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Replacing Trans Fat: The Argument for Palm Oil with a Cautionary Note on Interesterification

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To replace dietary trans fatty acids (TFA), two practical options exist: revert to a natural saturated fat without cholesterol (most likely palm oil or its fractions) or move to a newer model of modified fat hardened by interesterification (IE). This review summarizes the relative risks for cardiovascular disease inherent in these options. Interestingly, both types of fat have been the subject of nutritional scrutiny for approximately the last 40 years, and both have positive and negative attributes. Only during that period has palm oil production developed to the point where it has become the major edible oil in world markets, making clinical studies of it an important objective. On the other hand, approximately 25 human studies have fed interesterified fat in one form or another over this period, some for weeks, some as a single meal. Two types of diet designs exist. Several fed a small amount of interesterified fat, usually incorporated within a margarine, and stayed below the radar of biological detection of any abnormal metabolism. A few fed interesterified fat that incorporated stearic acid, as interesterified 18:0 (IE-18:0), even comparing it to trans fat and saturated fat, as a major part of total daily calories to assess its metabolic impact per se. These latter 5 to 6 studies clearly reveal negative biological effects on lipoproteins, blood glucose, insulin, immune function, or liver enzymes when relatively high intake of IE-18:0 or palmitic acid (IE-16:0) were fed in fats with sn2-saturated fatty acids. High intake of 18:0 in natural fats can depress total lipoproteins, while IE-18:0 and IE-16:0 at high levels adversely affect lipoprotein metabolism.

Still other studies have supplied interesterified fat as a single meal or fed such fat daily only in a single snack, as opposed to incorporating the fat into the entire fat pool consumed at all meals in association with most foods (which is the more physiological approach and more apt to elicit effects). Even in meal studies, IE-18:0 typically delayed fat absorption postprandially, indicating its effect on fat metabolism originating, in part, in the intestine.

Mainly 2 saturated fatty acids (18:0 or 16:0) have been interesterified to harden oils, using the 16:0 from fully hydrogenated palm oil or 18:0 from fully hydrogenated soybean oil as the source material. It is not clear that IE-16:0 is as problematic as IE-18:0, but IE-16:0 has been studied less. Levels between 8% energy (%E) and 12%E from 18:0 as interesterified fat (the typical diet provides about 2%E–4%E as 18:0 from natural fats) show the most effect. Detection of adverse effects would seem to start around 7%E–8%E as IE-18:0, but one can assume that effects are initiated, even if undetected, at a lower intake, similar to the situation with TFA. Thus, although an intake of 1%E to 4%E from IE-18:0 does not appear to influence lipoproteins, it is not necessarily the only system affected. The negative effects of IE-18:0 may be alleviated or masked by dilution with other fats, especially by adding 18:2-rich polyunsaturated oils to the diet. This is similar to the trans fat story, i.e., if a limited intake of TFA is heavily diluted with other oils, the consumption of TFA fails to be detected as an adverse effect.

Accordingly, more research is warranted to determine the appropriateness of interesterified fat consumption, particularly before it becomes insidiously embedded in the food supply similar to TFA and intake levels are achieved that compromise long-term health.

Key teaching points:

- Certain food applications will continue to require solid fat products to replace TFA in the diet.
- The 2 viable sources of solid fat for applicability and cost are unmodified saturated fat (the family of palm oils) and vegetable oils interesterified or blended with tristearin (or tripalmitin).

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Abbreviations: SFA = saturated fatty acids, TFA = trans fatty acids, IE = interesterification, %E = percent energy, CVD = cardiovascular disease, sn2 = carbon 2 on glycerol

Dr Hayes has received support for study of palm oil and its fractions in animals and serves as an ad hoc advisor to MPOC on occasion.

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- IE rearranges triglyceride structure (not unlike TFA production with partial hydrogenation) and removes or substitutes the sn2—fatty acid with 18:0 or 16:0, which alters lipid metabolism negatively at high intakes (>7%E of interesterified fat).
- More research is recommended to establish the level of interesterified fat consumption that compromises public health.

INTERESTERIFICATION (IE) OF FAT

Background

Although unsaturated fats (especially essential fatty acids of n6 and n3 origin) favor energy metabolism and are desirable for health reasons [1,2], current evidence suggests that natural saturated fats do not introduce a risk for chronic disease, such as coronary heart disease (CHD), on their own, but rather reflect the fact that such fats lack sufficient n6 and n3 polyunsaturated fatty acids (PUFA) [2–4]. Furthermore, certain technical constraints in product applications require that some fats be more plastic, less unsaturated, and more heat stable than naturally occurring vegetable oils. Shortenings for baking, as well as spreads or margarines for use on breads or cooked vegetables, are examples. Trans fatty acids (TFA) were incorporated into vegetable oils for this very reason, but TFA are now taboo in most well-versed nutrition circles [5,6]. So what to do now with all the vegetable oils? One solution fast becoming the norm is to fully hydrogenate an oil to make a fully saturated hard stock, which is then interesterified with additional vegetable oil. This replaces one or more of the unsaturated fatty acids normally on the glycerol molecule of the oil with a saturated fatty acid(s) (SFA) from the hard stock to generate a new, hardened plastic fat with a higher melting point, not unlike the TFA concept for hardened oils.

Although monounsaturated oils may be preferable as frying oils, their supply is limited and their application comes up short in many situations. In reality, 2 replacement possibilities exist. One is to utilize a natural saturated fat, most likely palm oil and its fractions because of world supply and cost, while the other possibility would be to harden vegetable oils by the aforementioned process of IE, incorporating either 18:0 or 16:0, to generate the degree of plasticity required. An abundant supply of these fatty acids is made by fully hydrogenating soybean oil or palm oil, yielding an 18:0:16:0 ratio of 85:15 for soybean oil and 55:45 for palm oil.

The potential problem with IE, especially when randomly applied, is the introduction of SFA as 18:0 or 16:0 into the sn2 position on the glycerol molecule, previously noted for its potentially untoward biological impact [7], or affecting triglyceride (TG) clearance from lipoproteins [8]. An overview of TG structure and its general implications for metabolism is also useful [9]. As TFA are replaced in foods, interesterified fat has been a preferred substitute, although cause for concern can be justified above a certain level of consumption.

Thus, the IE approach works technically, but inadequate data exist on the biological function(s) of such substitutions when significant intakes are achieved, as might occur with a 1-

to-1 replacement of TFA in food products. One could argue that the impact of IE may be no different in the long term than that of TFA. Both processes modify the basic molecular structure of fat in a similar fashion, i.e., by altering the degree of saturation and/or structure of the sn1,2,3 fatty acids in a random fashion and disrupting the natural composition of the TG molecule, typically increasing the melting point of the oil in the process. One major difference is that IE typically results in a SFA (18:0 or 16:0) inserted randomly at sn1, sn2, or sn3, whereas partial hydrogenation converts a PUFA (at sn1, sn2, or sn3) into a straight-chained fatty acid that is basically monounsaturated (e.g., elaidic acid, t18:1).

To examine the possible effects in some detail, this review summarizes the published reports on the human response to various amounts and forms of interesterified fat. The main emphasis is on the lipoprotein response, since that was the general focus of most such studies. Each reviewed study, including design and results, has been reduced to a single expository figure with compacted details that are described in the accompanying text. Thus, a written narration accompanies each figure to summarize the published report, with a critique of the salient design points and amplified interpretation in each case.

It is apparent from this analysis that interesterified fats can modulate the lipoprotein profile adversely, but the outcome can vary depending on which SFA is incorporated into the fat during the IE process, how much of the SFA is inserted into an unsaturated oil, and how much of the interesterified fat is ultimately consumed per day by individuals with presumably varying sensitivity to an interesterified fat. Currently, these variables are poorly understood.

These results offer additional insight and newer perspective to a review of the topic done almost a decade ago on the impact of IE in both animals and humans [10]. The conclusion at that time, admittedly with fewer data for evaluation, was somewhat more sanguine than the current findings would seem to allow.

Figures and Supporting Text

For orientation purposes, each of the following figures is designed to orient the reader to the key information presented in each published report. The figure number is located in the upper left corner. The literature reference is abbreviated, usually in the lower right corner, along with its citation in the text and full reference in the bibliography. The accompanying text also highlights the major design and results of the study as reported in the article and summarized by the graphics. The columns in each figure depict the total blood cholesterol value

subdivided into cholesterol fractions transported by the 3 lipoprotein classes (VLDL indicates very-low-density lipoprotein, in green, a minor fraction that also carries most of the TG; LDL, low-density-lipoprotein, the major cholesterol carrier for humans, in red; and HDL, high-density lipoprotein, the desirable cholesterol carrier, in orange, which usually transports about one-fourth of the total cholesterol in humans).

Each column represents the average values reported for total cholesterol (TC), VLDL, LDL, and HDL for the dietary treatment under discussion, i.e., the average for all subjects fed any given diet. The small number to the right of each column between LDL and HDL represents the average LDL/HDL ratio in response to that diet. Ratios above 4.0 have been associated with a high risk for CHD, while ratios under 2.0 represent a very low CHD risk and are most desirable. Actual cholesterol numbers (as mg/dl) are added to each lipoprotein fraction and final column to facilitate interpretation at a glance. An asterisk with a number indicates statistical significance, generally relative to the control value. A double asterisk represents another level of significance, particularly in regard to the biological importance of the response.

The reader is referred to the original publications for more specific details of the response, as necessary, as this review represents an attempt to compare the various studies on the basis of design and general implications of the lipoprotein response. The intent is to reduce the confusion that arises from comparison between these studies as a collective whole, when one may be unaware of certain design caveats within a given study. Without this overview, interpretation is clouded by the fact that studies often had substantially different objectives and designs. Even though most studies included here have utilized interesterified fat in at least one diet, some contained minimal interesterified fat or presented it in such a way that any adverse response would likely fall below detection, i.e., the design failed to very rigorously test the acceptability of the interesterified fat by avoiding upper levels of intake. This, of course, has the advantage of making the food product appear "safe" but fails to consider the impact of the modified fat when consumed at higher intakes if the interesterified fat were present in a wide variety of foods eaten daily, as opposed to a single product or a single meal.

Beneath each column is a condensed code that nutritionists typically use to characterize a dietary fat as applied to cardiovascular disease (CVD) research. The first line is the code name for the diet; the second line indicates the percent of the dietary calories contributed by the fat, most typically ranging between 30% and 40% of daily energy intake (%E). The third line denotes the overall ratio of S:M:P (saturated, monounsaturated, polyunsaturated) fatty acids contributed by summation of the various fatty acid molecules consumed in the total diet on a %E basis. 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, e.g., when the P/S ratio is 0.5, SFA are twice as prevalent as PUFA, and the blood cholesterol may be high, 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 currently about 0.4. If Americans could raise it to about 1.0, major health benefits would follow [1–3,11,12].

By applying the aforementioned codes while reading the study summaries, the reader should gain substantial insight concerning the relationship between the human lipoprotein response and changes in specific classes, as well as individual dietary fatty acids (S:M:P), and how their inclusion in interesterified fats might affect that relationship.

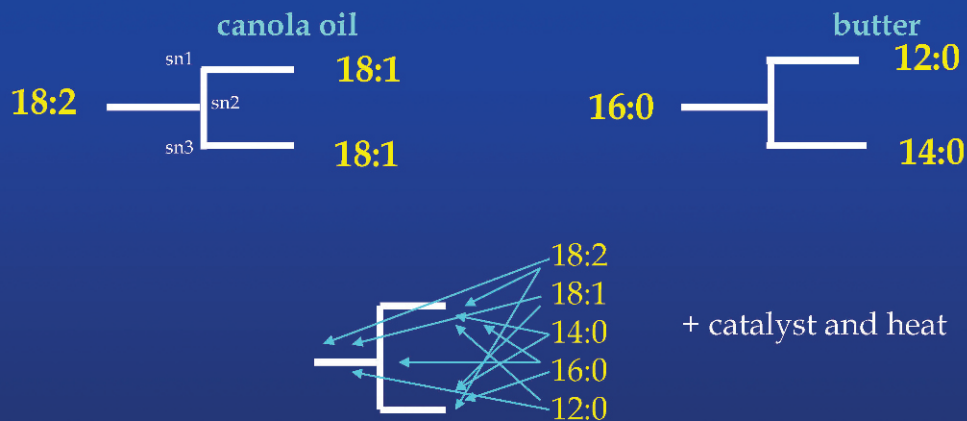
TG structure represents a second important consideration (Fig. 1). For orientation purposes, the reader should remember that each TG molecule includes 3 fatty acids esterified to the 3 carbons in a molecule of glycerol, referred to as the sn1, sn2, and sn3 carbons. Nature typically assigns specific fatty acids to these carbons in natural fats and oils, depending on the plant/animal source. Almost all fats from any natural source have TG molecules that have more than one type of fatty acid (SFA, monounsaturated fatty acid [MUFA], PUFA) in combination to make the complete TG molecule. Also, usually the sn2 fatty acid is either monounsaturated or polyunsaturated; seldom is it saturated unless isolated from milk fat, coconut oil, or palm kernel oil, a point with biological implications for lipoprotein metabolism, which will be discussed later.

When interesterified fat is formed with a chemical catalyst, the most common approach, the objective is to insert an SFA into a vegetable oil to harden it, much like partially hydrogenating a fat would do. The assumption has been that since 18:0 seems neutral toward blood cholesterol when consumed in natural fats (see following), it should be neutral when randomly inserted into oils. Thus, it has become the SFA of choice for IE and hardening of vegetable oils. Some have also modified (hardened) fat by inserting 16:0 during IE.

In this review, the assumption that IE of SFA is without effect is examined by assessing the dietary fat designs carefully for fat composition and comparing between diets, especially the fatty acid relationships, and assessing results as expressed in lipoproteins. Certain global assumptions are in place in the scientific and, by extension, in the lay community that certain fatty acids are either problematic or healthy, based on what we think they do to lipoprotein metabolism. This includes 12:0, 14:0, and 16:0, which are considered cholesterol-raising; 18:0 as neutral; 18:1 as neutral to cholesterol-lowering; whereas 18:2 and 18:3 are assumed to have the ability to lower cholesterol [3,12–14]. However, this expression of fatty acid potential is not cast in stone, as many exceptions exist, especially when other aspects of the diet are considered, including cholesterol intake [3,11,12].

Fig 1

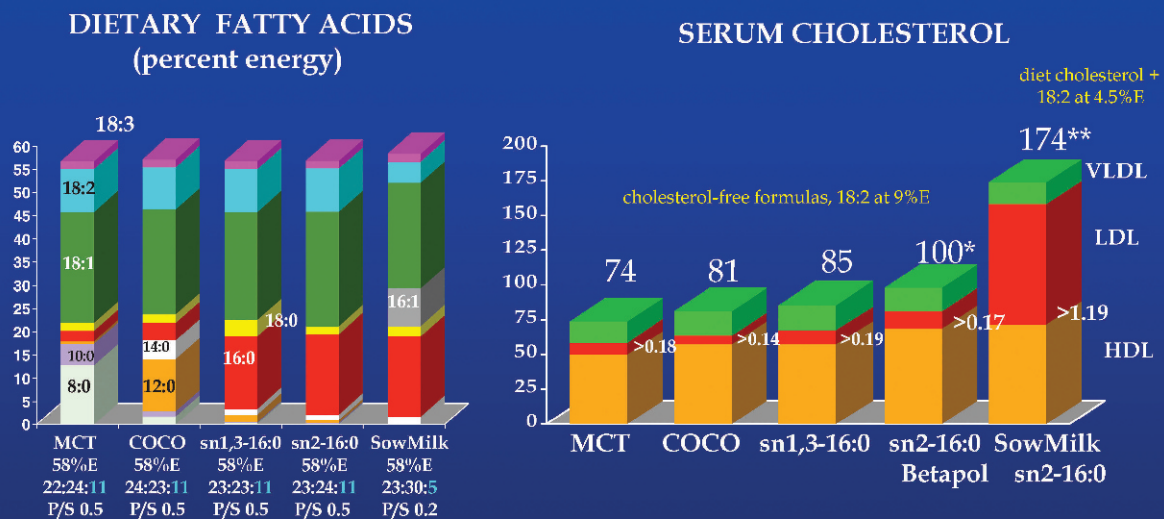
Triglyceride structure is important.... evidenced by interesterification (IE) processing



Random interesterification causes “unnatural reshuffle” of FA, which means new, artificial TG molecules are formed.

Fig 2

Newborn piglets cholesterol response to 8:0 thru 16:0-rich fats after 18d

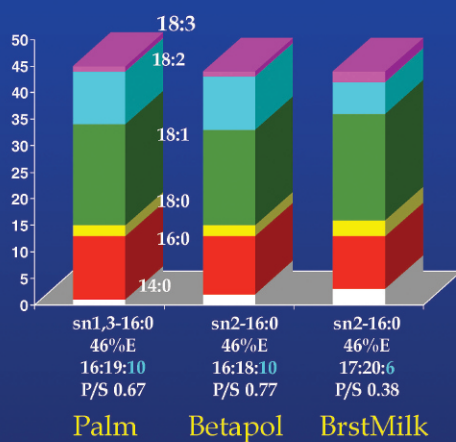


Innis et al, AJCN 57:382, 1993

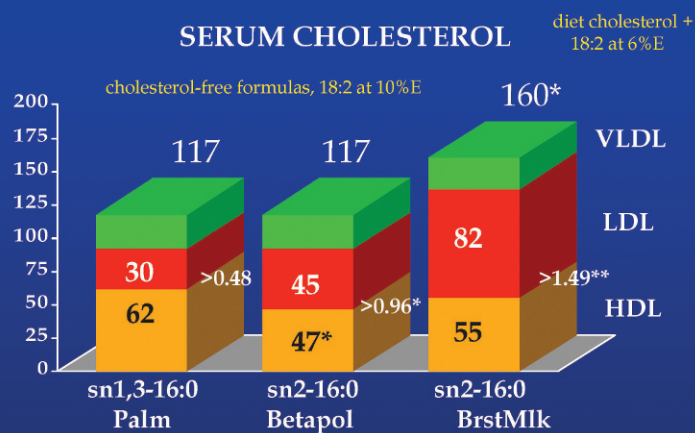
Fig 3

Human Infant cholesterol response (120d) to sn1,3 or sn2-16:0 formulas vs breast milk

DIETARY FATTY ACIDS (percent energy)



SERUM CHOLESTEROL

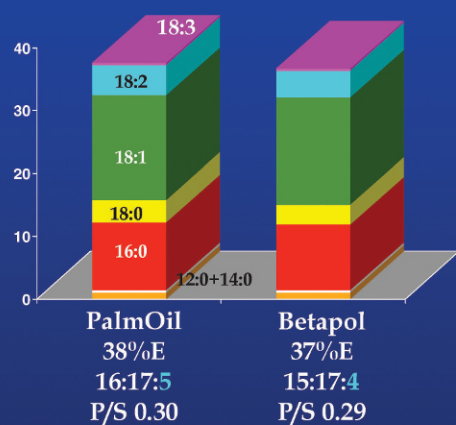


Nelson + Innis, AJCN 70:62, 1999

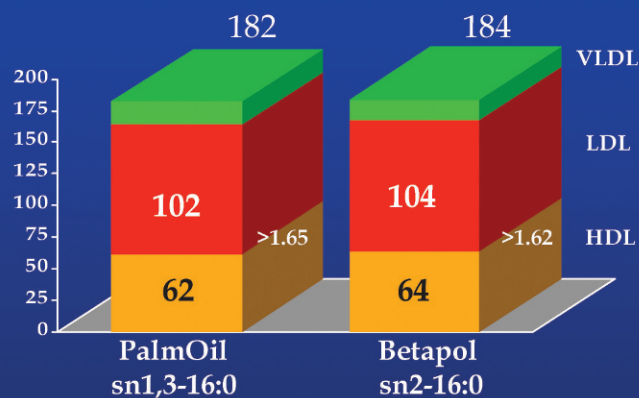
Fig 4

Adult human response to dietary fats with sn1,3-16:0 vs sn2-16:0

DIETARY FATTY ACIDS (percent energy)



SERUM CHOLESTEROL



Zock et al., AJCN 61:48, 1995

Fig 5 **Adult human response to dietary fats with sn1,3-16:0 vs IE sn2-14:0**

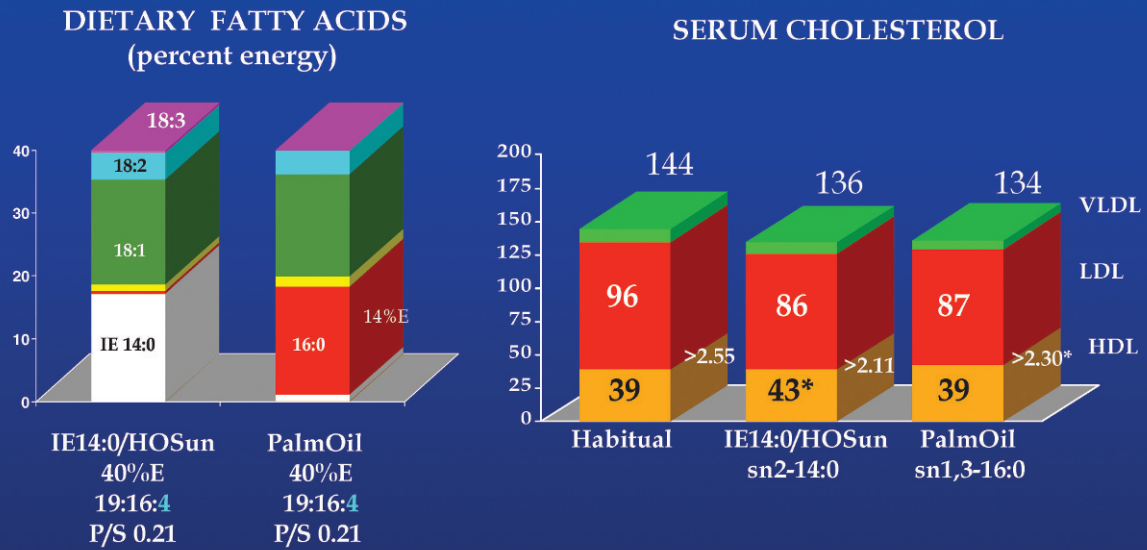


Fig 6 **Fat-modified margarines compare 14:0, 16:0 to c18:1 in HUMANS**

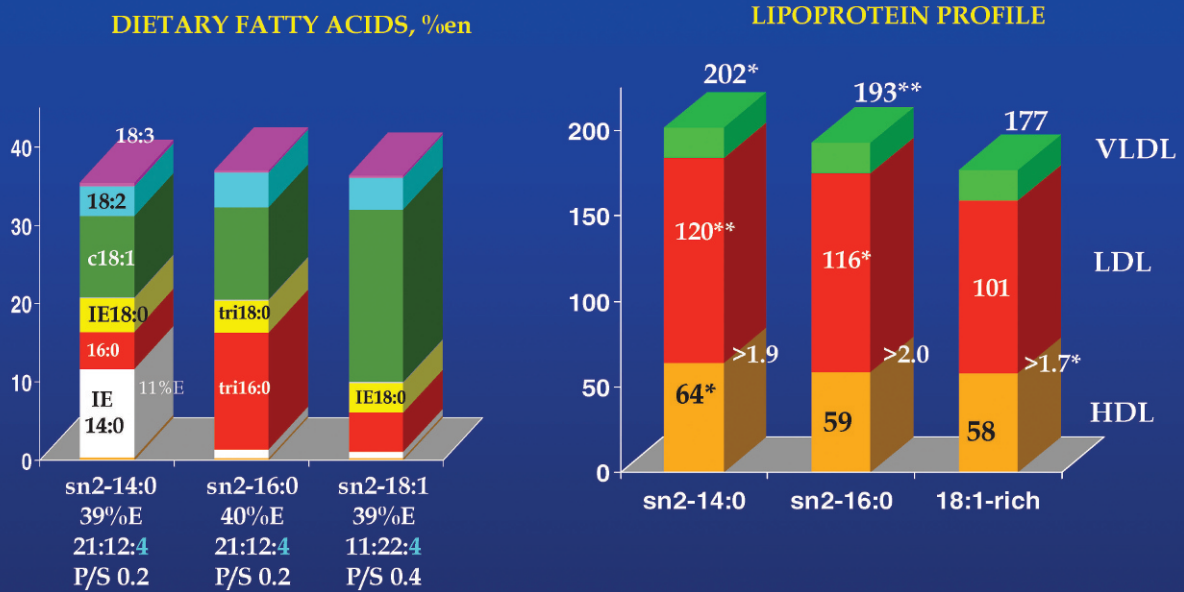
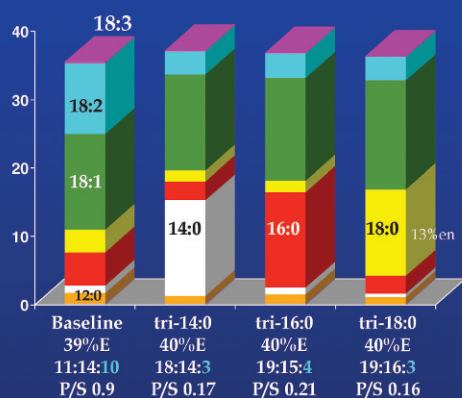


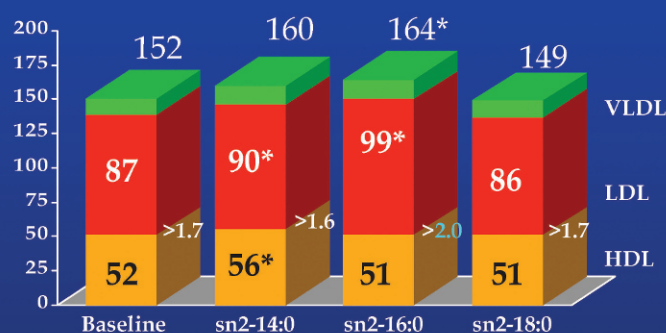
Fig 7

Human response to triSFA (14:0, 16:0, 18:0) in blends of oils

DIETARY FATTY ACIDS
(percent energy, 36%en)



SERUM CHOLESTEROL

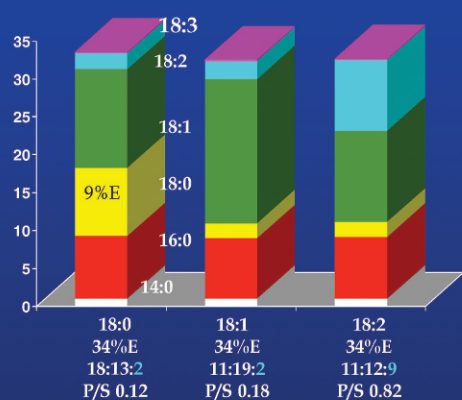


Snook et al., EJCEN 53:597, 1999

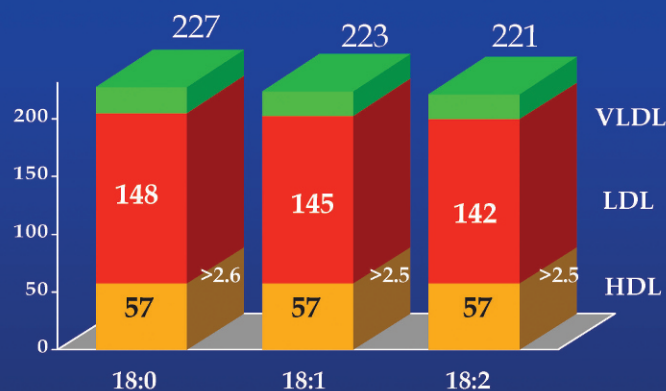
Fig 8

Human cholesterol response to tri-18:0, 18:1, or 18:2

DIETARY FATTY ACIDS
(percent energy)



SERUM CHOLESTEROL

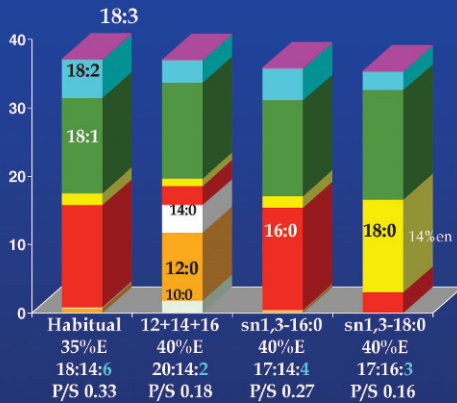


Thijssen AJCN 82:510, 2005

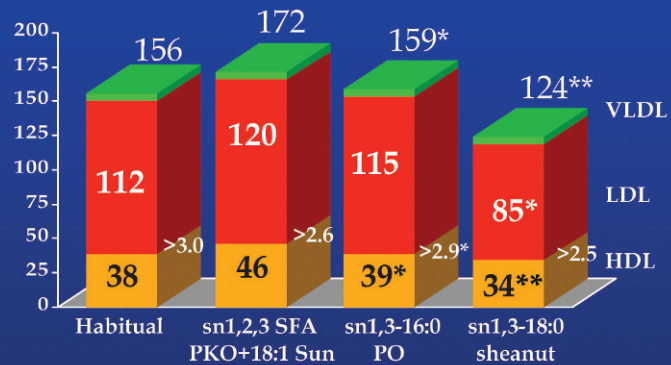
Fig 9

Human response to natural saturated fats rich in 12:0+14:0, 16:0, or 18:0

DIETARY FATTY ACIDS
(percent energy, 40%en)



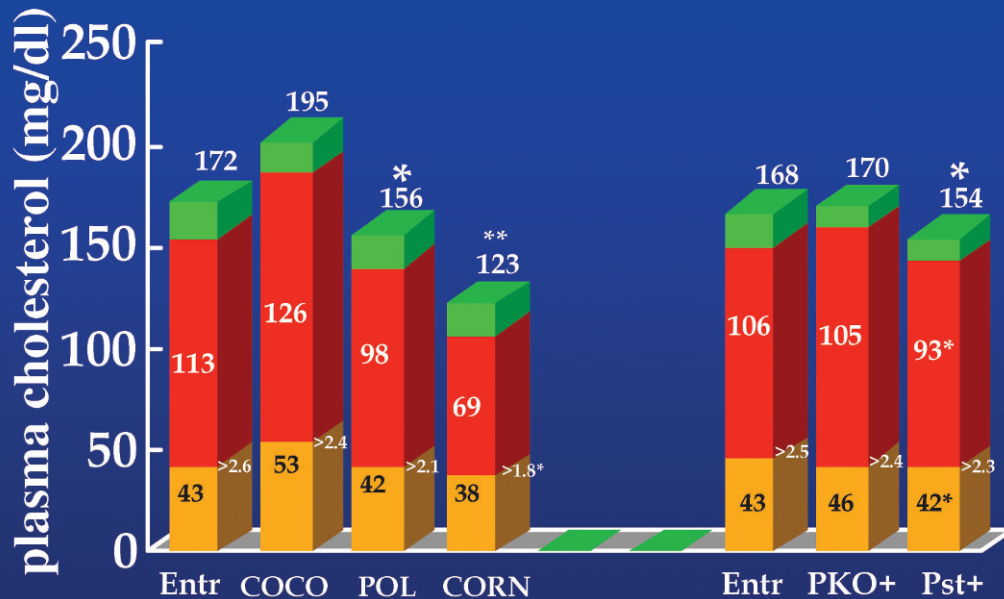
SERUM CHOLESTEROL



Tholstrup et al., AJCN 59:371, 1994a

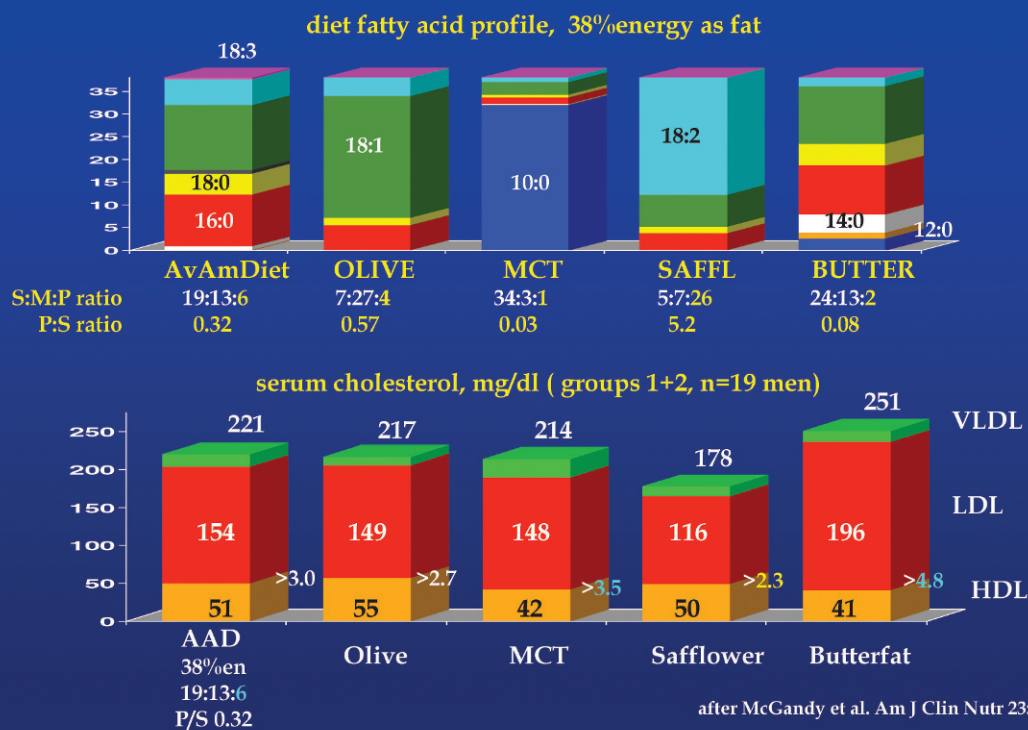
Fig 10

HUMAN TC RESPONSE TO 14:0 +16:0 FATS



Ng et al. AJCN 53:1015S, 1991

Sundram et al. AJCN 59:841, 1994

Fig 11 **Cholesterol response to fats of different saturation in humans**Fig 12 **Cholesterol response to IE fats based on MCT + tri14, tri16, tri18 in humans (combo)**

after McGandy et al. Am J Clin Nutr 23: 1288, 1970

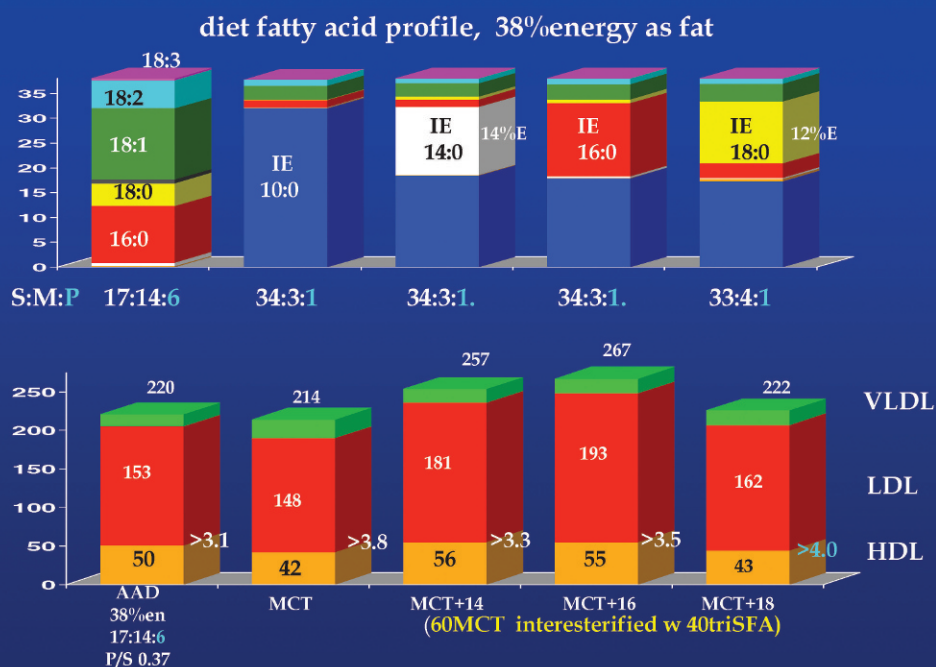


Fig 13

Cholesterol response to IE fats based on Olive Oil + tri12, tri14, tri16, and tri18 in humans

after McGandy et al. Am J Clin Nutr 23: 1288, 1970

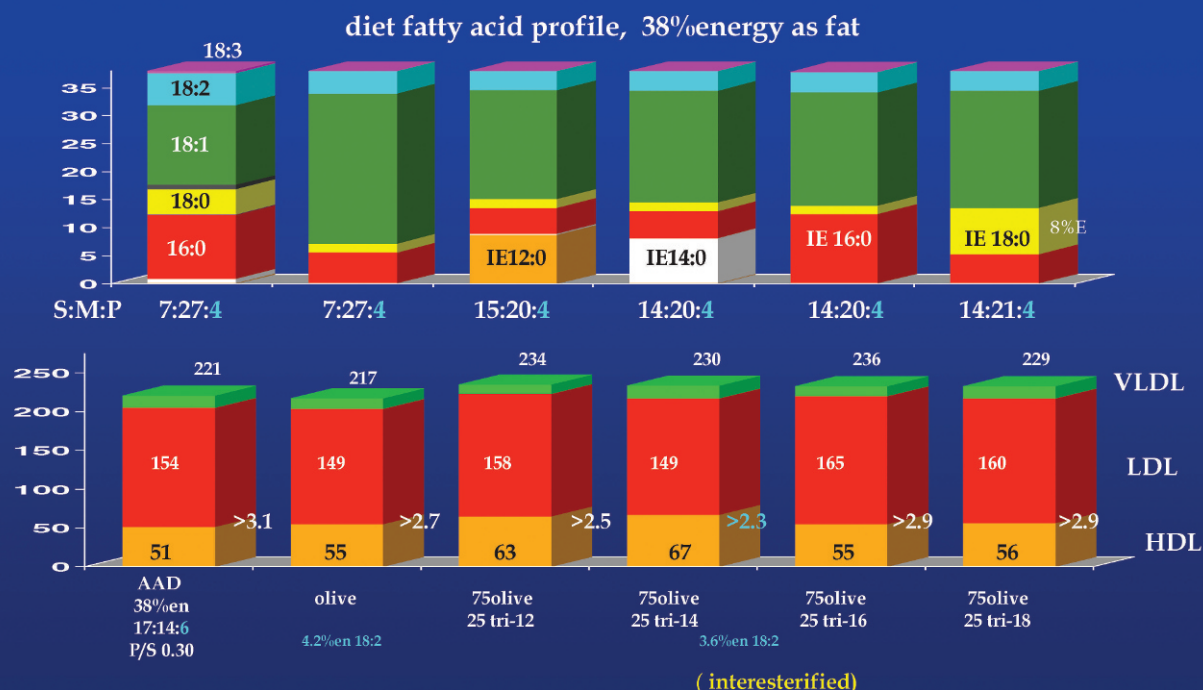


Fig 14

Cholesterol response to IE fats based on SAFFL OIL + tri12, tri14, tri16, tri18, and MCT in humans

after McGandy et al. Am J Clin Nutr 23: 1288, 1970

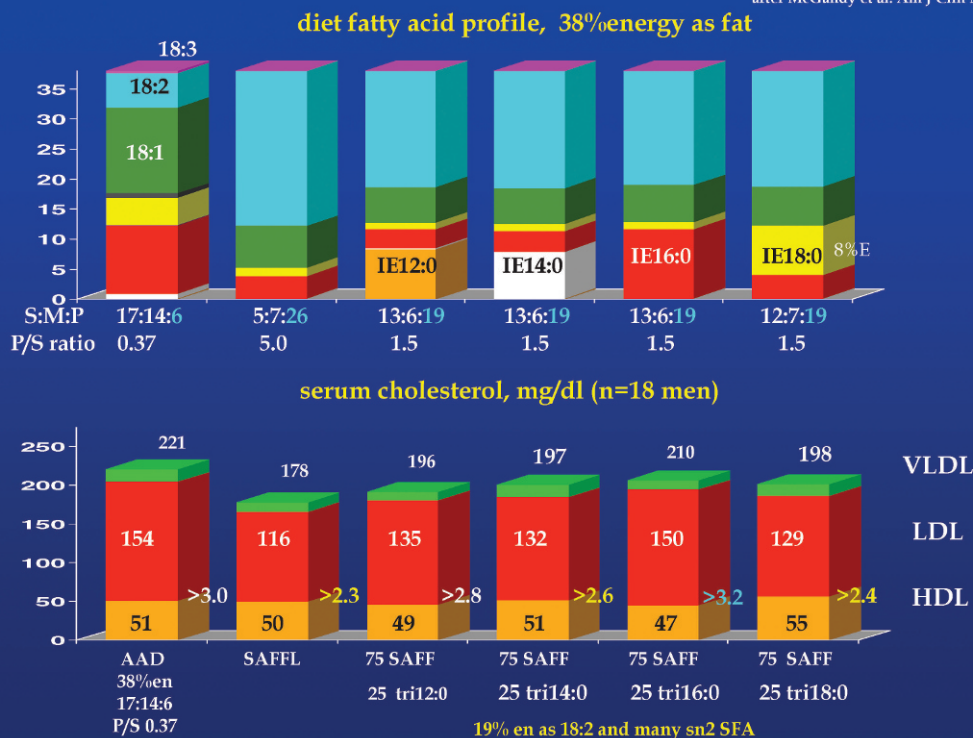


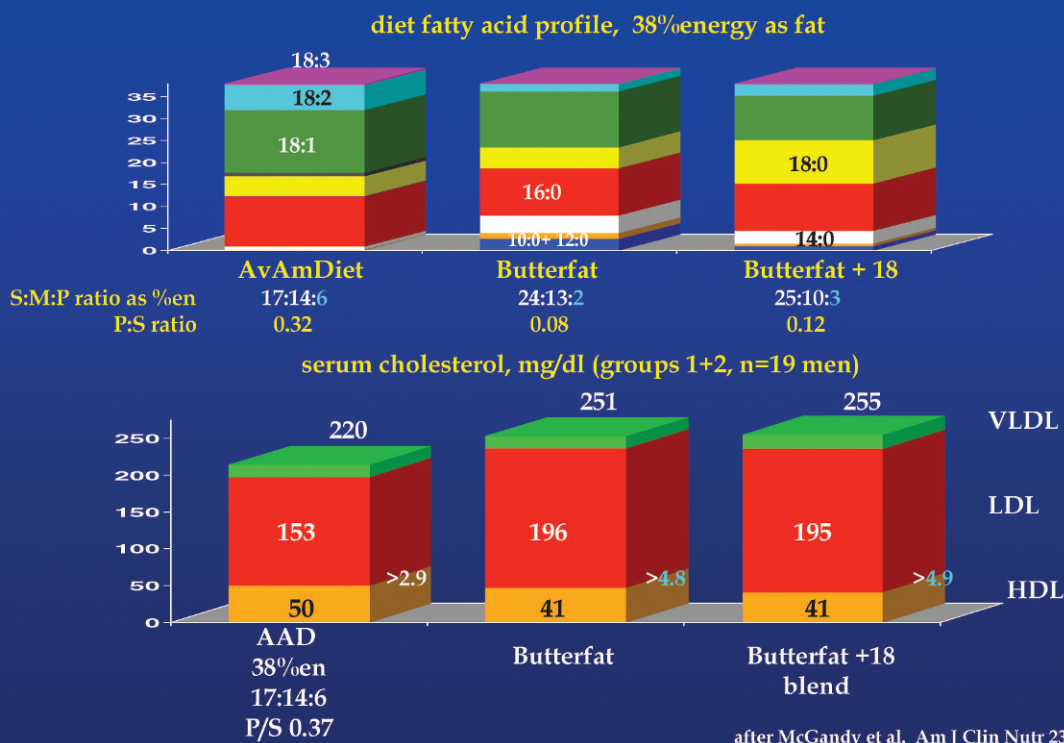
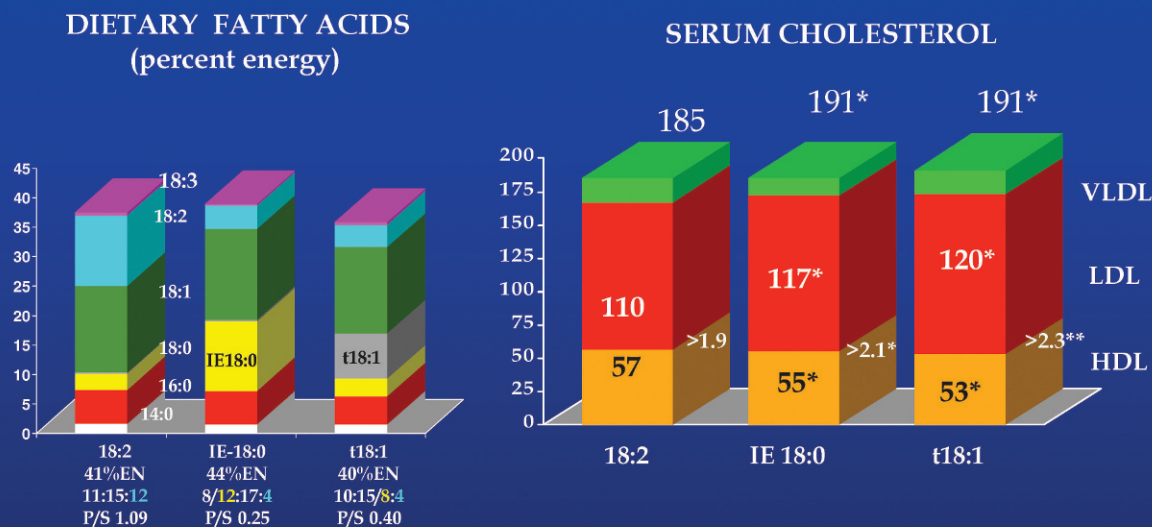
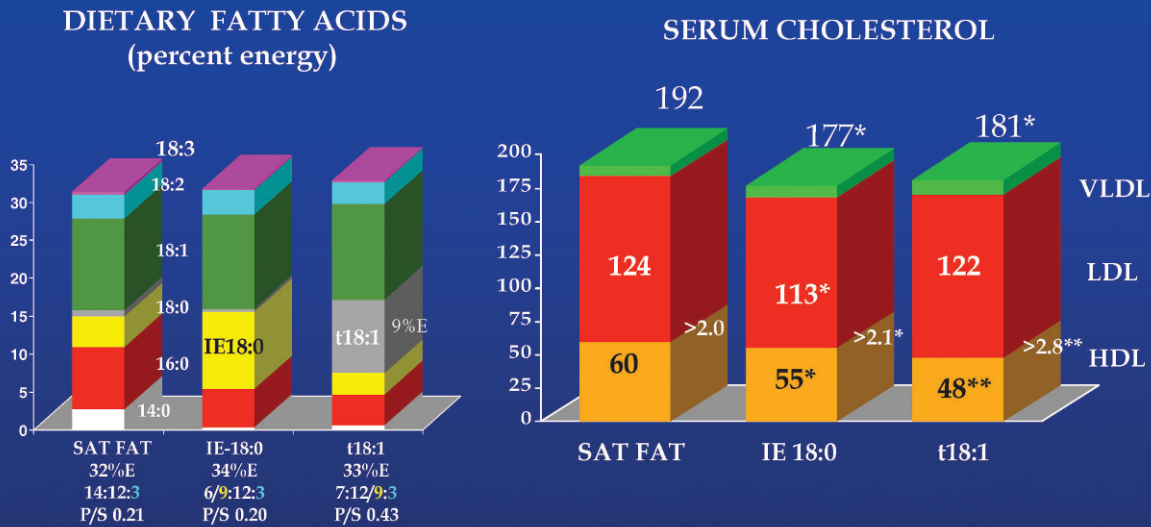
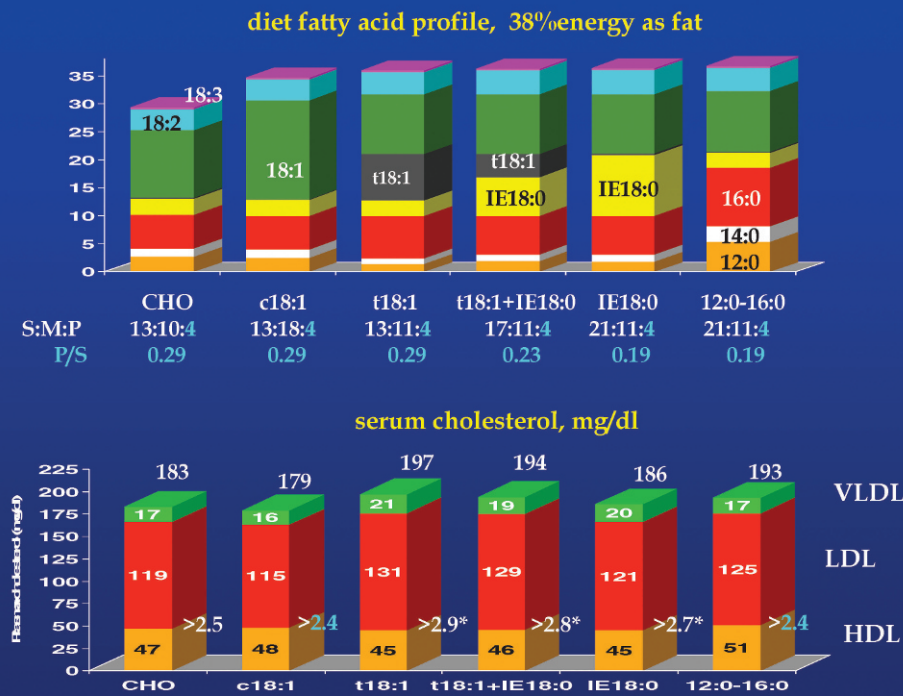
Fig 15 **Cholesterol response to butterfat +/- tri-18:0 in humans**Fig 16 **Human cholesterol response to IE-18:0 or TFA**

Fig 17 Human cholesterol response to IE-18:0 or TFA



Aro et al. AJCN 65:1419, 1997

Fig 18 Cholesterol response to t18:1 + IE-18:0 fats in humans

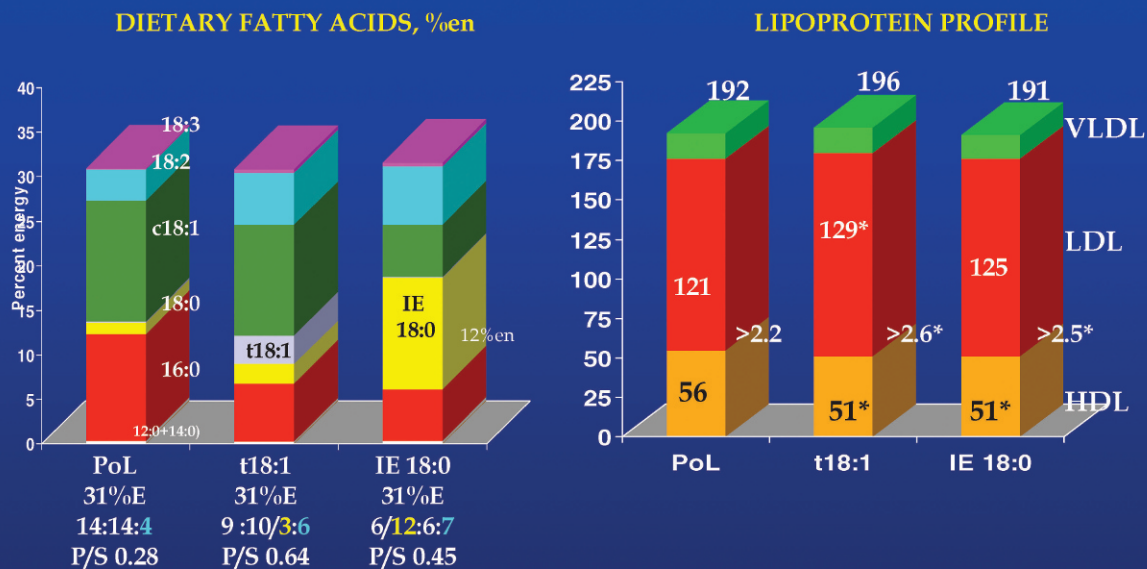


50 men@ 42y, 26 BMI/ 5wk periods/crossover

Judd et al. Lipids 37:123, 2002

Fig 19

Natural 16:0 vs TRANS 18:1 and IE 18:0 in HUMANS



Sundram et al., Nutr Metab 4:3, 2007

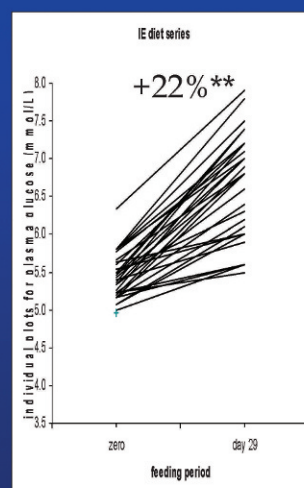
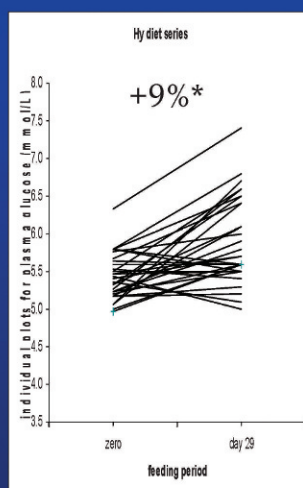
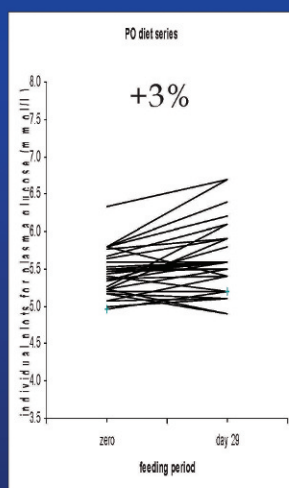
Fig 20

Individual fasting glucose response in humans to three test fats from 0wk to 4wk

insulin (μ Units/ml): 10.1(control)

9.1 (-10%)

7.9 (-22%)



Sundram et al., Nutr Metab 4:3, 2007

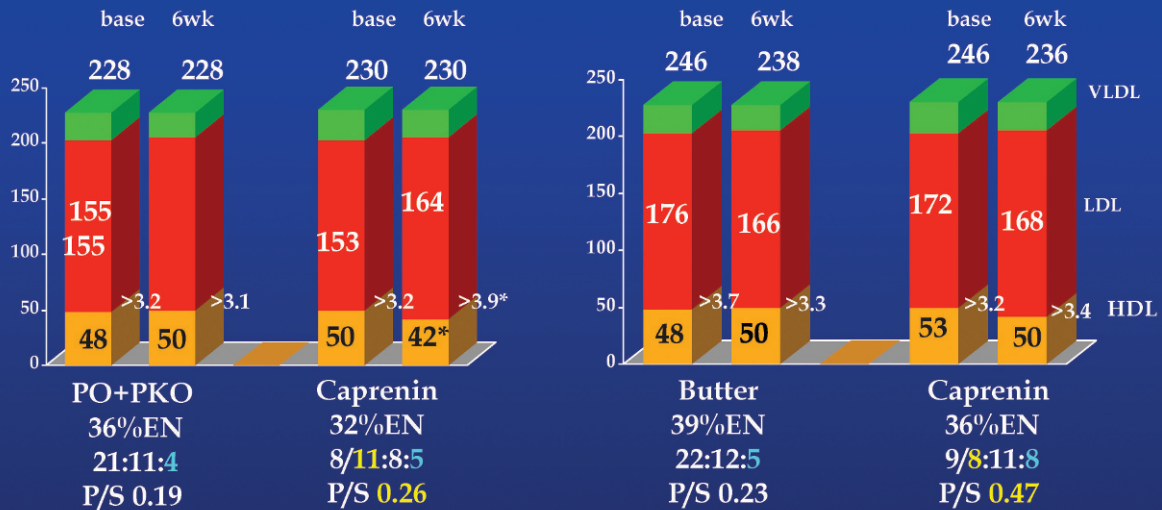
Fig 21

Human plasma cholesterol response to PKO or Butter vs Caprenin at two P/S ratios

Study 1 (n=17M+17M)

Study 2 (n=7M+7M)

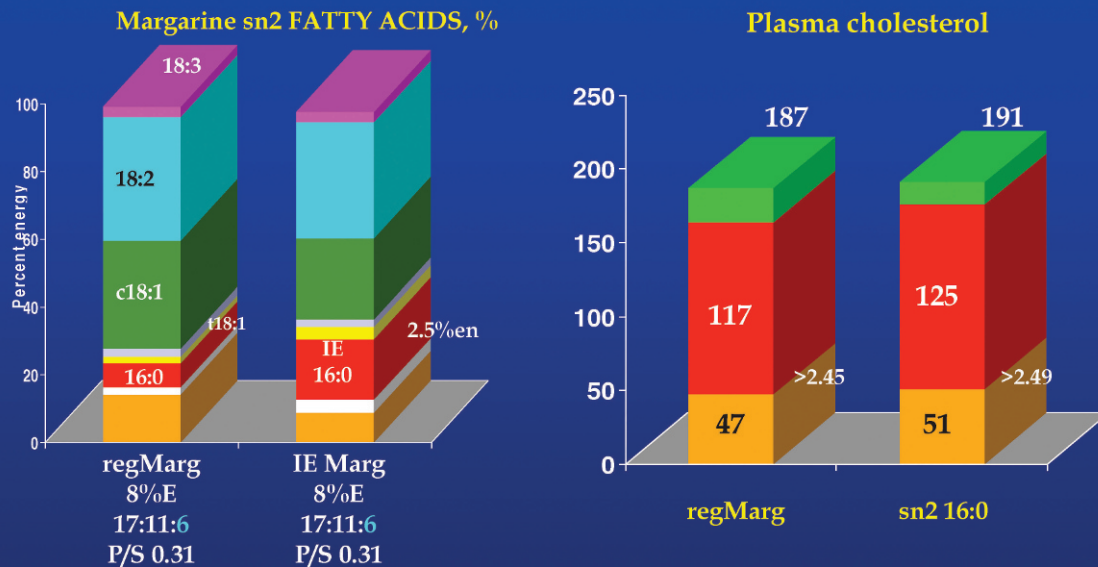
hyper-responders from Study 1



Wardlaw et al, AJCN 61:535, 1995

Fig 22

Effect of IE sn2-16:0 in MALES fed margarines providing 14%en fat in whole food diets with 34%E as fat



Meijer and Westrate, EJCEN 51: 527, 1997??

Fig 23

Cholesterol response to modPalm, IE-modPalm, and hi-18:2+TFA in humans

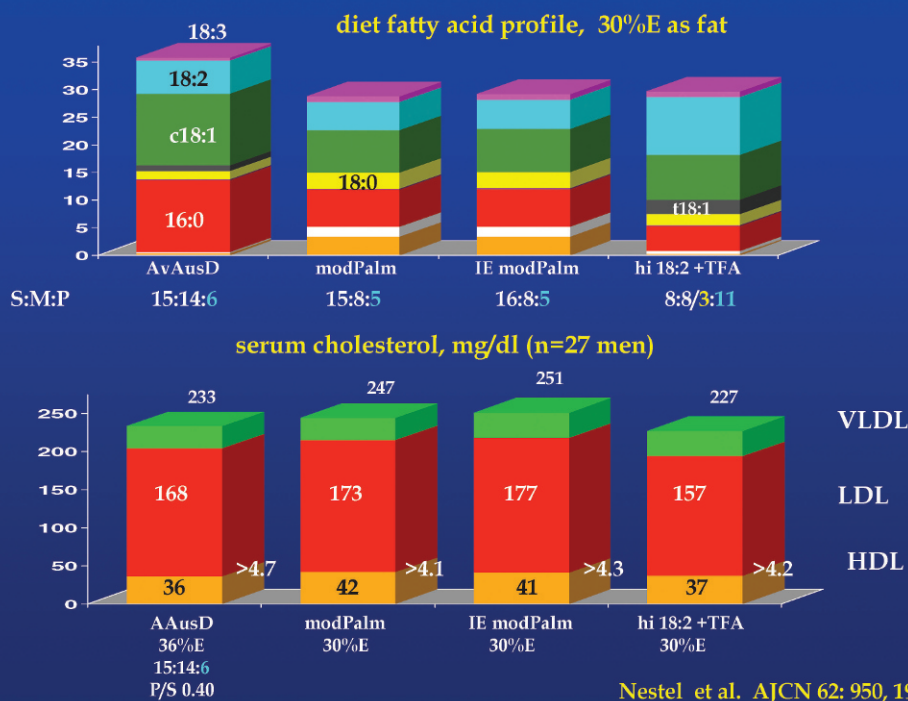


Fig 24

Palm oil 16:0 vs 18:0 (w IE sn2-18:0 as Salatrim) in HUMANS

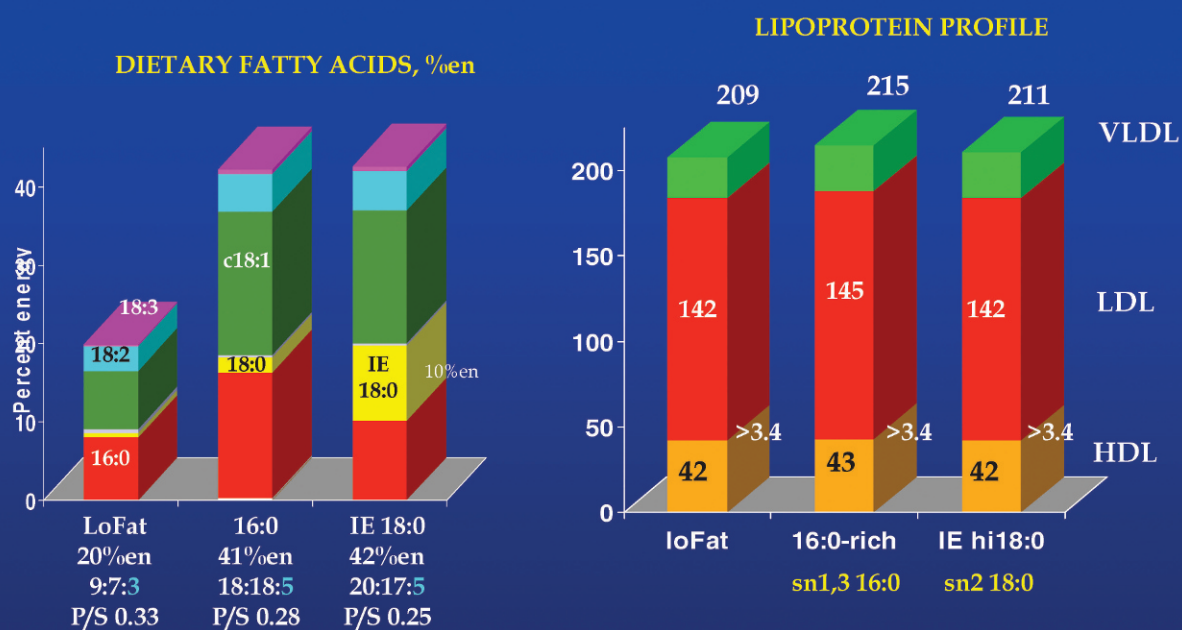
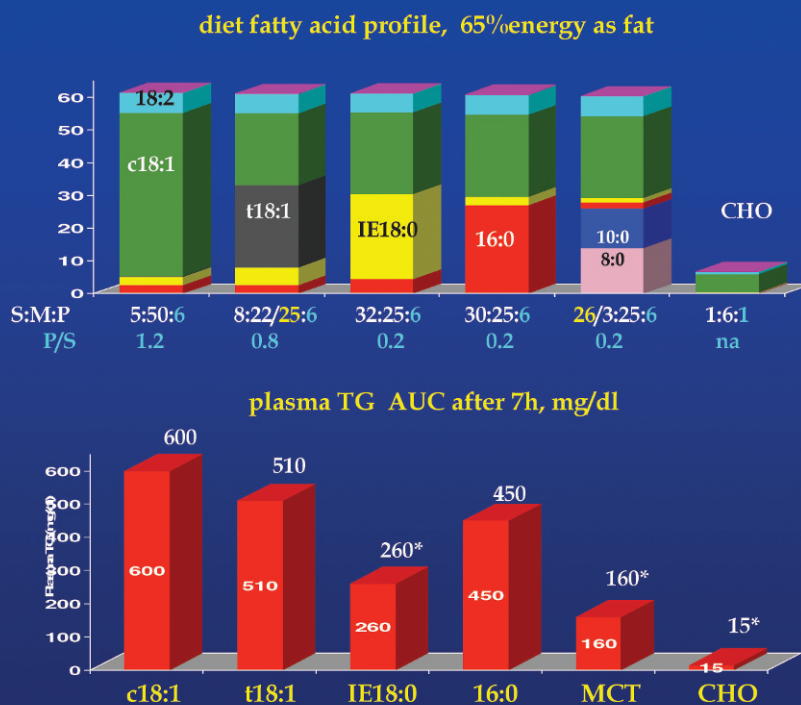
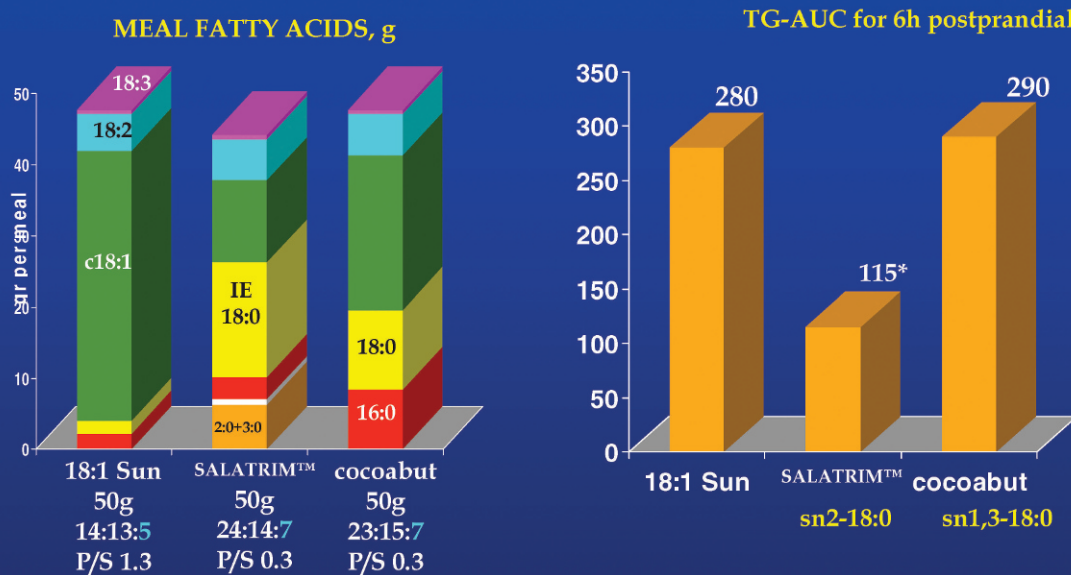


Fig 25 **7h TG-AUC response to IE fatty acids in meal-fed males**

Sanders et al. Athero 149: :413, 2000

Fig 26 **Natural cis18:1 vs sn2-18:0 vs sn1,3-18:0 as 50g fat in meal-fed HUMANS**

Sanders et al. , AJCN 73, 715, 2001

Fig 27 Acute meal effect of natural sn1,3-18:0 and IE sn2-18:0 in HUMANS fed natural cocoabutter or randomized cocoa butter

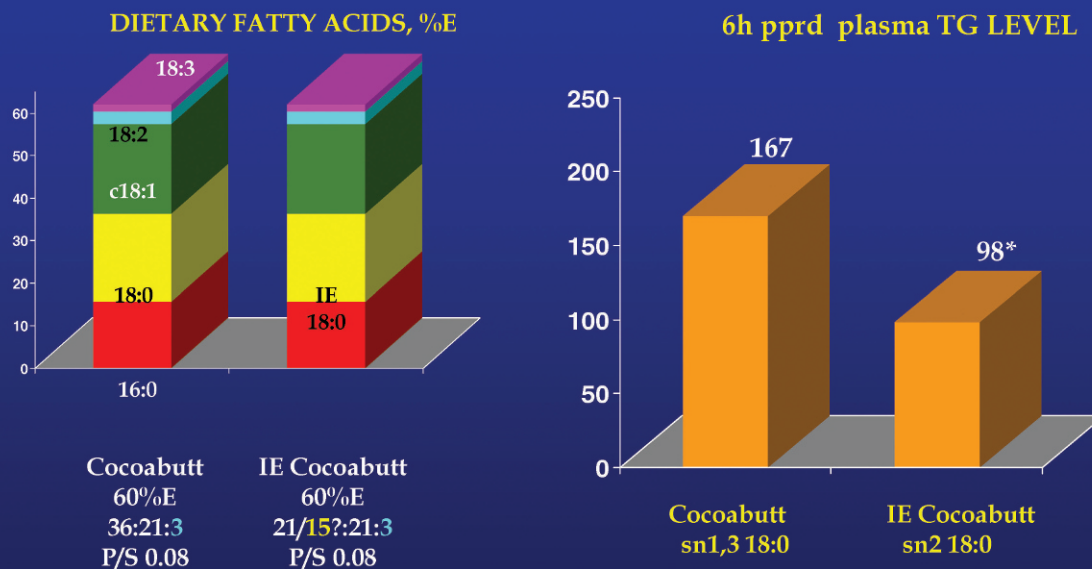


Fig 28 Natural 16:0+18:1 vs sn1,3-18:0 and IE sn2-18:0 in HUMANS

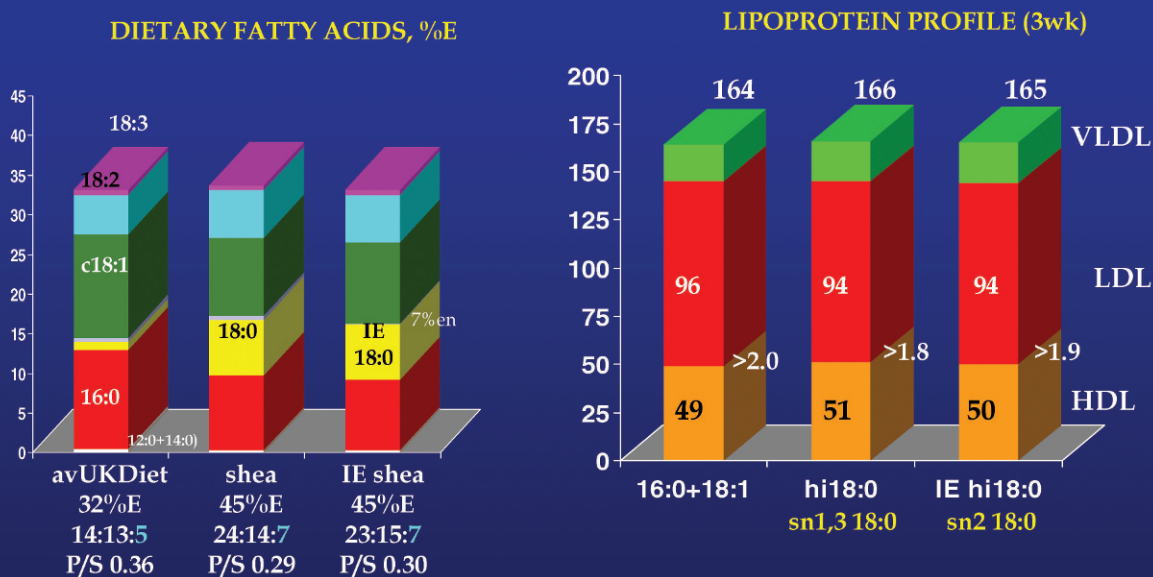
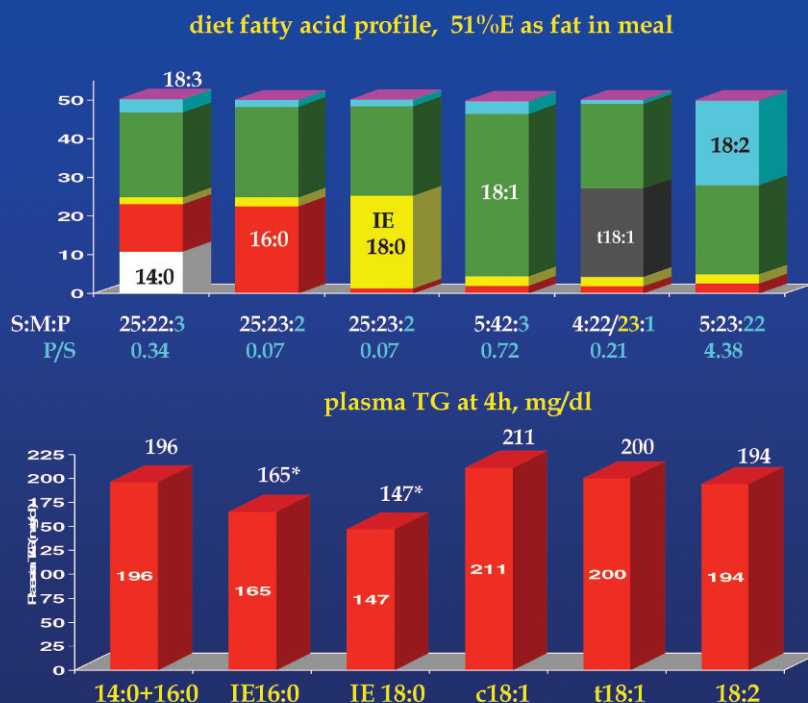


Fig 29 TG response to IE triSFA into hi-18:1 sun oil in meal-fed humans

Tholstrup et al. AJCN 73:198, 2001



STUDY SUMMARIES

Interesterified Fats

Betapol in Piglets. Because breast milk from humans and other species contains appreciable saturated fat and SFA, infant formulas for human infants have long been formulated to include saturated fat. But natural milks also provide fat with substantial sn2-SFA. For example, palmitic acid (16:0) in human breast milk is predominantly present as sn2-16:0. Thus, for infant formula production, palm oil was redesigned from its natural sn1,3-16:0 to incorporate substantial sn2-16:0 by IE, generating a milk TG structure more in keeping with human breast milk. The effect of this restructured fat (Betapol) was tested initially in piglets by Innis et al. in 1993 [15] (Fig. 2). Piglets (6 per group) were fed infant formula from birth for 18 days. Formulas were based on medium-chain TG (MCT) with 8:0+10:0, coconut oil, or palm oil (sn1,3-16:0), or on Betapol (where the 16:0 at sn1,3 in palm oil was mainly relocated to sn2 by IE; sn2-16:0). All these formulas were compared to sow milk, which is also naturally structured with sn2-16:0. Note from Fig. 2 that 11%E was provided as 18:2 in all diets except sow milk, which only provided 5%E as 18:2. Total fat provided 58%E for all formula diets to mimic sow

milk, which had a measurable content of palmitoleic acid (16:1), and like all natural milks, it contained cholesterol. Because of the higher PUFA in formulas, the P/S ratio of the formulas was more than twice that of sow milk (0.5 vs 0.2). Note that total SFA and MUFA were essentially constant across all milks; only the type of SFA differed.

The results are informative. First, all the cholesterol-free formulas induced a plasma TC response that was about half that of the natural sow milk. Second, under conditions of their high PUFA intake, all 4 formulas produced similar LDL/HDL ratios, and the first 3 had comparably low TC values. Only sn2-16:0 as Betapol raised both LDL and HDL proportionately compared to the sn1,3-16:0 of palm oil, indicating that the interesterified fat, with sn2-16:0 and essentially the same fatty acid profile as the natural palm oil formula, modestly raised plasma cholesterol in all lipoprotein fractions. The point is that the introduction of sn2-16:0 by IE in Betapol caused the plasma cholesterol to rise in piglets in a cholesterol-free formula. This was exacerbated by sow milk (especially the LDL increase relative to HDL), in part because of its lower 18:2 content and the presence of cholesterol. The cholesterol-raising capacity of dietary cholesterol in concert with consumption of saturated fat at low 18:2 intakes (and possibly exacerbated by sn2-16:0, as seen here) has been discussed

previously [11,12,14] but is illustrated dramatically by this example.

In addition to TG structure and sn2-SFA differences, 3 other factors likely contributed to the striking impact of dietary fat composition on lipoproteins in this instance. First, the total fat calories in sow milk (and replacement formulas) were high, at 58%E, so the metabolic impact of the dietary fatty acids and TG structure were accentuated. Second, these piglets were fed a fixed fat source, with a constant food (and fat) composition with every swallow. No exceptions or extraneous fat sources were introduced, so the true impact of a specific dietary fat composition on plasma lipids was realized by redundancy. Third, unlike adults, growing newborns have minimal adipose (fatty acid) reserves to mix with incoming dietary fat (especially not 18:2n6 or essential fatty acids, in general), so their diet represents the sole fat (fatty acid) source influencing lipoprotein metabolism during rapid growth, amplifying the metabolic impact of the fatty acid mixture being consumed.

Betapol (sn2-16:0) in Human Infants. Following the piglet study, a similar experiment was conducted in human infants by the same lab (Fig. 3) [16]. Here, palm oil with sn1,3-16:0 was compared again to Betapol (sn2-16:0 by IE) and breast milk (sn2-16:0) at 46%E from fat for 3 groups (22 to 40 infants per group) in a parallel study for 3 months. Results were somewhat similar to those in piglets. Although the TC did not differ between the 2 formula diets, the LDL almost doubled and HDL decreased substantially with Betapol, adversely increasing the LDL/HDL ratio in the infants receiving this fat formula, with its restructured TG containing sn2-16:0 compared to the same amount of natural sn1,3-16:0 in the palm oil formula. This effect of interesterified fat in infants is reminiscent of the LDL/HDL response associated with TFA intake in adult humans (see later text). Human milk, with 40% less 18:2 than formulas, coupled with its cholesterol content, caused a striking rise in TC similar to that seen in piglets while increasing the LDL/HDL ratio almost threefold over the palm oil formula and 50% over the Betapol formula. It is noteworthy that lower dietary 18:2 (6%E) plus the cholesterol in breast milk, like sow milk in piglets, once again exacerbated the increase in the plasma LDL/HDL ratio. This increase presumably reflects nature's way to insure the high level of LDL cholesterol that is needed for rapid expansion of cell membranes during the development of infant organs and brain, but it would not be desirable in adults.

Betapol in Adult Humans. Subsequent to the infant study, Betapol with its enzymatically engineered sn2-16:0 was again compared to palm oil with sn1,3-16:0 (both were liquid at 37°C) in diets fed to 60 adult men ($n = 37$) and women ($n = 23$) in a 3-week crossover study (Fig. 4) [17]. Unlike infant formulas, in which all TG molecules were modified by IE, these fats were fed

as margarines made from either palm oil or Betapol, both as 93 parts oil blended with 7 parts of an interesterified hard stock made from fully hydrogenated sunflower oil. These oils and margarines were used to prepare daily foods that provided 70% of all fat calories. Intake of 18:2 was modest and constant at 4%E to 5%E and the total fat intake was essentially the same between diets (38%E), except that 16:0 was located at sn2 in the interesterified fat and sn1,3 in palm oil when fed.

No differences were noted in plasma lipids in these adults, suggesting at least 3 possibilities. First (most likely), lipid metabolism in adults may be less sensitive to IE than in infants for reasons related to lack of growth and substantial adipose reserves of 18:2, already discussed with the piglet study. Second, the total amount of fat in the diet (38%E), and therefore the level of sn2-16:0, was appreciably less than the 58%E in piglets and the 48%E in the infant study. This may have lowered the interesterified fat component below a detectable threshold for eliciting a lipoprotein response. Third, sn2-16:0 may not be problematic in terms of lipoprotein metabolism in human adults. Any combination of the 3 could also apply.

These Betapol experiments make several points that are relevant to interpretation of the studies that follow. First, infants and piglets are uniquely sensitive in their responsiveness to interesterified fats because they represent newborn, naïve systems without essential fatty acid reserves, the lack of which would amplify dietary SFA effects [3,11,12]. Their rapid growth places a greater demand on dietary essential fatty acids to make phospholipids, as well as more sophisticated fatty acid-containing molecules (e.g., sphingolipids), needed for their developing nervous system and metabolism. Adults, by contrast, are endowed with essential fatty acid reserves in their adipose pools, which serve to buffer any deficit from dietary sources that might occur in the short term (i.e., for a few weeks). These differences between newborns and adults likely explain the greater sensitivity of infants to dietary interesterified fat. Another factor is the totality of the formula diet for infants. Formula was all they received, and the total daily fat composition was fully controlled. The only fats were either Betapol or palm oil, and every swallow of formula was exactly like the previous one, which is so unlike most adult studies, where the test fat represents some fraction of the total diet fat, allowing significant chance for dilution or modification of the test fat by extraneous fat consumption. Although it is true that people eating real foods assimilate fat from different sources, this more casual approach to regulating fat consumption addresses a different question than “what effect does this fatty acid or this TG structure have on metabolism in the purest sense?” Test fat dilution is especially troublesome if the %E intake of 18:2 is allowed to “drift” between diets, as 18:2 availability dictates how all other fatty acids are metabolized [11,12].

Interesterified 14:0. Two human studies focused on interesterified fats enriched with myristic acid, with the idea that 14:0 might be the cause of hypercholesterolemia associated with saturated fats. The first study [18] examined 12 men, age 24 years, averaging 79 kg and 3300 kcal daily intake. They were fed 2 diets in a crossover design for 3 weeks each (Fig. 5). The first diet provided about 17%E as myristic acid incorporated by IE of tri-14:0 into hi-18:1 sunflower oil, which was compared to natural palm oil, providing 17%E as sn1,3-16:0. Both diets had 3.5%E as 18:2, so the major difference was sn2-14:0 vs sn1,3-16:0 in an equal 14%E exchange of these 2 fatty acids. Test fat represented 90% of all daily fat, but protein intake was only 11%E. Surprisingly, neither diet raised relatively low baseline cholesterol levels in these young men, and the TC and LDL were the same for both diets. However, the HDL was 10% higher in sn2-14:0, so that the LDL/HDL ratio was lower. Low protein intake may have depressed all lipoproteins, making fatty acid effects less detectable. The exceptionally high SFA exchange at 14%E is somewhat unique, like the findings of McGandy et al. [19] (see below), who also found no significant differences between IE-14:0 and IE-16:0 in MCT at 14%E exchange, but the 18:2%E was <1 in that case.

A study by Zock et al. [20] also incorporated IE-14:0 at 11%E for comparison with diets enriched with either 16:0 or c18:1, with about 10%E exchanged among the 3 fatty acids across diets (Fig. 6). To generate an exact substitution of 14:0 or 16:0 for c18:1, complex margarines were formulated, each modified in some aspect. The first fat introduced 50% 14:0 (11%E) made possible by IE with pure 14:0 as the fatty acid and a complex fractionation process to reach the desired mix of fatty acids. The 16:0-rich fat was based on palm stearin (naturally rich in tri-16:0) and included fully hydrogenated sunflower blended in. Even the c18:1 fat was made with added 18:0 by IE, so some TG in all 3 fats had sn2-18:0. Thus, none of the fats can be considered natural, and no true standard reference was fed as a control for the 3 modified fats, as the underlying assumption was that TG structure was unimportant to fatty acid performance and therefore was not necessary to control with a naturally sourced fat. The 18:2 content was held constant at 4%E.

Results (Fig. 6) revealed that the high 14:0-enriched fat with sn2-14:0 generated the highest TC, LDL, and HDL compared to the most normal of the 3 fats, i.e., that rich in c18:1. The modified 16:0-rich fat was intermediate in terms of lipoprotein increases. However, interpretation is complicated within the context of this review, because none of the 3 fats were naturally sourced, and the 2 saturated fat preparations contained sizable amounts of tri-14:0 or tri-16:0, respectively. These tri-monoacyl molecules likely contributed to the cholesterol increases, based on sn2-SFA independent of their 14:0 or 16:0 content. Plus, although 14:0 represented 47% of the fatty acid in one test fat,

the test fat itself represented less than one third of total fat %E in the diet, with about 40% of dietary fats derived from extraneous foods. Thus, despite the implied conclusion, the report does not contribute robustly to our understanding of the contribution between sn2-SFA and natural fats rich in 12:0, 14:0, or 16:0 to lipoprotein metabolism. Nonetheless, it is interesting that 14:0 in this matrix again raised HDL substantially more than the 16:0-rich fat.

Fats with Tri-SFA Blends. Another study [21] with modified fats (Fig. 7) fed 18 premenopausal women (average age 28 years) diets that were based on blends of saturated fats that emphasized specific SFA, incorporated mainly as the tri-SFA by blending, without IE. Thus, TG structure (and sn2-SFA) was a lesser factor in the experiment and varied from about 18%–22% of TG molecules for 16:0 and 18:0 to more than 40% for the 14:0 diet. Subjects rotated through 3 diet periods of 5 weeks each with diets providing 35%E–38%E as fat in a repeated-measures design. One diet emphasized 14:0 from butter, coconut oil, and a high proportion of trimyristin. A second was enriched with 16:0 as tripalmitin mixed with coconut oil, butter, and lard as sources of SFA. The third diet was rich in 18:0 from shea nut oil, tristearin, and coconut oil. In part because of the tri-SFA admixture, the test SFA presented in the sn2 position varied widely between diets, even though the fat blend was not interesterified. The amount of specific tri-SFA was appreciable at about 14%E of total energy for the 14:0-rich diet, or slightly more than a third of total fat calories. The 18:2 intake was constant and low at 3%E, but followed a week with a preloading diet containing 10%E as 18:2. Dietary cholesterol was about 320 mg/d.

Results (Fig. 7) indicated that both 14:0 and 16:0 as tri-SFA raised TC above baseline values, while the 18:0-rich diet had no effect on TC, LDL or HDL compared to the 1-week baseline diet that preloaded 3 times the amount of dietary 18:2 (rationale not provided, but it may have biased the minimal response observed with any fat). The tri-14:0 diet increased HDL, so the LDL/HDL ratio was unchanged from entry, whereas only the tri-16:0 fat increased LDL more than HDL to raise the LDL/HDL ratio in these women with low blood lipid values. The 18:0 diet also excreted more SFA in feces. The point is that sn2-18:0 when fed as a tristearin (20% of the total 18:0) can be without effect on blood lipids, even at low 18:2 intake, but after a preload diet for 1 week with 10%E as 18:2. These rather modest differences may have occurred because the tri-SFA were blended into other fats, limiting the sn2-SFA incorporation that would have occurred among all TG molecules had the entire fat blend been exposed to IE. Also, the total SFA supplied about half of all fat calories in women (estrogen) with very low plasma lipid values, and both factors served as a buffer against a dietary saturated fat impact. Thus, the target population needs to be considered when interpreting

the response. Practical implications are difficult to assess because such large quantities of tri-SFA were blended into the diet fat, unlike any natural fat humans would consume, which also differs from using IE to distribute the SFA evenly among TG molecules, as occurs in most fatty acid enrichment applications. Nonetheless, it is noteworthy that 14:0 increased HDL under these circumstances and 16:0 increased LDL.

A similar experiment was published more recently [22]. In that study 45 subjects (18 men, 27 women) were fed the 3 different fats in 5-week rotations in a random crossover design. Here again, blending, rather than IE, was used to compare saturated 18:0 with monounsaturated oleic acid (c18:1) or polyunsaturated linoleic acid (18:2) in a 7%E exchange between these 3 fatty acids across diets (Fig. 8). All other fatty acids remained constant, with the limitation that 2 diets contained only 2%E as 18:2, while the 18:2-rich diet provided this fatty acid at 9%E. The 9%E as 18:0 was derived from 29% tri-18:0 and 34% cocoa butter in the test fat matrix, and the tri-18:0 was incorporated by blending rather than subjecting the entire blend to IE. The diets supplied 38%E as fat (only 34%E accounted for by fatty acid distribution), and 60% of the fat was derived from the experimental margarines, which represented blends of 4 to 6 fats each.

The response of these moderately cholesterolemic subjects was similar for all 3 diets, i.e., no changes were noted in TC, LDL, or HDL, which is rather surprising, given the well-documented lowering potential of 18:2. The other 2 diets may have been unresponsive as a result of their limited 18:2 content, distorting the responses seen relative to expectations. This was similar to Snook et al. [21], who blended tri-18:0 into the fat rather than by IE, as those subjects also did not alter their lipid profile from baseline (Fig. 7). However, their subjects did show increased cholesterol when tri-SFA from 14:0 or 16:0 was ingested. It may be that the failure to manipulate the blends by IE limited the sn2-18:0 distribution among total fats ingested, thereby limiting the negative impact of sn2-18:0 demonstrated in other studies, where IE was applied.

Naturally Saturated Fats and the LDL/HDL Ratio.

Beyond experiments with Betapol and interesterified or atypical fats with sn2-SFA (incorporated as in tri-SFA blends), a direct comparison of natural saturated fats and their specific SFA on the lipoprotein response is worth noting. This allows for comparison with interesterified fats, where processing causes some SFA to be situated unnaturally at sn2 in potentially all of the dietary fat consumed. For example, only a few natural fats contain SFA at sn2 in addition to sn1,3 (coconut oil, palm kernel oil, milk fat) and simultaneously when we have a very low 18:2 content, and all 3 raise plasma cholesterol substantially relative to all other fats. This presumably reflects their minimal 18:2 among their TG-MS.

Thus, a second study by Tholstrup et al [23] emphasized the difference in TG structure by simultaneously investigating 3 natural saturated fats having either sn1,2,3 endowed with 12:0, 14:0, or 16:0 (a palm kernel oil base mixed with hi-oleic sunflower oil as a 55:45 mix), sn1,3-16:0 (palm oil), or sn1,3-18:0 (shea nut oil), each of which was compared to the habitual diet at entry to the study (Fig. 9). Fat supplied 40%E. The exchange between 12:0+14:0, 16:0, and 18:0 between diets was about 14%E, and the test fat was 90% of the total fat ingested, meaning that the other dietary components were very low in fat. These other components also supplied only 10%E as protein, which may have biased results somewhat. Total amounts of SFA and MUFA were about the same between diets; only the quality (chain length) of the SFA differed, along with the location of the SFA on the fat molecule. However, the palm oil-based fat provided 50%–80% more 18:2 than the other 2 test fats, but all provided less than 4%E from 18:2, and the 14:0-rich and 18:0-rich diets were sufficiently low in PUFA to possibly have biased results also. The addition of a PUFA-rich fat to ensure equal PUFA across all 3 diets would have facilitated interpretation. The 15 young men were rotated through 3 diet periods of 3 weeks each.

Not surprisingly, like studies by Ng et al. [24] and Sundram et al. [25], which examined coconut oil or various palm oil products, the palm kernel oil–high-oleic sunflower oil fat, with the lowest P/S ratio and with many of its TG molecules having all 3 fatty acids as SFA, caused TC to rise the most, followed by the fat that was rich in sn1,3-16:0. Despite its low 18:2 and P/S ratio, the shea nut oil with sn1,3-18:0 actually produced the lowest TC, lowering both LDL and HDL substantially (Fig. 9). It is not obvious why this extraordinary decline occurred, but the low protein and low 18:2 intakes may have played a role, plus the fact that fats with unusually high 18:0 and low 18:2 seem to depress overall lipid absorption (not measured here) and metabolism. This result with shea nut oil is a good example of why 18:0 in natural fats is not considered cholesterol-raising. With the natural sn1,3-18:0 configuration in shea nut oil or cocoa butter [26], neither TC nor LDL is raised, but high intakes of sn1,3-18:0 can depress HDL [27], as observed here and similar to interesterified fats enriched in sn2-18:0 (see following). These data also demonstrate that natural fats having sn2-SFA (e.g., palm kernel oil) raise LDL and HDL, i.e., both lipoproteins increase simultaneously when consuming such fats, especially at lower 18:2 intakes, as seen here.

Fig. 10 illustrates 2 of the original studies describing the plasma lipoprotein response to diets comparing coconut oil or various palm oils, either alone or mixed with other oils and fed as typical Asian dishes in a native population. Ng et al. [24] compared coconut oil with palm olein and corn oil as natural oils, i.e., 2 natural saturated fats versus a highly unsaturated oil, and found that coconut induced the highest TC, LDL, and HDL, with the response to palm olein intermediate between

much lower values for corn oil. The LDL/HDL ratio did not change from the study's onset with coconut oil, but it was decreased progressively by palm olein and corn oil as the %E of 18:2 increased. Note that this ratio following corn oil was even lower than that for shea nut oil in the Tholstrup et al. study [23] (Fig. 9).

A second similar study [25] fed subjects a 16:0-rich fat based on a blend of natural saturated fats such as palm stearin, palm oil, palm kernel oil, and corn oil. This was compared, in a crossover design of 4 weeks each, to a second 12:0+14:0-rich blend of coconut oil, palm kernel oil, and canola oil, which produced an exact exchange of 12:0+14:0 for 16:0+18:0 of about 4%E. The 18:2 was low at 3.5%E for both fats. The results (Fig. 10) indicated a modest decrease in TC, LDL, and HDL with the 16:0+18:0 blend that did not alter the LDL/HDL ratio. The cholesterol-raising capacity of the 12:0+14:0-rich fat was consistent with earlier publications on coconut oil and butterfat. Results emphasize the higher HDL with 12:0+14:0, but LDL also increased with that fat, as is customary with natural saturated fats at a low 18:2 intake.

Revisiting the Initial IE Study

Studies such as Zock et al. [20] and Tholstrup et al. [23] raise the issue of which SFA (in a saturated fat) is most responsible for inducing hypercholesterolemia. This question indirectly led to one of the earliest studies of interesterified fats in humans [19]. These investigators were among the first to suspect that individual fatty acids were important in plasma cholesterol regulation; they reasoned that this could be tested by simply incorporating more than normal amounts of a single SFA into another fat, thereby enriching an otherwise non-cholesterolemic oil with SFA ranging from 10:0 to 18:0. Their results were prophetic regarding the possible use of interesterified fats for general application to foods. However, it is fair to say that their enrichment of an oil with a given SFA did not anticipate the impact of TG structure on lipoprotein metabolism but rather addressed the simple notion that superabundance of a single SFA would reveal which SFA was exerting the predominant effect on plasma cholesterol.

For these studies, a stable population of 21 men (about 50 years old) with an average body mass index of about 24 was studied for a 4-year period in an institutional setting. In this case, subjects consumed a series of 26 fat preparations sequentially in whole diets during 4-week periods for 1.5 years to assess specific fatty acid effects on serum cholesterol. All foods were prepared in conjunction with the house chef, and specially made fats (Procter & Gamble Co.) were incorporated into the diet to determine whether all SFA were equally cholesterol-raising, when each was added separately in excess to a specific fat or oil. Addition of the SFA took 2 forms: IE of a blend of 75 parts natural oils (olive or safflower) with 25 parts tri-SFA (e.g., tri-12:0, tri-14:0, tri-16:0) or fully hydrogenated soybean oil that

generated tri-18:0+16:0 containing an 85:15 ratio of 18:0 to 16:0. As additional fat sources, MCT containing only 10:0 on the glycerol molecule were interesterified with the various tri-SFA in a 60:40 ratio in another dietary series, whereas in another dietary challenge, butterfat (rich in SFA from 2:0 to 18:0) was simply blended with the tri-18:0 in a 80:20 ratio. Since butter already had a high proportion of its TG-MS as tri-SFA, it rendered the IE procedure less unique or necessary. Thus, the other fat blends were chemically interesterified to randomize the SFA among the sn1, 2, or 3 positions in conjunction with the natural unsaturated fatty acids present in the original fat. These interesterified fats were then incorporated into regular foods as shortening to provide menus that allowed 38%E from total fat, approximately 80% of which was provided by the test fats and the rest of which came from the foods in the menus. The natural fats and oils themselves were also fed for comparison with their SFA-enriched variations.

Substitutions with additional interesterified fats were more subtle and complex and are not detailed here, as our main concern is simply whether IE makes a difference on blood lipids and whether one can detect a difference in the influence of an SFA. Once interesterified, the whole diet was analyzed for fat and its individual fatty acid content, which was highly prescient for that era. Again, there was no attempt to control the dietary fat design for TG structure in these pioneering studies.

Although the study predated the modern era of lipoprotein profile analysis, the data generated do allow us to retrospectively approximate values for LDL, HDL, and VLDL, in part because each subject was his own control and all samples were processed in a similar manner throughout the series of diet changes. This was also possible because the total serum cholesterol was provided along with the beta-lipoprotein fraction, an estimate of LDL based on acetate electrophoresis in the early days of lipoprotein analysis. In addition, the serum TG can be used to calculate VLDL with the Friedwald equation ($\text{total TG}/5 = \text{VLDL}$). The HDL was then calculated as the difference between TC minus LDL+VLDL. Another limitation was the fact that the 19 men finally included had their values reported as the mean, group TC response to a fat, rather than as individuals, so statistical analysis is not possible. Also, the 19 men were divided into 2 groups, so the data sometimes represent the mean for the 2 groups when they consumed the same fat or a subpopulation of only 9 or 10 when only 1 group received the specific fat in question. Despite these limitations, the fact that each man served as his own control throughout all diet rotations makes the data revealing and clearly indicates interesterified fats as potential modulators of serum cholesterol in humans when consumed in significant amounts.

The first data from this study (Fig. 11) compare the lipoprotein response of these men to each of 3 natural fats versus their habitual diet and MCT. Note that the men were

moderately cholesterolemic (about 220 mg/dl) at the outset. The safflower oil diet, which included an inordinate amount of 18:2 and had the highest P/S ratio, lowered cholesterol the most and produced the lowest LDL/HDL ratio of all fats because LDL declined appreciably. Also note that butter, with essentially no 18:2 and the worst dietary P/S ratio (like MCT), was the only natural fat to raise cholesterol above baseline levels, which is represented by the "average American diet." Note, too, that a large monounsaturated fat intake (cis18:1) during olive oil consumption had no impact and that MCT without appreciable 18:2 had no effect on TC but decreased HDL substantially, in the manner of a high-carbohydrate (CHO) diet. This raised the LDL/HDL ratio intermediate to butter, which increased the ratio by raising LDL without raising HDL. Since MCT absorb rapidly and go directly to the liver via the portal vein like CHO, this might suggest that HDL in humans may depend more on fat absorption and gut production of HDL (apolipoproteins in chylomicrons) than on hepatic production from CHO (as VLDL). Dietary cholesterol was constant at 325 mg/day, so it may have interacted with SFA to adversely influence lipoprotein metabolism [12,14]. None of these results with individual natural fats are surprising, and all generally have been noted by others [3,24,25]. However, a novel observation for the time was the impact of the specific SFA interesterified into these base oils/fats.

MCT. In the MCT oil series (Fig. 12) the IE of SFA as either tri-14:0, tri-16:0, or tri-18:0 (at a 60:40 ratio of MCT to tri-SFA) inserted 14%E into the MCT diet in the form of the selected SFA. This had an adverse influence in all cases compared to MCT alone, at least for TC, i.e., when 18:2 intake was very low (1%E). However, whereas 14:0 and 16:0 caused major increases in both LDL and HDL, IE of tri-18:0 into MCT increased LDL without increasing HDL, so this fat actually resulted in the highest LDL/HDL ratio. The point is that increasing the longer-chain SFA content of an 18:1- and 18:2-deficient oil (MCT) by IE to replace an equivalent amount of 10:0 (at 14%E in the diet) raised TC by exerting a variable effect on LDL and HDL, which in turn had either positive or negative effects on the LDL/HDL ratio in these adult men. Furthermore, the data indicate that longer-chain SFA (14:0 to 18:0) are responsible for increasing LDL when inserted into a non-cholesterolemic saturated fat (MCT with tri-10:0); additionally, the data indicate that only 14:0 and 16:0 also increase HDL. This presumably occurred because IE processing caused many of the TG molecules to have 14:0 or 16:0 at sn2. Apparently, IE-14:0 exerted the most favorable influence (as previously emphasized), but IE-16:0 had a favorable outcome for HDL as well.

Olive Oil. When olive oil was subjected to the same IE process by inserting LC-SFA ranging from 12:0–18:0 in a

75:25 ratio of oil to tri-SFA, the final sum for 12:0, 14:0, 16:0, and 18:0 combined represented about 14%E, but the olive oil also quadrupled the 18:2 to 4%E compared to 1%E in MCT preparations (Fig. 13). In this series, each specific interesterified SFA replaced about 8%E for cis18:1 in the olive oil. In the lipoprotein response, the benefit of cis18:1 (MUFA) and additional 18:2 (PUFA) over 10:0 in MCT is noticeable because, although the TC and LDL responses to olive oil were similar to MCT, the HDL increased and LDL/HDL ratio decreased substantially. Again, the insertion of 14:0 into olive oil induced the best response, raising HDL appreciably while generating the lowest LDL/HDL ratio, including comparison with olive oil alone. IE with both 16:0 and 18:0 generated the highest LDL/HDL ratios, although the 18:0 insertion was an improvement over that seen following its IE into MCT, and 12:0 insertion was intermediate between olive oil alone and olive oil after IE with 14:0. Thus, feeding an oil with cis18:1 (and modest 18:2) as the main fatty acids improved results compared to the MCT oil in terms of the LDL/HDL ratio, but only the random insertion of 14:0 was an improvement over olive oil itself, at least when considering the lipoprotein response and LDL/HDL ratio.

Safflower Oil. Similar to the olive oil effect, a 75:25 blend of polyunsaturated safflower oil interesterified with tri-SFA resulted in the replacement of many 18:2 fatty acids with about 8%E from the individual SFA in question (Fig. 14). In this case, none of the LC-SFA inserted by IE proved advantageous because none improved the LDL/HDL ratio compared to safflower oil alone. With abundant 18:2 present, IE-18:0 caused an increase in HDL but also caused an increase in LDL. Thus, TC rose, but the LDL/HDL ratio was unchanged relative to safflower oil alone. Inserting 16:0 was least desirable.

Butterfat. When butterfat was the stock fat (Fig. 15), the addition of tri-18:0 (in an 80:20 blend) was without effect, but butterfat caused a major decrease in HDL and major increases in LDL and TC over entry levels, so that the LDL/HDL ratio increased by approximately 65%. The other tri-SFA were not fed as a blend with butter, presumably because butter is already enriched with 12:0, 14:0, and 16:0, many in the sn2 position. Thus, it is apparent that butterfat on its own (with the most sn2 SFA and minimal PUFA) increased CVD risk about 65% over entry values based on the rise in TC/HDL ratio (see Lewington et al. [28]).

The point is that all 3 tri-SFA esterified into the various oils had a distinctive effect on LDL and HDL. With the exception of 14:0, which seemed to have the capacity to preferentially increase HDL, IE of SFA into natural oils or as a mixture of tri-18:0 with butter had a negative impact, especially on the LDL/HDL ratio. In contrast to 14:0, the main effect of added 18:0 at about 12%E intake was to decrease HDL, whereas 16:0 tended

to increase LDL. Also, 18:0 appeared closest to 16:0 in raising LDL, and both seemed less desirable than either 12:0 or 14:0. The trend induced by feeding an interesterified oil containing an inserted SFA (random IE generating about one third sn2 as SFA) was consistent, irrespective of the background fat used for IE or its degree of unsaturation, i.e., whether it was saturated (10:0 as in MCT), monounsaturated (18:1 as in olive oil), or polyunsaturated (18:2 as in safflower oil) fat receiving the inserted SFA.

Other points are noteworthy. In comparison to olive oil alone, it would seem better (in terms of HDL and the LDL/HDL ratio), to have 75 parts olive oil interesterified with 25 parts tri-14:0, and to a lesser extent tri-12:0, but not IE with tri-16:0 or tri-18:0. This was not the case for safflower oil, however, presumably because its high 18:2 content prevented the increase in HDL that was observed by inserting 14:0 into the high 18:1 content of olive oil.

Trans Fat vs Interesterified Fat

The urgency for replacing trans fat in the diet has been reviewed recently [5,6], but the initiative began with the observation that TFA had a negative impact on the lipoprotein profile [29], a point that had been overlooked in the early days of trans fat introduction. In the 1960s the significance of lipoproteins in CVD was not fully appreciated, and TC was the standard used to assess the impact of dietary fat on blood lipids.

One of the initial studies to examine this issue further with lipoproteins as a focus [30] fed 56 normolipemic subjects a full menu diet in which the fat was altered to incorporate high levels of either linoleic acid, stearic acid, or TFA (as elaidic acid from partial hydrogenation). About 8%E as each specific fatty acid was exchanged between diets in a crossover design, such that all subjects received all 3 diets (Fig. 16). All other fatty acids were maintained at a constant level, with PUFA representing about 4%E in 2 of the diets and 12%E in the third. These normolipemic subjects varied considerably in their individual responses, but collectively the IE-18:0 diet depressed HDL significantly to a point that was intermediate between 18:2 and trans-18:1, with the latter inducing the greatest rise in LDL and the greatest depression in HDL. The point is that partial hydrogenation with TFA formation or full hydrogenation and random insertion of 18:0 by IE negatively impacted LDL and HDL, as well as the LDL/HDL ratio, not unlike McGandy et al. [19]. Trans-18:1 also raised triglyceride levels.

Aro et al. [31] conducted a similar study but compared a saturated fat (from dairy fat, mostly butter) with either TFA or IE-18:0 (as a 9%E exchange) (Fig. 17). The study included 80 subjects (31 men and 49 women), with all 80 consuming the saturated fat diet initially for 5 weeks before dividing into 2 groups of 40 to consume either the diet with 18:1 or IE-18:0. Total fat provided was 33%E, with 3%E from polyunsaturated fat. Both the TFA and IE-18:0 decreased TC relative to the

saturated dairy fat during the run-in period, but trans fat decreased HDL by about 17%, while IE-18:0 caused an 8% decrease in HDL. With trans-18:1 the LDL/HDL ratio increased 20%, as did the apolipoprotein B/apolipoprotein A1 ratio, while the atherogenic lipoprotein (a) (Lp[a]) increased by about 30%. Lp(a) increased by 10% with IE-18:0. In many respects these results are comparable to those cited earlier, indicating that high IE-18:0 intake can depress HDL and raise Lp(a), although somewhat less than TFA.

Again, it would appear that distributing sn2-18:0 across all TG molecules in dietary fat by IE is more detrimental than incorporating the 18:0 as tri-18:0 by blending [21,22]. The latter approach would concentrate the 18:0 in fewer molecules, with two thirds at sn1,3, and seemingly reduce the negative impact of 18:0 on metabolism. This point seems to have been overlooked in a recent review on stearic acid [26], where meta-analysis simply assessed total 18:0 intake without separating responses based on whether the 18:0 was derived from natural fats, tri-18:0 blended into other fats, or incorporated as IE-18:0 to convert many TG molecules to sn2-18:0. One can make the point that, in fact, distortion of lipoprotein metabolism is minimal when 18:0 is consumed in natural fats as sn1,3-18:0 but becomes progressively more evident as sn2-18:0 is consumed either as tri-18:0 or more widely distributed among all TG at sn2-18:0 by IE.

One of the best study designs and executions of a clinical comparison between fats incorporating TFA, IE-18:0, and natural saturated fats (SFA) in humans was conducted by Judd et al. [32] (Fig. 18). Six dietary fat compositions were compared in 50 men, each receiving all 6 diets during 5-week rotations for 8 months. The fats were specially prepared by industrial partners to ensure quality control for testing specific fatty acid effects of interest, based on their practical application in food products. Thus, the study compared a CHO diet (i.e., asking whether less fat would be better) that provided only 30%E from fat by replacing 8%E as c18:1 from fat with CHO. This diet was tested against 5 other diets that provided 38%E as fat. In each of the 5 higher-fat diets, the CHO was replaced by 8%E as fat that targeted a specific fatty acid. This was achieved by using specific fat compositions based on special fat blends that isolated the impact of the individual fatty acid in question. The first fat diet incorporated MUFA-rich cis18:1 at 18%E; the second inserted 8%E as trans-18:1 (via partially hydrogenated soybean oil) to replace some cis18:1; the third had 4%E as TFA + 4%E from IE-18:0 (interesterified from fully hydrogenated soybean oil) to replace some cis18:1; the fourth fat diet replaced 8%E as IE-18:0 for some cis18:1; and the final fat diet substituted 8%E as SFA for some cis18:1, using all natural saturated fats that included a blend of palm stearin, coconut oil, cocoa butter, and butterfat. All diets were adjusted by blending in the proper amount of canola or corn oil to provide 4%E as 18:2n6 across all diets, representing

an excellent reference amount of 18:2 intake [11,12], and unlike other studies [21–23] in which it was low or varied widely between diets, while other fatty acids were being compared.

Because of the exemplary dietary fatty acid control, the total SFA, total MUFA, and total PUFA intake was similar across diets, so that the final P/S ratios for diets were comparable, with slightly lower ratios for the last 2 fats as TFA were removed and replaced by SFA. Thus, in the final analysis, 8%E as CHO, TFA alone, TFA + IE-18:0, IE-18:0 alone, or natural 12:0+14:0+16:0, were substituted for an equal amount of cis18:1 (control fat), while all other fatty acids were held constant. All diets were consumed under cafeteria supervision or on weekends with take-home meals previously prepared and portioned according to need. The crossover design and execution of the study were remarkable for their detail involving regular whole-food diets and comparison between fatty acids in clinical studies of this duration.

Plasma lipids and lipoproteins revealed that the CHO and cis18:1 diets were similar, generating the lowest total cholesterol and best LDL/HDL ratios (Fig. 18). Interestingly, the natural SFA diet produced a LDL/HDL ratio equal to that of the first 2 diets, even though total TC was elevated. The ratio was the same because the increase in LDL was matched by an increase in HDL (consistent with the impact of fats rich in 14:0; see earlier text). By contrast, the other 3 fats containing TFA or IE-18:0 all raised TC similar to the SFA diet, but unlike the natural SFA or cis18:1, all 3 of these exchanges adversely affected the LDL/HDL ratio by raising LDL and reducing HDL. The trans fat was most offensive, with IE-18:0 being somewhat less, and the mix of the 2 being intermediate in terms of the final LDL/HDL ratio. Even though HDL decreased only slightly during the IE-18:0 intake, a trend for IE-18:0 to raise the LDL/HDL ratio was apparent and similar to other studies of IE-18:0.

In addition, publication of additional data from the same study revealed that TFA and IE adversely impacted certain aspects of immune cell function and inflammatory markers [33]. The point is that both types of restructured fats, whether as TFA insertion into the TG by partial hydrogenation of an oil or 18:0 insertion by IE, impacted lipoprotein metabolism adversely in normal subjects fed under highly controlled conditions of fat composition while ingesting whole foods.

These data support several other reports on IE-18:0 described earlier [19,30,31] and earlier Betapol studies in neonates with IE-16:0 [15,16], indicating that modification of TG-MS by IE with different SFA can have an adverse impact. In effect, both TFA and IE-18:0 can alter lipoprotein metabolism when all fatty acid intakes are accounted for and specific fatty acid exchanges are precisely executed between diets. Again, unlike the propensity for an HDL-depressing influence of 18:0, the presence of 14:0, and possibly 16:0, in natural saturated fats fed here raised HDL along with LDL, as noted earlier [23–25].

In a report by Sundram et al. [34], both a trans-rich and an interesterified fat (as IE-8:0) were compared to an unmodified saturated fat for their relative impact on blood lipids and plasma glucose. Each fat had melting characteristics, plasticity, and solid fat content suitable for use as hard stock in margarine and other solid fat formulations. Unlike the studies supplying only modest amounts of margarine as test fats (see later text), these modified fats were fed directly as the entire test fat and were not diluted by additional fat other than that present in low-fat foods in the diet. Thirty human volunteers were fed complete, whole-food diets during 4-week periods, where the total fat (~31% daily energy, >80% from the test fats) and fatty acid composition were tightly controlled. A crossover design was used with 3 randomly assigned diet rotations and repeated-measures analysis. One test fat rotation was based on natural palm olein (POL) and provided 12.0%E as palmitic acid (16:0); a second contained trans-rich, partially hydrogenated soybean oil (PHSO) and provided 3.2%E as TFA plus 6.5%E as 16:0; while the third fat was interesterified with 18:0 to provide 12.5%E as stearic acid (IE-18:0). After 4 weeks, the plasma lipoproteins, fatty acid profile, and fasting glucose and insulin were assessed. In addition, at 2 weeks into each 4-week period, an 8-hour postprandial challenge was initiated in a subset of 19 subjects who consumed a breakfast containing 53 g of the test fat.

After 4 weeks, both PHSO and the interesterified fats significantly elevated both the LDL/HDL ratio (Fig. 19) and fasting blood glucose, the latter by about 20% in the interesterified fat group relative to POL. Fasting 4-week insulin was 10% lower after PHSO ($p > 0.05$) and 22% lower after IE ($p < 0.001$) compared to POL, an unmodified saturated fat. For the postprandial study, the glucose incremental area under the curve (AUC) following the IE-18:0 meal was 40% greater than after either of the other meals ($p < 0.001$) and was linked to relatively depressed insulin and C-peptide secretion ($p < 0.05$) (Fig. 20).

As in previous studies [30–32], where the test fats represented a high percentage of the total fat, both PHSO and IE-18:0 fats altered the metabolism of lipoproteins relative to an unmodified saturated fat when fed under identical circumstances, as in these humans. Criticism of these findings [35] suggested that results were simply an effect of differences in fat saturation between diets, not an effect of IE. However, the design examined 3 types of fat containing roughly equal approximations for both total SFA+TFA (12%E–18%E) and PUFA (3.5%E–7%E). Not only was the fatty acid exchange of interest, but the structure of TG was a consideration as well, because both partial hydrogenation and IE modify the molecular structure of TG.

TG structure repeatedly surfaced as the most logical explanation for the observed results, as differences in fat saturation between diets did not adequately account for the findings. First, although the IE-18:0 diet provided more total

SFA than POL or PHSO, most of this was as 18:0, which is often deemed to be neutral or even cholesterol-lowering [11,23,26]. If the latter is true, then 18:0 should not be counted as an SFA, thereby eliminating the argument that LDL was higher during interesterified fat intake because it contained more SFA. Also, palmitic acid, described as the most cholesterol-raising fatty acid [36], was much greater in the POL diet, yet it produced the lowest LDL and highest HDL. It has also been pointed out that the effects of natural fats rich in stearic acid on the serum lipoprotein profile may differ from the effects of 18:0 in synthetic fats [22] in an 18:0 vs 18:2 vs 18:1 comparison. Thus, application of usual fatty acid associations with responses in the LDL/HDL ratio are suspect at best under conditions of interesterified fat consumption. Furthermore, the interesterified fat diet had twice the amount of PUFA as the POL diet, which by most counts and regression analyses would clearly favor LDL lowering by the interesterified diet [4,11,12,14]. Thus, if interesterified fats and TFA appear similar in their metabolic outcomes yet differ from POL, and if differences in dietary fatty acid saturation/unsaturation do not apply, it seems most likely that IE with 18:0, and TG structure in general, was the issue in the cited study, rather than fatty acid saturation.

Finally and more importantly, a focus on lipoproteins and fat saturation misses the real message in the report, i.e., modifying TG structure by IE or partial hydrogenation is seemingly able to alter glucose and insulin metabolism in a surprisingly short period relative to a natural saturated fat. Altered glucose metabolism may be most dramatic after TG restructuring, as observed with the interesterified fat fed here. Further evaluation seems warranted because interesterified fats continue to raise questions about atypical outcomes, whether in the interpretation of a regression analysis involving 18:0 and the lipoprotein response [22] in 18:0 vs 18:2 vs 18:1 or the plasma glucose/insulin outcomes reported in the Sundram et al. study [34]. With more clinical studies it should be possible to determine at what level interesterified fats alter metabolism, thereby allaying concerns that will continue in the absence of such studies.

Other Interesterified Specialty Fats: Caprenin and SALATRIM

In another study [37], a modified specialty fat, Caprenin, was developed based on MCT (8:0+10:0) that was randomly interesterified with behenic acid (22:0) along the lines of McGandy et al. [19]. This novel fat with IE-18:0 was similar in concept to SALATRIM fats, containing TG molecules made by interesterifying 18:0 and short-chain fatty acids (2C and 3C) [38]. In the case of Caprenin, the assumption was that the long-chain saturated fatty acids (22:0) would both harden the MCT oil and not be absorbed well, thus rendering it a reduced-calorie fat, similar to SALATRIM. The influence of this fat on

blood lipids was examined in 2 studies in which Caprenin was compared to natural saturated fats for 6 weeks. The first incorporated Caprenin at 11%E in one diet and compared it to a highly saturated fat based on a palm kernel oil/palm oil blend, presumably of comparable plasticity. Fat supplied 36%E in the first study of 17 men and 17 women, and it incorporated a lower intake of 18:2 at 4.5%E and higher Caprenin (11%E) than the second study. Neither the natural palm kernel oil + palm oil blend nor Caprenin induced a change in TC from baseline during the 6-week study period, but Caprenin caused a significant (25%) rise in the LDL/HDL ratio compared to the natural fat blend, which was rich in 12:0+14:0, by raising LDL and decreasing HDL (Fig. 21).

The second study had only 7 participants per group after the greatest responders to fat were selected from the first study. Some important fatty acid changes were also incorporated into the design of study 2 that reduced the negative response to Caprenin seen in study 1. Specifically, the fatty acid profile was altered by decreasing Caprenin to only 8%E (30% decrease) while increasing 18:2 to 8%E (60% increase). This dual fatty acid adjustment in the diet of these hyperresponder subjects resulted in Caprenin having no significant effect from baseline over the 6-week study period: TC, LDL, and HDL all declined slightly, while the LDL/HDL ratio rose slightly because the HDL decline was proportionately greater than that for LDL. Butterfat fed as the control fat in study 2 actually improved lipoproteins relative to Caprenin, in the sense that TC and LDL decreased slightly, while HDL was actually higher, so the LDL/HDL ratio was lower, even with less 18:2 intake in the butter group (Fig. 21).

In essence, 22:0 randomly inserted into a TG with MCT, similar to 18:0 and TFA in other studies [19,30–32,34], produced an unfavorable result on the LDL/HDL ratio. This unfavorable influence was improved somewhat by decreasing the Caprenin load and increasing the protective intake of dietary 18:2. Collectively, these studies indicate that random insertion of a long-chain SFA (in this case 22:0) including the sn2 position can adversely impact lipoprotein metabolism and the LDL/HDL ratio.

SALATRIM itself has been studied extensively [38]. In a 7-day clinical trial it provided about 70% of the fat at 25 mg/kcal/d, i.e., with IE-18:0 representing about 12%E. The acute response was compared to entry values and a group of subjects who consumed fully hydrogenated coconut oil (which would have all sn-SFA) for blood lipids. Unfortunately, no natural fat control was represented, so baseline lipids were the *de facto* control. The blood lipid response was not remarkable, except that the coconut oil raised lipids about 20% from baseline, while SALATRIM did nothing. It was noteworthy that liver enzymes for hepatocyte integrity were elevated significantly by SALATRIM from control values but remained within the clinically normal range. A longer study of 3 to 4 weeks might have been more revealing.

A general thought is that using lipoproteins as an endpoint of the metabolism of interesterified fats or TFA may be relatively insensitive and not necessarily indicative of what has transpired in other cell membrane functions. Lipoproteins are easy to sample and eventually reveal an effect, but they may be an obtuse measure. Also it seems possible that 8%E as interesterified SFA may be required to detect a lipoprotein response, so lesser amounts go undetected, but not necessarily without biological effect in systems other than lipoprotein metabolism. It is likely that we do not usually measure the best system. How much, and which, interesterified SFA is required to initiate a response still remains to be delineated.

Spreads with Interesterified Fat or TFA

Although spreads and margarines are logical delivery modes in tests for interesterified fat function, by themselves they may result in low intakes of interesterified fat relative to the entire daily fat intake as other dietary fats dilute their relative abundance. For example, one of the first studies using dietary margarines containing interesterified fats examined the use of an interesterified hard stock blended with soybean oil (58:42 ratio) and 20% water to make the final margarine [39]. The interesterified hard stock itself was a blend of saturated fats (36% coconut oil, 33% palm oil, 22% palm stearin, and 9% partially hydrogenated canola oil) prior to IE to form the first hard stock. The original blend of 4 fats was also mixed with soybean oil in a 58:42 ratio to serve as a second, noninteresterified hard stock for comparison. The final margarines thus contained considerable 18:2 from soybean oil (26% wt/wt), such that the percentage of sn2 in all TG occupied by 18:2 (35%) was equal for both margarines, and only sn2-16:0 was doubled (to 18% of the TG molecules) in the fat exposed to IE, while sn2-18:1 decreased by a roughly equivalent 8%E. Those were the only appreciable differences in the margarines. In fact, IE-18:0 was not a factor in this study, occupying only 4% of the sn2 fatty acids in the interesterified margarine and 2% in the control. These margarines were fed as part of a carefully executed whole diet program, such that the hard stock portions (as opposed to specific interesterified fatty acids in other studies included here) of the margarines represented either 4%E or 8%E when fed to different sets of men and women (7 to 8 per group; 60 subjects total) in a parallel design for 3 weeks. The 2 types of hard stock margarines were then fed in a crossover series for 3-week periods to the respective groups. The actual dietary 18:0 provided by the hard stock never exceeded 0.5%E.

Many cardiovascular and general physiological parameters were measured without incident or differences in any parameter, including blood lipids (Fig. 22). Serum fatty acids revealed no changes between groups, and TC (190–220 mg/dl), LDL (120–140 mg/dl), and HDL (50–60 mg/dl) were the same.

In fact, not one statistically significant difference was found in the entire study!

Two points are relevant here. First, the amount of IE hard stock fat at 4%E and 8%E (consumed as part of the margarine) was a relatively small subportion of the total dietary fat content at 34%E, which included about 5%E–6%E as PUFA in the total dietary fat profile of 16:11:5 for S:M:P. Second, although IE served to increase the sn2-SFA content at the expense of cis18:1, the increase occurred only in a small increment of sn2-16:0, contributing only 1.5%E in the final diet, while sn2-18:0 contributed less than 0.5%E. Thus, the low challenge with IE-sn2 SFA would not be expected to affect the physiology here. Other than the infant studies with Betapol, or adults with high intakes of sn2-16:0 as interesterified fats [20,21], other studies using sn2-16:0 have failed to show an effect with low intake of IE-16:0, suggesting the possibility that IE that moves 16:0 to sn2 is not as deleterious as sn2-18:0 or longer-chain SFA, at least in adults. More studies are needed with different intakes of sn2-16:0 to confirm this, as the infant formula data would indicate that it may have a negative effect at some level of intake in adults.

Australian Margarines. A similar study with a crossover design compared 3 experimental stick margarines composed of special fats against a typical Australian fat blend in 27 men, average age of 49 years, for 3-week periods (Fig. 23) [40]. However, the so-called control margarine (modified palm oils) was atypical in itself, because it did not contain all natural fats but rather a blend of 35% fully hydrogenated palm kernel oil, 40% palm olein, 20% sunflower oil, and 5% palm stearin. Even though it had not been subjected to IE, more than 40% of the fat molecules in this “control” saturated fat blend would have sn2-SFA, resulting from modification by full hydrogenation of palm kernel oil, coupled with the tri-16:0 in the TG solids from palm stearin. Accordingly, this fat and its related data represent a questionable normal standard for comparison of an IE effect, because the same fat blend following IE (IE modPalm) had about the same degree of sn2-SFA (47%), although the main effect of IE was to increase the sn2-16:0 threefold. The third test fat (hi18:2+TFA) included a blend that incorporated partially hydrogenated soybean oil and partially hydrogenated cottonseed oil to supply 3%E as TFA in the diet, but this fat contained only half the total SFA and more than twice the 18:2%E of the other 2 margarines. Test fats provided about 62% of all fat consumed. Thus, the study was not a reliable test of fatty acid exchanges or TG structure per se, but rather represented a comparison of 2 novel hard-stick margarines against a more conventional one having a modest content of dietary TFA and appreciable PUFA.

Not surprisingly, the diet with modest TFA, low SFA, and high PUFA performed the best; but because no true “reference standard fat” or fatty acid profile was included, the

comparison does not represent a TFA exchange for equivalent SFA or c18:1. Therefore, the true effect of TFA or interesterified SFA was not tested by the other diet designs. The same can be said for the interesterified fat because it, too, was compared to the atypical control saturated fat, which had almost as much sn2-SFA as the interesterified fat but favored 16:0. And although IE of the control saturated fat (IE modPalm) did not produce a measurable change in TC, the shift to slightly increased LDL and slightly decreased HDL with an increase in the LDL/HDL ratio was nonetheless consistent with tendencies observed in other studies cited earlier that more rigorously tested IE-18:0 ingestion (Fig. 23). The higher HDL observed following both the “control,” modPalm, and IE modPalm fats than with either of the other 2 test margarines may reflect the greater content of 12:0+14:0 in control and interesterified fats, as suggested by earlier studies [19,20,23–25,36], all of which described a propensity for fats containing 14:0 to raise HDL. Thus, this margarine comparison tested neither an IE nor a TFA effect in the true sense, even though all margarines resulted in slight improvement of the LDL/HDL ratio relative to the native Australian diet.

Salatrim Margarine (IE-18:0) Acts Like sn1,3-16:0. A second study from the same Australian group [41] examined 15 middle-aged men and women to compare a palm oil-based margarine rich in 16:0, as a natural TG source, with an interesterified margarine containing 18:0 randomly interesterified with short-chain fatty acids (SCFA) in the form of SALATRIM (Fig. 24). These 2 diets were compared to a low-fat (hi-CHO) diet containing only half the fat (20%E) of the other 2 diets (at 40%E), where the test fats reportedly represented more than 60% of the total fat intake. The fatty acid profile of the 2 high-fat diets was similar, except that the 8%E as IE-18:0 in SALATRIM was substituted for the sn1,3-16:0 in the palm oil diet. The high-CHO diet had about half (3%E) of the 18:2 content in the high-fat diets (5%E). Surprisingly, and contrary to most published results, all 3 diets produced the same plasma lipid results after 5 weeks in a crossover design. In fact, TC, LDL, and HDL were identical for all 3 diets, leading to 3 possible conclusions: (1) total fat intake (i.e., 20%E or 40%E) made no difference in this case; (2) IE-18:0 was not any better than sn1,3-16:0 at lowering cholesterol (unlike Tholstrup et al. [23] or McGandy et al. [19], where 16:0 appeared to raise TC relative to 18:0); or (3) both 16:0 and 18:0 were as cholesterol-neutral as CHO. When considered against the cholesterol-lowering effect of shea nut oil [23], this result might suggest that randomizing 18:0 with SCFA, including sn2-18:0, is not as favorable and cholesterol-neutral as natural fats rich in 18:0 with sn1,3-18:0 (shea nut oil).

Thus, one could conclude that neither IE-18:0 from SALATRIM nor 16:0 from palm olein could be considered cholesterol-raising relative to CHO, contrary to general

findings [14,32] and reports such as Tholstrup et al. [23]. Or, one might conclude that IE-18:0 in SALATRIM is less detrimental than might be expected from other reports on IE-18:0 associated with long-chain fatty acids [30–32,35]. One potential limitation is that the test fats herein represented a lower percentage of the total daily fat (60%E) than several other studies, and thus had their effect diminished by other fats in the diet. For example, compare this to Tholstrup et al. [23] and Sundram et al. [34], where the test fat made up >80% of total dietary fat. The outcome can differ and interpretation can suffer when extraneous dietary fat intake is left to chance. The blood lipids in these subjects at entry were normal, too, making it more difficult to elicit differences [12].

Postprandial Effect of Fatty Acid Types from a Single Meal

Meal-Fed Tests of Fats and Postprandial Lipids. Other studies have attempted to compare fat (fatty acid) effects, including interesterified fats, by feeding a single meal on the assumption that postprandial absorption and metabolism of chylomicrons would reveal the main disturbances in fat utilization attributable to TG-MS of specific fatty acids fed in the meal (see Karupaiah and Sundram [8] for review).

Acute Meal Effects of IE-18:0. The possibility that differences in dietary fat (fatty acids) might be detected in lipid metabolism immediately following a single meal was tested in the United Kingdom in a series of studies that considered TG structure as well as specific dietary fatty acids with 18C chain length. It is obviously easier to execute a postprandial experiment of 6 to 8 hours than a long-term diet plan, and the assumption was that the postprandial lipemic response might provide data to predict lipoprotein modifications that occur after much longer periods. The latter experiment requires that the test fat be incorporated into the entire diet for several weeks, but it also provides a longer time for lipoprotein response to adjust to any given dietary fat, which takes into account the capacity of adipose reserves to contribute to dietary fatty acid imbalances that might occur.

In the first study [42], the test breakfast was a milkshake that incorporated fat along with polydextrose and sucrose as carriers and a source of CHO energy. Six variations in the breakfast meal fat composition were compared: CHO and MCT (the latter with 8:0+10:0, which behaves much like CHO rather than fat); 16:0 from palm oil; IE-18:0 from fully hydrogenated sunflower oil IE into hi18:1-sunflower oil; trans-18:1 from partially hydrogenated sunflower oil; and cis18:1 from regular hi-cis18:1 sunflower oil mixed with MCT. The 5 fat meals contained 90 g of fat (65%E as fat in the meal) while the low-fat, CHO meal had 10 g of fat (7%E). The high-fat meals used fat blends to exchange about 25%E between 16:0,

trans-18:1, cis18:1, IE-18:0, and MCT (8:0+10:0). The 18:2 intake was constant at 6%E for all high-fat meals but less than 1%E for CHO. Following the challenge breakfast, the postprandial lipemia (AUC for plasma TG) was determined over 7 hours, with a low-fat lunch provided at the 3-hour point.

Among the results for the 11 male and 5 female subjects (average age 25 years), it was interesting that the AUC for TG in the young female participants (presumably reflecting a depressed rate of fat absorption) was less than half that of the young male subjects for each of the diets. And, as one might expect with minimal intestinal fat absorption, CHO had no impact on raising the postprandial plasma TG level, with MCT being only slightly more triglyceridemic than CHO (Fig. 25; males). This difference most likely reflected the presence of added sunflower oil in the MCT challenge meal and lack of 18:1 and 18:2 (and other long-chain fatty acids) in the CHO meal. The IE-18:0 meal also depressed the AUC by about 50%, as did 16:0 (25% depression), relative to the cis18:1 control fat. The responses to cis18:1 and trans-18:1 were not significantly different; both generated normal TG-AUC profiles.

Thus, in this postprandial model, the IE-18:0 induced atypical delayed fat absorption compared to an unsaturated fat as cis18:1, or even natural sn1,3-16:0, for that matter. The reader should bear in mind that this was an exceptional level of fat intake (90 g/meal) and presumably pushed the limits of fat absorption, especially for the women, where the large fat load for body size appeared to reduce stomach emptying and decreased the fat absorption rate by about 50% relative to the men. The observed differences in postprandial absorption rate of an oil interesterified with 18:0 likely reflects the gut mucosal cell function and a compensatory adjustment in metabolism related to the previous meal and its fat content. But the apparent failure to absorb all the IE-18:0 in the postprandial period exposes certain caveats and questions the relevance of meals containing various fats to long-term lipoprotein effects.

In a second postprandial experiment, Sanders et al. compared another IE-18:0 fat discussed earlier (SALATRIM) to natural cocoa butter and hi-18:1 sunflower oil [43]. Older men and women (50 years on average) were challenged with 50 g of fat (a much smaller dose than in the previous study [42]) in a meal of a muffin and a milkshake to assess postprandial lipemia as before. Again the IE-18:0 (16%E, one third as sn2-18:0) as SALATRIM with its SCFA (2:0–3:0) produced less than half the TG-AUC at 6 hours compared to either cis18:1 or sn1,3-18:0 (11%E) from natural cocoa butter. The cis18:1 diet was unique because it caused the plasma HDL to rise after 6 hours, while HDL declined after the cocoa butter meal. The sn2-18:0 provided by SALATRIM did not alter the postprandial plasma HDL level. Thus, because fat absorption after delivery of the sn1,3-18:0 in cocoa butter was greater than

that following a larger mass of sn2-18:0 delivered in SALATRIM, both TG structure and fatty acid saturation affected the nature of the postprandial absorption and metabolism of fats in this study. No difference was noted between sexes in these older subjects given a much smaller fat load, which suggests that either postmenopausal women are more like men than young women in their fat absorption or that the fat load was not so large as to delay absorption in these women (Fig. 26). The point is that IE-18:0 in SALATRIM, as in the first Sanders study [42] with IE-18:0 in a conventional oil, depressed TG-AUC when replacing cis18:1, while natural 16:0+18:0 as sn1,3-18:0 in cocoa butter was similar to cis18:1 regarding the TG-AUC.

To further address the issue using this type of postprandial challenge, a third Sanders study subsequently explored the impact of interesterified fat in more detail [44]. They fed either regular cocoa butter (as earlier) or interesterified cocoa butter to 17 young men, again using a breakfast milkshake and a muffin to provide 50 g of fat in a single meal. The fatty acid profiles of the 2 meals were identical, but the natural cocoa butter had mostly 18:1 at sn2 and 18:0 at sn1,3, while the interesterified version randomized about half the sn2-18:1 present in the natural fat with sn2-18:0. A low-fat lunch was again provided 3 hours into the postprandial period. Under these circumstances in men, no effect was noted on plasma TC or HDL up to 6 hours postprandially, but the AUC for TG (chylomicrons + remnants pool) was again significantly depressed by the interesterified fat (like SALATRIM earlier), including a transient increase in TG following lunch. The HDL response after 6 hours was identical and unaffected over time for both fats (Fig. 27). Thus, again, one can conclude that IE with sn2-18:0, compared to a natural fatty acid profile and a TG-MS having sn1,3-18:0, appeared to depress fat absorption during the 6 hours following a meal but had no effect on postprandial lipoproteins, unlike the long-term response, where HDL tends to be depressed. Note well that the test fats represented the only fat consumed (somewhat akin to the infant formula studies) and were given in one meal, mixing their fatty acids with those in the mucosal cells, etc. These TG-AUC data showing reduced TG absorption during sn2-18:0 studies are in agreement with the perturbed lipoprotein profiles noted when fats containing IE-18:0 are fed for several weeks [30–32,34].

IE of 18:0 vs 16:0 at sn1,3. A fourth study of IE from the Sanders group was conducted in 16 young men [45] and served to confuse the issue (Fig. 28). Two separate but related experiments examined the effect of stearic acid location on the TG molecule, using a different natural source of 18:0, shea nut oil. In the first experiment, 14%E as fat was provided to normolipemic subjects as either a shea nut oil/sunflower oil blend (53% 18:0) in either native TG form (SOS) or after

random IE (SSO, SOS, OSS) in a crossover design of 3 weeks each. Thus, the exchange compared the same fatty acid profile, either as natural shea nut oil with sn1,3-18:0, or as the interesterified fat with significant sn2-18:0. These 2 fats were compared to a "low-18:0" British diet that was presumably rich in 16:0+18:1 (fatty acid profile not provided). Once fed this background diet, subjects then consumed 2 muffins daily to provide 30 g extra fat from the shea nut oil/sunflower oil blend, causing the daily fat intake to increase from 66 g/d to 96 g/d when the muffins were added. Both before and after the 3-week feeding trials with muffins, subjects fasted overnight, then were challenged with a breakfast meal of a muffin and a milkshake that contained 50 g of the test fat. Blood lipids were then followed postprandially for 8 hours.

Fasting plasma lipids, including glucose and insulin responses, were compared at the end of the 3-week test periods. No differences were found in the fasting blood lipid profile or glucose/insulin parameters as a function of the 3-week treatments. Like Nestel et al [40], who compared sn1,3-16:0 with interesterified sn2-18:0 in margarines, the lack of effect probably reflected test fat dilution by extraneous dietary fat during the 3-week crossover periods and the low percentage of test fats (30%) provided by the muffins. Once again, one is led to believe that 16:0+18:1 (from the run-in diet) was no more or less cholesterol-raising than 18:0 presented either as a natural fat having 18:0 at sn1,3 or as 18:0-IE at sn2 after IE. Such data are contrary to most studies, where 18:0 in natural fat proved neutral and IE-18:0 exerted a negative impact on the LDL/HDL ratio. In more revealing studies by others, the test fats provided a greater percentage of total fat, suggesting that the test fats herein provided either an insufficient mass or were offered in an atypical fashion (muffin snacks outside meals?) that precluded the fats being detected as unusual.

Surprisingly, the randomized (interesterified) oil appeared to produce a greater postprandial lipemia than natural shea nut oil (not significant), which is in contrast to all previous results of Sanders et al. (the authors were also puzzled). This is the only such report in the literature indicating this surprising effect for IE-18:0. But this may have been biased by only reporting the lipemias after correcting for the initial postprandial lipemia prior to the 3-week feeding trial (initial unadjusted data not presented). It is possible that the 3-week exposure to the test fat biased the second postprandial trial at its conclusion, which could happen if the gut mucosa absorbing interesterified fat was still loaded with TG containing IE-18:0 that then flushed postprandially after the meal challenge, making the IE-18:0 fat seem more lipemic.

A follow-up second experiment in that report simply fed 2 test meals (50 g fat) to fasting subjects; one meal had native shea nut oil and the second had hi-18:1 sunflower oil. The question was whether native shea nut oil with sn1,3-18:0 would differ from sn1,3-18:1 in sunflower oil. The more

liquid, hi-18:1 sunflower oil caused a greater postprandial lipemia, as expected, in keeping with studies on the relative rate of absorption for different unsaturated fats, rendering the findings in the first experiment even more confusing.

Again, the point is that one can feed a small percentage of daily fat as a bolus (muffins) and see no change if an ample amount of nontest fat dilutes the test fat (or fatty acid) or if the previous meal fat is stored in the mucosal cells and mixes with the meal fat. On the other hand, one can feed a large percentage of test fat that is included in every bite of the food consumed daily to enhance the chance of observing a true effect. Another caveat here is that only 30 g of test fat was fed daily in the 3-week clinical test, and it was provided as 2 servings with 15 g fat each in muffins (timing of muffins not designated), so the absorption and metabolism of test fat might have been independent of the other fat metabolized during the day, i.e., 2 separate mini-metabolisms possibly operative, unlike other studies [17,31,32,34] or tests with infant formulas [15,16], where the fat in each bite of food all day long was the same as that in the previous bite. The latter type of design is more conducive to discerning how a specific fatty acid behaves in a relatively fixed pool of other dietary fatty acids. This issue becomes very complex when comparing both fatty acids and TG structure, because the appropriate designs are more demanding for testing hypotheses around this more complicated relationship. Higher standards of fat delivery and overall composition are required than is possible in meal studies, which are by nature transient events.

The authors remark that the main physical difference between the 3 fats tested was their solid fat content (melting point profile), with the randomized fat being the worst (highest melting point), native fat intermediate, and sunflower oil the lowest (at 0% at room temperature). They argue that fats with high melting points absorbed more slowly. Thus it was counterintuitive that the randomized shea nut oil, with the highest solid content, should absorb more readily than native shea nut oil, contrary to previously published data from that lab. Thus, the entire data set around postprandial studies and their interpretation is suspect in terms of relevant physiology to long-term lipoprotein metabolism.

Several questions emerge concerning the relevance of these postprandial studies, i.e., it is unclear what is being measured. Is prolonged absorption of a fat (presumably related to fat solids) good or bad if the apparent effect is lower postprandial triglycerides? Since no fat was lost in feces, all the fat was absorbing eventually, just not as fast as typical fats and undetected during the postprandial window observed. Are the 50-g test loads of fat practical and a useful predictor of fat metabolism, in general, or is 50 g too high for a single dose? After all, 50 g represents the total fat calories for the entire day in a woman on modest fat intake and even two thirds of the usual total daily fat intake for these men. If one is trying to evaluate

randomized 18:0 for its typical metabolic impact, perhaps 2 or 3 levels of intake are required, both in the challenge dose of a meal (25 g and 50 g) as well as in the daily muffin intake (2, 3, or 4 muffins per day?). Where does the lipid processing become depressed or otherwise altered, and if extended over time, is it detrimental to lipid metabolism in general?

An additional postprandial study [46] fed 15 young men a breakfast meal containing 75 g fat (representing 40% of the calories in the meal) after an overnight fast (Fig. 29). Six fats (3 interesterified) were tested with this model, without major effects on the postprandial lipoprotein profile. As in the Sanders et al. studies, one end point was the rate of TG absorption, measured as the TG peak at 4 hours after the meal (Fig. 29). The first 2 diets was based on IE of tri-14:0+tri-16:0 into hi-18:1 sunflower oil; a second represented tri-16:0 interesterified into sunflower oil; a third provided interesterified fat from tri-18:0 into sunflower oil; TFA was inserted in a fourth diet; and the final 2 diets provided an 18:2-rich or c18:1-rich fat. Fat absorption was depressed only by the IE-18:0 diet, and to a lesser extent by IE-16:0-rich fat. As with most other studies, the interesterified fats with either 16:0 or 18:0 seemed to reduce the rate of fat absorption compared to other fatty acids, such as cis18:1 or even trans-18:1 and cis18:2, following a meal.

CONCLUSION

IE of fat with sn2-18:0 (and to a lesser extent tristearin blended into a fat) exerts a negative influence on lipoprotein metabolism at high intakes and has been shown to affect glucose and insulin metabolism, immune function, and liver enzymes in isolated situations as well. Two tablespoons of an interesterified margarine will not be detected as harmful, particularly if the fat includes significant polyunsaturated oil. Any problem will arise (as with trans fats) if IE becomes the process of choice for hardening oils and is consumed in many foods throughout the day (for example, if it fully replaces TFA in food processing). By comparison, when a solid fat is required, the application of an unmodified saturated fat, such as palm oil, palm olein, or possibly even palm kernel oil or coconut oil, would seem preferable to interesterified fat based on the long-term biological implications. Because palm stearin contains a significant amount of tri-palmitin, with sn2-16:0, that fraction should be utilized sparingly until its impact can be further clarified.

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