA is not prudent because their deletion from the diet surprisingly exerts an adverse effect on the LDL/HDL ratio. Even the "less than 7% of calories from SFA" now proposed by NCEP and AHA can prove deleterious for HDL if total fat is allowed to drift towards 40%en, thereby resulting in an imbalance towards only MUFA and PUFA (see examples that follow).

TRANS ARE BAD. So what should be the approach to SATURATED FAT, and what dietary combination of SFA should we allow in our diet? In recent years one mistaken answer has been to utilize synthetic SFA-like molecules manufactured by "hardening" vegetable oils through hydrogenation. This process makes a stiff, plastic fat that is rich in so-called TRANS fatty acids (TFA). But it turns out that certain of these TFA can be worse than any of the individual natural SFA because they not only raise LDL but also lower HDL, leading to an unfortunate and exaggerated increase in the LDL/HDL ratio (unfortunate in terms of cardiovascular risk). Trans fatty acids also increase a highly atherogenic lipoprotein in the LDL fraction called Lp(a), and they elevate the blood TG pool when the 18:2 PUFA intake is low. An alternative to this predicament is to provide a reasonable level of SFA in our diet by careful selection of naturally available SFA. Our research with monkeys and humans indicates that the guidelines are best tempered by the original AHA Step I diet (30%en from dietary fat, and approximately 1:1:1 for S:M:P) and that the best SFA are 16:0 and 18:0 from natural fats. Recall that not much 18:0 is found in natural fats, so that leaves us with 16:0 from natural sources. This conclusion results from carefully analyzing all aspects of the NCEP-AHA recommendations coupled with analysis of the available blood lipoprotein data in relevant studies involving the controlled intake of dietary fat in humans (and experimental animals).

BALANCE AMONG PUFA. In selecting PUFA, the issue of whether to include linoleic acid (18:2n-6) or linolenic acid (18:3n-3), or longer n-3 like EPA and DHA from fish oil, must be considered. Both n-6 and n-3 families are essential fatty acids (needed in the diet because the body cannot synthesize them) and both are important to health, especially cardiovascular health. The linoleic acid (n-6) level has the greatest impact on regulating the LDL/HDL ratio, whereas linolenic acid (n-3) and its longer derivatives have a major influence on clotting mechanisms, as well as stabilizing the heart against abnormal beating, called arrhythmias, that can lead to sudden death. Diets enriched in 18:3n-3, or even better, 22:6n-3 (DHA) have been shown to exert a significant anti-CHD effect in humans, both in clinical and epidemiological studies. Smart Balance™ contains a good balance (7:1) of linoleic (n-6) to (n-3) linolenic acids. This balance is unlike partially hydrogenated margarines, in which most of the linolenic acid has been destroyed by processing, and is also unlike most vegetable oils, which contain only a small amount of this important fatty acid (soybean and canola oils being exceptions).

DIETARY CHOLESTEROL. Dietary cholesterol is very important in this scenario, as evidenced by the NCEP-AHA diet recommendations to reduce daily intake below 300mg or even 200mg, depending on individual risk. In fact, dietary cholesterol increases the body's sensitivity to SFA, particularly 16:0, so that maximizing its removal can substantially reduce much of the negative influence of SFA on the LP profile. Polyunsaturated fatty acids, on the other hand, are the major FA able to actually offset the negative impact of dietary cholesterol because linoleic acid (18:2n-6) increases the removal of plasma LDL, the main LP that is increased by dietary cholesterol.

MONOUNSATURATES. From our results and the analysis of others, monounsaturated fatty acids (MUFA) have been found to be essentially neutral in terms of the LP profile, and thus, perhaps, are the best source of FA to use as extra "filler" in the dietary fat load. To an extent that is true, but the critical issue remains as to how much SFA and PUFA should be consumed to achieve the best LDL/HDL ratio? As our comparison between olive oil and balanced fat revealed in cynomolgus monkeys, a high MUFA intake at the expense of PUFA and SFA does not counter the presence of dietary cholesterol very well and leads to an increased LDL/HDL ratio relative to a balanced S:M:P ratio that allows for a higher PUFA intake. Thus, for example, Smart Balance™ fatty acids incorporate a better FA balance than olive oil alone.

THE LIPOPROTEIN PROFILE. How the individual LP profile (normal cholesterol versus high cholesterol) fits into this story is an important consideration. Obviously more research will be needed, but it appears that the fundamental response to the current AHA balanced fatty acid diet is similar in both categories of individuals. That is, the approximately 1:1.5:1 balance in S:M:P recommended appears to be important to both groups for generating the ideal LDL/HDL ratio. In absolute terms, the response by the high-cholesterol individual to changes in the diet fatty acid profile is more dramatic, but the person with a normal cholesterol value responds in the same manner, just not to the same degree.

THE LDL/HDL RATIO. It is true that an elevated cholesterol (TC >180mg/dl, LDL >110mg/dl) begins to increase risk for CHD. Most of any increase above 180mg/dl arises in the

LDL pool, and this lipoprotein is the one that is deposited during arterial cholesterol build-up. On the other hand, people (and essentially all animals) with naturally high levels of HDL do not develop CHD, primarily because this lipoprotein transports cholesterol back to the liver for excretion in bile. HDL in the arterial wall also blocks LDL deterioration, thereby preventing the local damage induced by LDL accumulation. Thus, the "bad" and "good" connotation for these two LPs becomes apparent, and it is easy to understand why one wishes to have the lowest LDL and highest HDL (ie. lowest LDL/HDL ratio) for any given TC value.

THE BRANDEIS-GFA CONNECTION. The novel finding from collaborative nutritional research at Brandeis University and the Palm Oil Research Institute of Malaysia (PORIM) resulted in technology for fat blends free of trans fatty acids and a Brandeis patent that was licensed to GFA BRANDS. The patent defines a means to reduce to practice the concept of approximately 1:1:1 balance in S:M:P recommended for many years by the AHA, and adjusted by trial and error to approximately 1:1.3:1 through Brandeis-PORIM experiments and product development by GFA. Adequate intake of natural fats blended to approximate this FA balance consistently elicits the best LP profile in animals and humans.

Review of the literature (see below) suggests that this would be true for all levels of fat intake normally consumed in Western Diets (20-40% of total calories). Significant deviation from a 1:1.3:1 ratio between S:M:P, such as too low S or too high M or P, induces a less than ideal LP profile, even if the total plasma cholesterol is lower. Licensing of the Brandeis –PORIM technology by GFA Brands, Inc. has resulted in Smart Balance / Earth Balance margarines and a family of related products for use in a total diet program specifically designed to approximate this 1:1.3:1 fatty acid balance from blends of natural oils, thereby removing all trans fatty acids. Several human studies and epidemiologic reports indicate that TFA are “equal to or worse than” the saturated fatty acids they were designed to replace. In fact, some of the deleterious effects attributed to saturated fatty acids over the years were probably the result of their substitution by TFA.

SUPPORTING LITERATURE. Although future research will undoubtedly examine and refine these points in more detail, the following published reports provide support for the major aspects of the above discussion.

Figures and supporting text. For orientation purposes, each figure is designed to orient the reader to the key information presented in each literature report. The figure number is located in the upper left corner. The literature reference is abbreviated in the lower right corner, with full citation in the text. The text also highlights the major results reported in the reference and summarized by the graphics. The columns represent the total blood cholesterol value subdivided into the 3 lipoprotein classes that transport cholesterol (VLDL, very low density lipoprotein in green...a minor fraction which also carries most the TG; LDL, low density lipoprotein, the major “bad” cholesterol carrier, in red; and HDL, high density lipoprotein, the “good” carrier in orange, which usually transports about 1/5 the total cholesterol).

Each column represents the average values reported for TC, LDL, and HDL for the dietary treatment under discussion, ie. an average for all subjects fed that diet. The small number to the right of each column between LDL and HDL represents the LDL/HDL ratio in response to

that diet. Ratios greater than 4.0 represent high risk for CHD, while ratios below 2.0 represent very low CHD risk. Thus, a low ratio is desirable. Actual numbers are added to each fraction and column to facilitate interpretation. An asterisk with a number indicates statistical significance, generally relative to the control value.

Beneath each column is a condensed code that nutritionists typically use to characterize fat diets applied to CHD research. The first line is the code name for the diet; the second line indicates the percent of the dietary calories derived from the fat, most typically ranging between 30-40% (often written as % energy or %en). The third line denotes the overall ratio of S:M:P (saturated, monounsaturated, polyunsaturated) fatty acids contributed by the various fat molecules consumed in the diet. The fourth line represents a shorthand (the P/S ratio) that quickly keys a nutritionist to the cholesterol-raising potential of a dietary fat composition, eg. when the P/S ratio is < 0.5, saturated fatty acids (SFA) are twice as prevalent as PUFA, and the blood cholesterol may rise, while a P/S ratio of 1.0 or more generally maximizes the lowering of TC by the fat component in the diet. The typical dietary P/S ratio in North America is about 0.4. If we could raise it to about 1.0, major health benefits would follow.

By using the above code while reading the study summaries below, the reader can gain substantial insight concerning the relationship between the lipoprotein response and changes in specific classes of dietary fatty acids (S,M,P).

HDL CAN INCREASE WHEN TOTAL FAT INTAKE DECREASES (ref 1). It is generally agreed that replacing fat with carbohydrate is associated with a decline in TC, but unfortunately HDL also tends to decrease. In retrospect, one of the first studies to show that this need not occur was a subgroup from the Oslo Study, which basically applied the AHA Step 1 diet approach to a large population. In actual practice, reductions in total fat, especially SATURATED and MONOUNSATURATED, and dietary cholesterol to slightly < 30%en and <300mg/day, respectively, greatly reduced TC and LDL without decreasing HDL in 18,000 men. To examine this response more closely, 3 diet-responders from the original study were subsequently compared with 23 controls who continued to eat the high-fat baseline diet. Both groups had identical, elevated blood lipid values initially. The test group was taught how to lower dietary fat from 44%en to about 30%en by focusing on removal of saturated fat. In the process, a good balance in S:M:P was achieved, decreasing from an imbalanced 18:19:7 to 8:12:8 %en. The data (Fig 1) demonstrate sharp declines in TC, LDL and TG (200 vs 129 mg/dl) with an equally robust *increase* in HDL (42 vs 50mg/dl). Thus, removing both SFA and MUFA from a high-fat diet to improve the overall FA balance can decrease LDL sharply, but may also increase HDL if the P/S ratio approximates 1.0 and total balance S:M:P approximates 1:1.3:1.

ref 1. Hjermann, I., Enger, S.C., Helgeland, A., Holme, I., Leren, P. and Trygg, K. The effect of dietary changes on high density lipoprotein cholesterol: The Oslo Study. *Am J Med* 66:105-109, 1979.

BOTH SFA AND PUFA ARE REQUIRED FOR THE BEST LDL/HDL RATIO (ref 2). This report tested the hypothesis that providing either too few saturated fatty acids (SFA) or polyunsaturated fatty acids (PUFA) in the diet (ie. an imbalance between S and P) would be

detrimental to the HDL or LDL level, respectively. Three fats were fed in whole-food diets, providing 2/3 of the daily fat load from the supplemented oil in each diet (with 31% of daily calories as fat) for 23 young men with normal cholesterol values.

The diet fat was initially balanced as AHA Step I with a 10:13:8 ratio of S:M:P in the final diet followed by a high-MUFA, low-SFA (6:17:8) or a high-SFA, low-PUFA (13:14:4) diet. The first fat represented a blend of soybean oil:palm oil: canola oil, whereas the other two fats were supplied as canola oil or palm olein alone.

All three fats produced about the same normal total cholesterol value, but the AHA blend produced the highest HDL and lowest LDL, so that the LDL/HDL ratio was significantly enhanced by the AHA balanced blend of S:M:P (see Fig.2).

Thus, neither too low SFA nor too low PUFA was adequate, and MUFAs were no substitute for either. Rather one needs a balance of PUFAs (to lower LDL) and SFAs (to raise HDL) for the best TC and LDL/HDL profile, at least when following an AHA Step I diet at 30% en from fat. The 9:12:9 balance for S:M:P inherent in the current NCEP and AHA recommendations for 30% en from fat appears to be the best advice for the average individual.

ref 2. Sundram, K., Hayes, K. C. and Siru, O. H. 1995. A balance between dietary 18:2 and 16:0 may be required to improve the serum LDL/HDL cholesterol ratio in normocholesterolemic men. J.Nutr. Biochem. 6,179-87.

PUFA INTAKE IS CRITICAL FOR THE BEST LDL/HDL RATIO (ref 3). Another study addressed two questions a) whether a low-fat diet (20% fat calories) would improve relatively normal TC values in 31 adult women, and b) whether it matters much if dietary fatty acids are balanced between SFA:MUFA:PUFA in either a high-fat (40% en) or a low-fat (20% en) diet situation, ie. considerably above or below the AHA Step I diet objective of 30% fat energy, and with or without the 9:12:9 balance in S:M:P which an AHA diet would support.

Several results were apparent (see Fig 3). The dietary P/S ratio was only 0.3 in group I (n=15) and 1.0 in group II (n=16) women. Fatty acid balance had little effect on LDL or HDL at 40% en, primarily because the basal (group 1) intake of PUFA (6% en) was close to the amount of 18:2 required for normal lipoprotein (LP) metabolism given the circumstances of these normolipemic women. But the superior balance (P/S 1.0) did tend to improve the LDL/HDL ratio slightly at this high-fat intake. However, when consuming the low-fat diet, balance in fatty acids was especially important because a balanced 1:1:1 ratio (group II) prevented the substantial decline in HDL seen with group I, where the typical American Fat imbalance (P/S, 0.3) resulted in higher LDL and lower HDL with a much improved LDL/HDL ratio. The undesirable impact on LDL and HDL in group I occurred primarily because the absolute intake of PUFA (@3% en) was too low for adequate lipoprotein metabolism when total fat supplied only 20% en. Thus, the LDL/HDL ratio was much improved by feeding the 1:1:1 fatty acid balance at the low-fat intake (group II) because the 6% en from PUFA was now adequate in absolute terms, ie. in total grams of 18:2/day.

Thus, with dietary fat somewhere between 40% en and 20% en a proper balance in fatty acid intake becomes exceedingly important for generating an optimal LDL/HDL ratio, ie. the lowest LDL and highest HDL values. Like Sundram et al (ref2), it would appear that a controlled intake of PUFA (18:2) is required to allow for the greatest decline in LDL without

also lowering HDL. The particular type of SFA fed in this study was not specified, although an amount of total SFA equal to the PUFA resulted in a very favorable LDL/HDL response.

Ref 3. Jones, D.Y., Judd, J.T., Taylor, P.R., Campbell, W.S. and Padmanabhan, P.N. 1987. Influence of caloric contribution and saturation of dietary fat on plasma lipids in premenopausal women. *Am. J. Clin. Nutr.* 45,1451-6.

FATTY ACID BALANCE SELECTIVELY LOWERS LDL BUT NOT HDL (ref 4). In a theme reminiscent of the above references, this report addressed the issue of whether simply improving the fatty acid balance in the diet of 30 normolipemic men fed a typical Western Diet fat intake (37%en) would enhance the lipoprotein profile, even after 3 months of comparison feeding and even if not including the typical goal of reducing fat intake to 30%en. The hypothesis was tested by switching from a P/S fatty acid ratio of 0.3 to a ratio of 1.0, ie. by adopting an AHA balance in S:M:P of 1:1.3:1. The average entry TC was upper-normal (200mg/dl), and the level of PUFA intake (5.6%en) is very typical of USA today. Balancing the P/S to 1.0 by shifting 6%en from SFA to PUFA caused a significant decline in TC and LDL without depressing HDL (see Fig.4). This resulted in significant improvement in the LDL/HDL ratio. A design flaw was the failure to designate the specific type(s) of SFA removed.

Thus, similar to a subsequent trial (Weisweiler, ref 5), balancing the dietary FA intake over a significant period of time is beneficial. Balancing FA is important if one wants to lower LDL without depressing HDL, even when consuming a somewhat elevated level of dietary fat (37%en) in normolipemic subjects.

Ref 4. Schwandt, P., Janetschek, P., and Weisweiler, P. 1982. High density lipoproteins unaffected by dietary fat modification. *Atherosclerosis* 44, 9-17.

FATTY ACID BALANCE IS MORE CRITICAL THAN THE AMOUNT OF FAT (ref 5). This report evaluated the importance of dietary fatty acid balance on the lipoprotein profile in 22 nuns (22-55yr, mostly post-menopausal) who had mildly elevated TC (224mg/dl at entry). They were fed three dietary fats for 6 weeks each: first, a high-level, saturated fat (42%en, P/S= 0.16); or second, that same level of fat with a balanced fatty acid profile (P/S, 1.0), which was accomplished by decreasing SFA (exact fatty acid profile not provided) and increasing PUFA. The third fat was close to the original AHA Step I (32%en with a 1:1:1 balanced fatty acid profile) and similar to the S:M:P balance in the second fat rotation.

The results (Fig 5) suggest that if one begins with a very unfavorable P/S ratio (only 0.16 because PUFA was too low) in a high-fat diet (42%en), balancing the P/S ratio along AHA guidelines improves TC and the LDL/HDL ratio. The new balance between SFA and PUFA decreased LDL and increased HDL slightly. However, dropping fat intake to 32%en with the AHA balance in place did not improve TC or the LDL/HDL ratio further. Thus, in the 30-40%en range, a balance (adequate PUFA, adequate SFA) seems more critical than total fat. Although the exact SFA profile was not described, other studies have found that decreasing 12:0+14:0 is more important than decreasing 16:0+18:0 if the best LDL/HDL ratio is to be achieved at a lower SFA intake (see ref 10).

Thus, the approximately equal balance of S:M:P (1:1.3:1) as recommended by NCEP-AHA is an important basic consideration *at any fat intake* for maintaining the best LDL/HDL ratio.

ref 5. Weisweiler, P., Janetschek, and Schwandt, P. 1985. *Influence of polyunsaturated fats and fat restriction on serum lipoproteins in humans.* *Metabolism.* 34,83-87.

TOO HIGH PUFA or TOO LOW FAT DEPRESSES BOTH LDL AND HDL (ref 6). This report demonstrates what happens to LDL and HDL in normolipemic (n=11) and hyperlipemic (n=19) subjects fed a very saturated, high-fat diet (P/S 0.2, 40%en) or a very polyunsaturated, high-fat diet (P/S 2.0, 40%en). Subjects were then compared to an almost fat-free saturated fat diet (P/S 0.2, 3%en). The questions addressed were two: does the response of people with normal cholesterol differ from those with high cholesterol?.. and does the LDL/HDL profile benefit more from a high-polyunsaturated fat approach to diet modification, or is it better to drastically reduce the fat intake by eating a high-carbohydrate (low-fat) diet without concern for the fatty acid balance?

The results (Fig 6) show that a high-PUFA diet (P/S 2.0) decreased both LDL and HDL in all subjects. Removing essentially all the fat (Lofat) decreased both LDL and HDL even further. The LDL/HDL ratio did not improve with either tactic, and the general response was similar for both groups of subjects, ie. normolipemics and hyperlipemics.

Thus, a very high-PUFA or an essentially fat-free diet will both decrease TC and LDL in both normolipemic and hyperlipemic subjects, but the decline in HDL is also substantial. The LDL/HDL ratio does not improve. Thus (as shown by Weisweiler et al, ref 5), if one wishes to maintain the HDL while selectively lowering LDL and thereby improve the LDL/HDL ratio, a balance between dietary SFA and PUFA is important. The same decrease in LDL obtained with very high PUFA can be achieved by simply balancing S:M:P, and this balanced approach does not depress HDL.

ref 6. Schaefer, E.J., Levy, R.I., Ernst, N.D., Van Sant, F.D., and Brewer, H.B. 1981. *The effects of low-cholesterol, high-polyunsaturated fat, and low fat diets on plasma lipid and lipoprotein cholesterol levels in normal and hypercholesterolemic subjects.* *Am. J. Clin. Nutr.* 34, 1758-1763.

FATTY ACID BALANCE IS ESPECIALLY CRITICAL IN LOW-FAT DIETS (ref 7). The objective of this study (ref 7) was somewhat similar to reference 3, emphasizing the importance of balance at any level of fat intake. Specifically, it determined whether the TC and lipoprotein profile would be altered by decreasing fat intake from a high level (39%en) to a low level (22%en) if the P/S ratio were held constant and balanced at about 1.0. Most studies show that switching to a high-carbohydrate (low-fat) diet lowers TC, including both LDL and HDL (eg. see ref 6). Nine normolipemic males were evaluated in a carefully monitored metabolic ward, but the S:M:P ratios were not totally balanced and were 1.2:1.5:1.0 (hi-fat) and 1:1.4:1 (low-fat), providing P/S ratios of 0.8 and 1.0, respectively.

The results (Fig 7) reveal that the TC, LDL, and HDL were not significantly affected by the fat load, although they tended to be slightly lower during the low-fat period without affecting

the LDL/HDL ratio. Thus, a low-fat diet (22%en) does not necessarily mean that HDL will decline during a high carbohydrate intake, provided that the balance between SFA and PUFA is maintained. However, the tendency toward slightly lower HDL at 22%en suggests that 30%en from fat might better sustain HDL (per Sundram et al, ref 2) or that the MUFA intake was allowed to drift up too far relative to SFA and PUFA for this low fat intake.

The results suggest that the dietary P/S ratio is important at any fat intake, but is especially critical for maintaining the best LP profile during low-fat intake (<20-25%en) because it dictates the absolute intake of 18:2. At low fat intakes, a low P/S ratio (<0.5) greatly limits the 18:2 needed to meet metabolic requirements for normal LP metabolism, especially for lowering the LDL, but also for sustaining HDL. As pointed out in other references, a dietary S:M:P ratio of 1:1.3:1 generally appears to be best.

ref 7. Nelson, G.J., Schmidt, P.C., and Kelley, D.S. 1995. Low-fat diets do not lower plasma cholesterol levels in healthy men compared to high-fat diets with similar fatty acid composition at constant caloric intake. Lipids 30, 969-976.

HIGH-MONO NOT AS FAVORABLE AS LOW-MONO DIET (ref 8). The original AHA recommendation called for an even balance between S:M:P at 30%en from fat. Recently, AHA has recommended approximately 50% more MUFA at the expense of SFA and PUFA, especially as fat intake rises above 30%en. However, a human study in 8 normolipemic males demonstrates the potential problem of over-exaggerating the M:P ratio, feeding either 0.5 or 3.0 M:P ratios in two diets in which the P/S ratio would be considered ideal and constant at 1.0 (Fig 8).

The high-MUFA diet produced a TC that was identical to the low-MUFA diet, but the LDL was elevated ($p < 0.05$) when SFA and PUFA intake became too low; the HDL was also lower (n.s.), so that the LDL/HDL ratio was significantly increased by high MUFA (Fig. 8). In addition, the high-MUFA diet induced a 20% rise in triglycerides. Thus, the high-MUFA diet proved inferior to the low-MUFA intake.

The implication is that a proper balance in all three fatty acid classes (S:M:P) is important for generating the best LDL/HDL ratio. Whereas keeping the P/S ratio about 1.0 may be the most critical relationship, it would appear that MUFA should not exceed 1.5 in their relative abundance with P and S.

ref 8. Chang, N.W. and Huang, P.C. 1990. Effects of dietary monounsaturated fatty acids on plasma lipids in humans. Lipid Res. 31, 2141-2147.

HIGH MUFA INFERIOR TO BALANCED S:M:P (ref 9). The objective of this study in cynomolgus monkeys more precisely explored the relative importance of the S:M:P balance in the regulation of TC and LDL/HDL ratio when consuming 30%en and less than 300mg/day cholesterol human equivalent (ie. AHA Step I diets). Diet fats were an Average American control at 35%en (AAD, with P/S 0.5) and 3 comparisons at 30%en. Two of these (AHA-1X, AHA-1H) blended different oil sources to reach about the same balanced S:M:P ratios while the third balanced S and P, but had much more MUFA (olive oil alone). As in ref 8 with humans, an unfavorable imbalance developed in the LDL/HDL ratio with olive oil when the SFA and PUFA were about equal (P/S 0.8), but too low relative to MUFA (see Fig 9). Diet AHA-1H with P/S of

1.1 represented a blend of 3 oils, whereas Diet AHA-1X represented 4 oils. The TC response, as well as the LDL/HDL ratio, were much improved when the relative intake of S:M:P was fully balanced in the two AHA diet blends.

Thus, the dietary P/S ratio is a rough index of how a fat will affect the plasma LDL/HDL ratio, but an approximate balance between all three FA classes (S:M:P) appears most critical, at least for 30% fat intake.

Ref 9. Hayes, K.C. et al. Lipoprotein response of cynomolgus monkeys fed AHA Step I diets having different fatty acids profiles (unpublished data).

PROGRESSIVE REMOVAL OF SFA LOWERS BOTH LDL-C AND HDL-C. This carefully executed first DELTA study examined the effect of a two-step selective removal of SFA (@4.5% each step) from a human diet containing 34% as fat, while keeping MUFA and PUFA constant (ref 10). Even though the P/S ratio increased to 1.0 in the process, MUFA intake equaled the other two FA classes combined in the low-fat diet (containing 25% as fat). This progressive removal of 9% as SFA decreased LDL by 12%, but HDL was depressed proportionally (Fig 10).

Thus, the indiscriminant removal of SFA (individual SFA not identified) lowers TC without improving the LDL/HDL ratio, at least when MUFA intake substantially exceeds that of SFA or PUFA.

Ref 10. Mustad, V.A., Etherton, T.D., Cooper, A.D., Mastro, A.M., Pearson, T.A., Jonnalagadda, S.S., and Kris-Etherton, P.M. Reducing saturated fat intake is associated with increased levels of LDL receptors on mononuclear cells in healthy men and women. J. Lipid Res. 38:459-468,1997.

SFA ARE BEST REPRESENTED BY 16:0 AND 18:0 (ref 11, 11a). The most recent NCEP and AHA diets recommend a fat intake of about 30% with a balance of approximately 7:15:8 % for S:M:P. As indicated by ref.10, this fat profile typically means reducing SFA in the average diet, but does it matter which of the major 4 SFA are removed? Our data from cebus and rhesus monkeys reveal that removal of fats containing 12:0+14:0 (leaving 16:0+18:0-rich fats) leads to a greater reduction in TC and LDL and results in a better LDL/HDL ratio (Fig 10), especially if the overall fatty acid profile is balanced instead of simply removing the SFA. The preference for 16:0+18:0 reflects the fact that 12:0+14:0-rich fats tend to increase LDL more than HDL.

Thus, when balancing the S:M:P ratio in a fat blend, it is preferable to utilize a natural 16:0+18:0-rich fat (eg. palm oil, beef tallow) rather than one rich in 12:0+14:0 (eg. milk fat, coconut oil, palm kernel oil) in terms of generating the best LDL/HDL ratio.

Ref 11. Ideal LDL/HDL ratio requires precise balance in dietary saturated and polyunsaturated fatty acids in cebus monkeys. Pronczuk, A., and Hayes, K.C. FASEB J. 6: A561, 1999

Ref 11a. Khosla, P., Hajri, T., Pronczuk, A. and Hayes, K.C. Decreasing dietary lauric and myristic acids improves plasma lipids more favorably than decreasing dietary palmitic acid in rhesus monkeys fed AHA Step 1 diets. *J. Nutr.* 127:525S-530S, 1997.

TRANS are WORSE THAN SFA in HUMANS. Trans fatty acids are generated when vegetable oils are hardened by hydrogenation in order to replace naturally saturated fat in the diet. Since they typically are monounsaturated, it was thought that *trans* exerted a neutral effect on cholesterol metabolism and other biological functions. However, more recent data suggested that they have a negative influence on lipoproteins and possibly other functions, as well. To examine this point more directly, *trans* 18:1n9 (elaidic acid) was compared head-to-head with the most cholesterol-raising saturated fatty acids and the neutral, cis18:1n9 (oleic acid) in humans. The four fats focusing on these fatty acids were tested in natural diets of normocholesterolemic subjects who each consumed all 4 diets over a 16 wk period (Fig 12). The data reveal that *trans*FA proved as cholesterol elevating as the worst SFA (12:0+14:0), and that *trans* had the most detrimental impact on LDL (greatest increase) while uniquely depressing HDL. Again, note that the 16:0-rich fat was neutral and comparable to the cis18:1-rich fat. Thus, when assessed by direct comparison with specific fatty acids, *trans*FA proved worse than the saturated fatty acids they were designed to replace.

Ref 12. Sundram, K., Ismail, A., Hayes, K.C. Trans (elaidic) fatty acids adversely affect the lipoprotein profile relative to specific saturated fatty acids in humans. *Nutr.* 127:514s-520s, 1997.