Human Studies Using Isotope Labeled Fatty Acids: Answered and Unanswered Questions
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Abstract: Human studies using deuterium-labeled fatty acids have answered many questions related to the metabolism and health effects of dietary fats. These studies also raised a number of unanswered questions and unresolved issues. For example, studies with cis and trans positional isomers dispelled concerns and allegations that the isomers in partially hydrogenated fats were poorly absorbed, accumulate in undesirable phospholipid acyl positions, mimic stearic acid and competed with oleic acid. Trans 18:1 isomers were metabolically intermediate between 16:0 and 18:0, so the unanswered question is why are the metabolic properties of trans fatty acids not consistent with their physiological effects? Results from 2H-18:0 studies address questions regarding stearic acid absorption and desaturation. Contrary to accepted dogma, stearic acid was well absorbed and less than 10% was desaturation to oleic acid. The still unanswered question is what is the metabolic basis for why 18:0 is less hypercholesterolemic than other saturated fatty acids? The question of whether humans convert 18:3n-3 to EPA and DHA was investigated by feeding male subjects a mixture of 2H-18:3n-3 and 3H-18:2n-6. The unequivocal answer was that 18:3n-3 is converted to EPA and DHA and the conversions for 18:3n-3 to 20:5n-3 and 18:2n-6 to 20:4n-6 were about equal. A major issue that remains unresolved is the wide variability between studies for the estimated conversion of 18:3n-3 to 20:5n-3 and 22:6n-3. The commercial availability of liquid oils hardened by interesterified with 18:0 has raised the question of whether fatty acids in the sn-2 and sn-1,3 TAG positions are metabolically equivalent. To answer this question, subjects were fed triglycerides containing 2H-16:0 and 3H-18:2n-6 at specific sn-1(3) and sn-2 acyl positions. The result was that dietary fatty acids at the sn-1(3) and sn-2 triacylglycerol positions are essentially metabolically equivalent.

Key words: saturated fats, trans isomers, triglyceride, linoleic acid, linolenic acid, metabolism, deuterium isotope, human, review

1 INTRODUCTION

The results from a wide variety of in vivo tracer studies using isotope labeled fatty acids have provided much of our basic knowledge about fatty acid metabolism. Nearly all isotope tracer studies use radioactive carbon-14 and tritium labeled fatty acids and are necessarily restricted to animal models because safety considerations precludes the use of radioisotope tracers in healthy human subjects. Our initial 1976 study with non-radioactive deuterium-labeled fatty acids was the first to use a multiple-stable isotope experimental protocol to investigate fatty acid metabolism in human subjects8. This and subsequent studies have provided much of the currently available human isotope tracer data for assessing fatty acid metabolism in humans and for comparison to results from animal studies.

The most unique feature of the multiple-stable isotope experimental design is that, by using fatty acids tagged with different numbers of deuterium labels, a control fatty acid and one or more experimental fatty acids can be fed to the same subject at the same time. This is an extremely important feature because it greatly reduces problems associated with subject variability since each subject now serves as his own control. This design allows the accurate comparison of results from a series of studies if the same internal control used. It also provides much more information than typical single labeled studies. The disadvantages are that synthesis of the differently labeled deuterated...
fatty acids containing different numbers of deuterium labels is a challenge and a sophisticated GC-MS system is required for sample analysis. In fact, the application of this multiple-labeled experimental design was only possible because we developed both the methods to synthesize a variety of fatty acid structures containing different numbers of deuterium atoms and the chemical ionization mass spectrometry methodology required to analyze samples containing a mixture of differently labeled fatty acids. The deuterated analogs of all the major fatty acids in normal diets were used to investigate their metabolism in human, animal and cell culture experimental models.

This review is limited to a brief overview of the following four topics based on results from our stable isotope tracer studies in human subjects: (1) metabolism of trans fatty acid isomers, (2) palmitic and stearic acid absorption and desaturation, (3) influence of triacylglycerol structure on fatty acid, (4) linoleic and linolenic acid metabolism and conversion to n-6 and n-3 metabolites. Results from these studies unequivocally answered a number of questions, resolved misconceptions and raised a number of unanswered questions.

2 Metabolism of cis and trans monoenoic isomers compare to non-isomeric fatty acids.

The metabolism of all the major isomeric and non-isomeric fatty acids present in typical US diets have been investigated in a series of human studies. A comparison of the metabolic data from these stable isotope tracer studies has been reviewed in detail\(^2\). These studies addressed concerns and allegations that cis and trans \(18:1\) isomers were poorly absorbed, accumulate in undesirable phospholipid acyl positions, mimic stearic acid, and competed with oleic acid.

The question of absorption is addressed by the chylomicron-triglyceride(TG)time-course curves shown in Fig. 1. These data compare the absorption of the 9t-, 12t-, 12c-, 13t-, and 13c-18:1 isomers to the internal control, 9c-18:1. Comparison of these curves shows that these 18:1 isomers were equally well absorbed. These chylomicron-TG data are representative of all the cis and trans positional 18:1 isomers investigated and showed that all of the 18:1 isomers were well absorbed.

![Fig. 1](image)

**Fig. 1** Chylomicron triglyceride time-course curves for subjects fed mixtures of triglycerides containing cis and trans 18:1 isomers and 9c-18:1.

were poorly absorbed, accumulate in undesirable phospholipid acyl positions, mimic stearic acid, and competed with oleic acid.

![Fig. 2](image)

**Fig. 2** 2A and 2B: Selectivity values for incorporation of isomeric and non-isomeric fatty acids into human plasma 1-acyl and 2-acyl phosphatidylcholine\(^2\). Positive selectivity values indicate preferential incorporation and negative values indicate discrimination relative to 9c-18:1. Selectivity values are relative values and are equivalent to percent difference divided by 100.
The question of unregulated incorporation of 18:1 isomers into phosphatidylcholine (PC) is addressed by the selectivity values in Figs. 2A & 2B for accretion of 18:1 isomers into the 1- and 2-acyl PC positions. Results for the fatty acids (palmitic, stearic, linoleic and linolenic acids) and the conjugated linoleic acids (9c,11t- and 10t,12c-18:2) are also included for comparison. All selectivity values are relative to oleic acid, which was included in all studies as an internal control. Selectivity values are equivalent to percent difference divided by 100 (i.e., a selectivity value of one represents a 100% difference). In general, cis and trans 18:1 positional isomers are selectively incorporated into the 1-acyl position of phosphatidylcholine and strongly excluded from the 2-acyl PC position relative to oleic acid. This selectivity pattern is consistent with the known specificity of phosphatidylcholine acyl transferase for selective acylation of saturated fatty acids at the 1-acyl PC position and unsaturated fatty acids at the 2-acyl PC position. The positive 1-acyl PC selectivity values for cis and trans 18:1 positional isomers indicate that cis and trans 18:1 isomers are metabolically more like 16:0 than 18:0 and correlate with the difference in their effect on LDL-c (trans 18:1 isomers and 16:0 raise LDL cholesterol but 18:0 does not). The incorporation of 18:0 into PC-1 was over 3 times greater than for 16:0. This strong preference for 18:0 suggests that 18:0 may have more biological importance than 16:0.

The selectivity values for 2-acyl PC indicate that the trans 18:1 positional isomers are strongly excluded and also indicate that they are metabolically more like the saturated fatty acids than unsaturated fatty acids. In contrast, most cis 18:1 positional isomers were not strongly excluded from the 2-acyl PC position which suggest that they are metabolically intermediate between oleic acid and saturated fatty acids. The clear exception is the 12c-18:1 isomer which is metabolically more like 9c,12c-18:2 than 9c-18:1 and uniquely different from oleic acid and stearic acid. The four fold positive value for the 12c-18:1 isomer illustrates the sensitivity and/or selectivity of the acyltransferases for n-6 fatty acids with a cis double bond. The extremely large positive selectivity value for 9c,12c-18:2 reflects the biological importance of 9c,12c-18:2. Note that 9c,12c,15c-18:3, 10t,12c -18:2 and 12c,15t-18:2 have a 12c double bond but they are not preferentially incorporated because they are not non-conjugated n-6 fatty acids. The slight negative selectivity for 9c,12c,15c-18:3 is surprising given that it is an essential fatty acid and has significant physiological / biological importance.

Plasma cholesterol ester (CE) selectivity values are summarized in Fig. 3. The extremely large negative selectivity values for the trans 18:1 positional isomers and the CLA isomers indicate that the lecithin cholesterol acyl transferase enzyme (LCAT) strongly discriminates against fatty acids containing a trans double bond and are consistent with the very low trans fatty acid content of tissue CE. In fact, most trans fatty acid isomers are essentially excluded from CE. LCAT also strongly discriminates against incorporation of the cis 18:1 positional isomers relative to 9c-18:1. This lack of incorporation argues that the cis and trans 18:1 positional isomers would not significantly contribute to the atherogenic properties of CE. Note that the selectivity values show a strong discrimination against 18:0 but not 16:0 and correlate with the difference in their effect on serum HDL-C levels. In addition, the extremely high linoleic acid selectivity value correlates positively with linoleic acid’s well known beneficial cholesterol lowering properties. In contrast to the 2-acyl PC data, the selectivity values in Fig. 3 for plasma cholesterol ester show that accretion of 12c-18:1 is not significantly different from other cis 18:1 acids, which are metabolically intermediate between 18:0 and 16:0. All the trans isomers, including the CLA isomers are strongly excluded from CE and some are essentially totally excluded. The lack of accretion into plasma CE indicates that the trans-18:1 positional isomers would have little impact on the physical or liquid crystalline properties of CE and would not influence its atherogenic properties.

These human isotope tracer studies answered several questions. For example: cis and trans 18:1 positional isomers are well absorbed and accretion into tissue lipid classes is well regulated. The strong exclusion from CE and
2-acyl PC position is consistent with low levels of trans isomers in tissues lipids. These results indicate that turnover is rapid, β-oxidation is higher than for 16:0 and 18:0 and the physiology effects should be minimal. Nearly all the selectivity values for the various plasma lipid classes (triglyceride, phosphatidylcholine, phosphatidylethanolamine, cholesterol ester) are negative. The conclusion is that accretion of cis and trans 18:1 positional isomers are well regulated and that significant physiological affects due to errant accumulate in cell membrane lipids is not likely to occur. The trans fatty acid content of human tissues lipids are much lower than the trans levels in dietary fat and reaffirms the negative selectivity values. For example, organ tissues contain mostly phospholipid and CE and have a trans content that is about 80% lower than dietary levels, which is consistent with the negative plasma PC and CE selectivity values. Adipose tissue contains mainly triglyceride and has a trans content that is about 50% lower than dietary levels which is consistent with the negative plasma TG selectivity values. In addition, the negative plasma triglyceride selectivity values indicate that postprandial hyperlipidemia would not be an issue because clearance and turnover is more rapid than for oleic acid which is consistent with the higher β-oxidation rates for 9t-18:1 compared to 16:0, 18:0 and 9c-18:1.

The unanswered question is: Why are the metabolic properties of trans 18:1 isomers not consistent with their observed influence on LDL cholesterol? All of the isotope tracer metabolic data indicate that trans isomers should be nutritionally benign. In general, trans 18:1 isomers are metabolically more similar to 16:0 than to 18:0 and 9c-18:1. Thus, they may be physiologically more like 16:0. This possibility is consistent with clinical studies that have observed substitution of dietary 16:0 for 18:0 increased serum LDL levels. In reality, trans isomers are not as bad as advertised in the popular press. For example, the 1990 Mensink study reported that substitution of 1 gm of trans isomers for oleic acid increased LDL-C by 0.43 mg/dl and decreased HDL by about 0.2 mg/dl. The 1994 and 2002 Judd studies reported that substitution of 1 gm of trans isomers for oleic acid increased LDL-C by about 0.6 mg/dl and decreases HDL by about 0.1 mg/dl. In comparison, substitution of 1 gm 16:0 for oleic acid increased LDL-C by about 1.8 mg/dl and did not raise HDL-C. Replacing all of the trans fatty acids (1.5 g) in the current US diet with oleic acid would reduce LDL-C by less than 1 mg/dl and have a non-measurable effect on HDL-C. Thus, low levels of dietary trans fatty acids intake would appear to be a non-issue.

3 Stearic and palmitic acid isotope tracer studies.

It has been 48 years since the 1965 landmark studies of Hegsted and Keys reported that stearic acid was less hypercholesterolemic than 16:0. Subsequently, a large number of human diet studies have reported that 18:0 does not raise LDL cholesterol and that it is essentially cholesterol neutral. At the time our human isotope tracer studies were initiated, the explanation for the lower cholesterol effect of 18:0 compared to other saturated fatty acids was based on results from animal studies that reported 18:0 was poorly absorbed and that stearic acid was extensively converted to oleic acid. To confirm the stearic acid absorption results in humans, young adult women were fed controlled diets enriched with canola and soy oil for 4 wks. The women were then dosed with a mixture of triglycerides containing 2H-16:0, 3H-18:0, 9t-18:1, and 18:2n-6.

The percent of each 2H-FA in the fed mixture are similar to the % in the 4 hr Chylo TG samples (Fig. 4) and clearly show that all four fatty acids were equally well absorbed. These results and an earlier study in male subjects were contrary to the accepted dogma that stearic acid was well absorbed. A comparison of the experimental design used in the animal studies to these human tracer studies identified an obvious difference. In the animal studies, stearic acid or tristearin was generally added to the diets as a powder. In the human studies, the mixture of deuterated triglycerides containing tristearin was melted and then emulsified with sugar-milk protein solution. The reason the animal studies reported poor absorption of tristearin studies was because the test diets contained microcrystalline particles of tri-

Fig. 4 Comparison of deuterated fatty acid absorption by young adult female subjects fed controlled canola and soy oil diets. Subjects were dosed with a triglyceride mixture containing deuterated 16:0, 18:0, 9t-18:1, and 18:2n-6.
stearin. The melting point of tristearin (72°C) is well above body temperature, which prevents complete hydrolysis by pancreatic lipase. These isotope tracer studies showed that tristearin was always well absorbed when it was fed as a mixed TG or when it was melted and absorbed on to a protein carrier, which prevents crystal formation. These results were subsequently confirmed by non-isotope tracer studies in humans.

The question of percent conversion of 18:0 and 16:0 to 9c-18:1 and 9c-16:1 was investigated by feeding a mixture of deuterium labeled tristearin and tripalmitin to young adult male subjects\(^{12, 13}\). An example of the plasma triglyceride time-course curves that compare the conversion of 18:0 and 16:0 to their respective desaturated metabolites and the chain-shortened product (16:0) of 18:0 are shown in Fig. 5 for a young adult male subject. Desaturation of 18:0 was ca 14% and was ca three times greater than desaturation of 16:0. Chain-shortening of 18:0 to 16:0 was about 9%. These conversion results are directly comparable because subjects were fed both fatty acids at the same time. The data from this individual represents the maximum amount of delta 9-desaturation observed in this study. For the five male subjects studied, the average % conversion of 18:0 to 9c-18:1 was about 10% and was significantly greater than the 4% conversion of 16:0 to 9c-16:1. However, the absolute difference in conversion of 18:0 and 16:0 to their monounsaturated metabolite is small and it would not be sufficient to explain the differences in the effect of 16:0 and 18:0 on LDL cholesterol levels.

So the unanswered question remains - What is the metabolic basis for why 18:0 is less hypercholesterolemic than 16:0? There is a large difference between the selectivity values for 18:0 and 16:0. These selectivity values are a reflection of LCAT and phospholipid acyltransferase specificity and these enzymes are involved in LDL and HDL metabolism. Thus, it might seem that PE, PC and CE selectivity values for 16:0 and 18:0 correlate with their cholesterolemic properties. For example, the difference between the phosphatidylethanolamine selectivity value for 18:0 and 16:0\([ +10 \text{ vs. } -0.4]\) is particularly striking. In addition, the selectivity value for incorporation of 16:0 into 1-acyl PC is roughly three times lower than for 18:0 and inversely correlates with the increased serum cholesterol levels produced by dietary 16:0 relative to dietary 18:0. In contrast, the CE selectivity value for 16:0 is dramatically higher (four fold) than for 18:0 and correlates with the higher serum cholesterol levels produced by increased intake of dietary 16:0 compared to 18:0. The inconsistency or problem with this scenario is that CE selectivity values for 9c-18:1 and 16:0 are about equal but they have different cholesterolemic effect. Another problem is that only the 1-acyl PC selectivity values for the \textit{trans} 18:1 isomers are similar to 16:0 selectivity values. A third problem is that the 1-acyl PC selectivity value for 16:0 is higher than the 9c-18:1 selectivity value.

4 Influence of triglyceride acyl position on fatty acid metabolism.

Commercially semi-solid fats produced from liquid oils interesterified with 18:0 are marketed as a zero-\textit{trans} alternative to partially hydrogenated vegetable oil. The interesterification process hardens liquid vegetable oils by incorporation of 18:0 into the TAG structure. This process rearranges the TAG structure and has lead to speculation that fatty acids in the \textit{sn}-1,3 and \textit{sn}-2 acyl TG positions may be metabolically and/or physiologically different. Examples are: \textit{sn}-1,3 and \textit{sn}-2 acyl triglyceride position influence fatty acid accretion into lipid classes, interesterified fats have negative atherogenic properties, \textit{sn}-2 18:0 triglycerides have a negative effect on glucose/insulin levels, and \textit{sn}-2 saturated fatty acids have a negative a postprandial effect on chylomicron TG levels. Most human diet studies have reported that saturated fatty acids in the \textit{sn}-1 and \textit{sn}-2 acyl TAG position have similar influence on cholesterolemic properties. However, many conflicting results have been published. An example is a 2007 study\(^{14}\) that compared a palm oil diet to an interesterified fat diet and reported the interesterified fat diet lowered HDL cholesterol by 9%, lowered insulin levels and increased fasting

![Fig. 5](image_url) Plasma triglyceride time-course curves for comparison of the conversion of 18:0 and 16:0 to their respective delta-9 desaturated and chain-shortened metabolites by a young adult male subjects fed a mixture of deuterated 16:0 and 18:0\(^{15}\).
blood glucose. One reason for the conflicting metabolic results is that the effect of triglyceride structure on fatty acid metabolism is an extremely difficult problem to study rigorously.

The protocol that we designed to investigate the effect of palmitic acid at the sn-2 acyl TG position was to provide subjects with diets containing native and interesterified lard and then dose the subjects with a mixture of triglycerides labeled with deuterated fatty acids at the sn-1,3 and sn-2 acyl position. Lard was used to formulate the diets because it is a commercially available dietary fat that contains about 70% of the 16:0 in the sn-2 acyl TG position. After interesterification, all fatty acids are equally distributed between the sn 1-, 2-, and 3-acyl positions.

A cross-over experimental design was used and middle-age male subjects were fed the native lard and interesterified lard diets for 4 week periods. Both diets produced similar effects on total and LDL cholesterol and had no effect on HDL cholesterol, which indicates that TG acyl position did not influence the cholesterolemic properties of 16:0\(^\text{15}\). Three subjects from each diet group were fed a mixture of two triglycerides (P-d\(_6\)/Lo-d\(_4\)/P-d\(_6\) and Lo-d\(_2\)/P-d\(_4\)/Lo-d\(_2\)) that contained differently labeled \(2\text{H}-16:0\) and \(2\text{H}-18:2\text{n-6}\) at the sn-1/3 and sn-2 positions.

The time course curves are shown in Fig. 6 for incorporation of \(16:0\) and \(18:2\text{n-6}\) into the chylomicron 2-acyl TG position\(^\text{15}\). The 4 hr chylomicron data show that 80-90% of the deuterated fatty acids in the sn-2 acyl position were retained during absorption. This was the expected result and is totally consistent with results from non-tracer human studies.

The unexpected result was that if \(2\text{H}-16:0\) and \(2\text{H}-1\text{8:2}\text{n-6}\) were in the non-preferred acyl TG positions, the chylomicron triglyceride structure was extensively rearranged to the preferred TG structure. In contrast, if the \(2\text{H}-16:0\) and \(2\text{H}-1\text{8:2}\text{n-6}\) were in the preferred chylomicron acyl TG positions, there was no rearrangement of the chyl TG structure. The practical ramification is that the rearrangement of non-preferred structures to preferred chylomicron TG structures would significantly reduce the physiological and metabolic impact of interesterified fats.

The time course curves in Fig. 7 show that TG acyl position did not influence accretion of the \(16:0\) and \(18:2\text{n-6}\) into PC. The preferential incorporation of \(18:2\text{n-6}\) relative to \(16:0\) is consistent with the known selectivity of phosphatidylcholine acyltransferase for \(18:2\text{n-6}\). These PC data are representative of the results for all the various neutral and phospholipid plasma fractions. This general lack of an effect of acyl TG position on accretion is consistent with the observation shown in Fig. 6 that rearrangement of the chylomicron TG structure would significantly attenuate the effect of TG structure on accretion into lipid classes.

The influence of triglyceride acyl position on conversion of \(18:2\text{n-6}\) to \(20:4\text{n-6}\) in male subjects pre-fed native and randomized lard diets is shown in Fig. 8. The native lard diet subjects were dosed with a mixture of two triglycerides containing \(18:2\text{d}_4\) in the preferred sn-2 acyl TG position and \(18:2\text{d}_4\) in the non-preferred sn-1,3 acyl TG position. Conversion of the \(18:2\text{d}_4\) in the preferred sn-2 acyl

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**Fig. 6** Time-course curves for the incorporation of deuterium labeled 16:0 and 18:2n-6 into the sn-2 acyl position of chylomicron triglycerides from a male subject fed a mixture of two specifically labeled triglycerides (Lo-d\(_4\)/P-d\(_4\)/Lo-d\(_4\) & P-d\(_4\)/Lo-d\(_4\)/P-d\(_4\))\(^\text{16}\).

**Fig. 7** Time-course curves for the incorporation of deuterium labeled 16:0 and 18:2n-6 into plasma phosphatidylcholine from male subjects fed a mixture of two specifically labeled triglycerides (Lo-d\(_4\)/P-d\(_4\)/Lo-d\(_4\) & P-d\(_4\)/Lo-d\(_4\)/P-d\(_4\))\(^\text{16}\).
position was about 30\% higher than conversion of the 18:2-d2 in the sn-1,3 acyl position. Conversion of 18:2n-6 in the sn-2 acyl position by subject pre-fed the randomized lard diet was about 30\% higher than the total 18:2n-6 conversion by the subjects pre-fed the native lard diet. These results suggest that the TG acyl position of 18:2n-6 in a TG structure and the TG structure of dietary fats may have an influence on the conversion of 18:2n-6 to 20:4n-6.

The answered questions from this isotope tracer/lard study are that TG structure does not influence fatty acids accretion into the lipid classes and the TG acyl position had no effect on LDL or HDL cholesterol. The observed rearrangement of non-preferred chylomicron TG structures to preferred TG structures provides a reasonable explanation for the lack of a TG structure effect. This study did raise several unanswered questions. Some obvious examples are: How are chylomicron TG rearranged? Is there a lipase imbedded in the chylomicron membrane? Why is 18:2n-6 conversion influenced by TG acyl position but not accretion? Would TG acyl position also affect conversion of linolenic acid to DHA? Is the metabolism of sn-2 acyl 18:0 similar to sn-2 16:0?

Fig. 8 Effect of triglyceride acyl position on conversion of \(^2\)H-18:2n-6 to \(^2\)H-20:4n-6 in male subjects pre-fed native and randomized lard diets\(^5\).

5 Conversion of 18:2n-6 and 18:3n-3 to long-chain polyunsaturated fatty acids (LCPUFA).

Time-course curves are shown in Fig. 9 for accretion of \(^2\)H-18:3n-3 and its n-3 metabolites into the 2-acyl position of plasma PC from a male subject. Note that 18:3n-3 and the n-3 metabolite data are plotted on different scales\(^17\).

\(^2\)H-22:6n-3 is equal to about 50\% of the maximum \(^2\)H-18:3n-3 concentration. This indicates that a considerable amount of conversion had occurred. Note that the data are plotted as ug/ml of plasma. The use of absolute concentration units is a rigorous approach that circumvents many of the interpretation problems that can occur when isotope data are plotted as % enrichments. Estimates for % conversion can be obtained using the area-under-the-curve method that the pharmaceutical industry routinely uses to estimating drug metabolism.

The results from five subjects pre-fed a high (P/S = 0.8) and low (P/S = 0.3) linoleic acid diet and then dosed with a mixture of deuterated 18:3n-3 and 18:2n-6 are summarized in Fig. 10. This study was the first to establish conclusively that humans can convert 18:3n-3 to n-3 LCPFA and was the first to compare directly the conversion of 18:2n-6 and 18:3n-3 to their respective metabolites\(^17\). The results clearly show that the high 18:2n-6 PUFA diet reduced the amount of 18:2n-6 and 18:3n-3 converted to their respective n-6 and n-3 metabolites. This is a classical example of substrate inhibition and partially explains why feeding high dietary levels of 18:2n-6 and 18:3n-3 does not result in a substantial increase of their metabolites in tissue lipids. The data in Fig. 10 also illustrates that one needs to be careful when interpreting percent conversion results using isotope tracer data. In this study, the PUFA diet clearly reduced the absolute concentration of the n-3 and n-6 metabolites. However, the conclusion from the data shown for percent conversion would be that diet did not have an
The reason for the difference is that the high 18:2n-6 diet reduced the concentrations of 2H-18:2n-6 and 2H-18:3n-3 by about 40‰ which affects the % conversion calculation. The bottom line is that % conversion and % enrichment data are useful but they need to be consistent with the more rigorous absolute weight or concentration data.

The effect of dietary docosahexaenoic acid on the conversion of deuterium-labeled linoleic and linolenic acid to their long chain fatty acid metabolites by human subjects is shown in Fig. 11 [19]. In this study a mixture of 2H-18:2n-6 and 2H-18:3n-3 was fed to male subjects that had been pre-fed Low and High DHA diets for 90 days. The High DHA diet significantly inhibited the conversion of 2H-18:3n-3 and 2H-18:2n-6 to their respective metabolites. In fact the High DHA diet almost totally shuts down 2H-18:3n-3 conversion to 2H-22:6n-3 and is a classical example of product inhibition. Comparison of the 2H-18:3n-3 Low and High DHA diet metabolite data, shows that dietary 22:6n-3 inhibited the elongation of 2H-20:5n-3 to 2H-22:5n-3. It did not inhibit delta-6 desaturation of 2H-18:3n-3 to 2H-18:4n-3, which is the normal rate-limiting step. In contrast, the 2H-18:2n-6 metabolite data indicate that dietary 22:6n-3 inhibited delta-6 desaturation of 2H-18:2n-6 to 2H-18:3n-6. The data plotted in Fig. 11 also reflect an interesting and unexplained difference between dietary 18:3n-3 and dietary 18:4n-3. The 2H-18:4n-3 metabolite of dietary 2H-18:3n-3 was converted to 2H-22:6n-3 but dietary stearidonic acid (18:4n-3) is reported to only be converted to 20:5n-3 and 22:5n-3 [19]. In fact, 22:6n-3 levels in erythrocyte phospholipids from subjects fed dietary 18:4n-3 were slightly decreased compared to an 18:3n-3 diet group.

The effect of dietary arachidonic acid on the conversion of 2H-18:2n-6 to its long chain n-6 fatty acid metabolites by male subjects is shown in Fig. 12. In this study, 2H-18:2n-6 was fed to three male subjects that had been fed a diets containing 0.2g/d (LOAA) and 1.7 g/d (HIAA) of 20:4n-6 for 50 days. The metabolite data show that dietary 20:4n-6 reduced 2H-18:2n-6 conversion to 2H-20:4n-6 by about 50%. The results are a classic example of product inhibition and are consistent with the product inhibition results observed in the DHA diet study (Fig. 11). These metabolite data provide some insight into why dietary 20:4n-6 reduced 18:2n-6 conversion. Dietary 20:4n-6 inhibited elongation of 18:3n-6 to 20:3n-6. It did not inhibit the rate-limiting

Fig. 10 Concentration and percent conversion data for deuterated n-3 and n-6 long chain-length fatty acid metabolites in plasma total lipid from male subjects fed a mixture of deuterium labeled 18:2n-6 and 18:3n-3 [17].

Fig. 11 Effect of dietary docosahexaenoic acid on conversion of deuterium-labeled linoleic and linolenic acid to their long chain fatty acid metabolites by male subjects [19].

Fig. 12 Effect of dietary arachidonic acid on conversion of 2H-18:2n-6 to its 2H-20:4n-6 metabolite by male subjects [20].
delta-6 desaturation to 18:3n-6 step nor the delta 5-desaturation of 20:3n-6 to 20:4n-6. These results are consistent with the results in Fig. 11 that showed dietary DHA did not inhibit delta-6 desaturation. The dietary 20:4n-6 and 22:6n-3 results were unexpected and they are difficult to explain since delta 6-desaturation is known to be the rate-limiting step in the conversion of 18:2n-6 and 18:3n-3 to their long chain metabolites. A possible explanation for these results is that competition from dietary 20:4n-6 inhibited the transfer of the H-18:3n-6 metabolite to the site where elongation to H-20:3n-6 occurs. These results are consistent with the results from the dietary DHA study and suggest that dietary 22:6n-3 also inhibited the transfer of H-20:5n-3 to the site where elongation to H-22:5n-3 occurs. An alternative explanation is that 22:6n-3 and 20:4n-6 inhibit elongase expression. Also, it is very interesting that dietary 20:4n-6 and 22:6n-3 inhibited 18:2n-6 conversion by different mechanisms. Dietary DHA reduced 18:2n-6 conversion by inhibition of delta 6-desaturase (see Fig. 11) but dietary 20:4n-6 inhibited the 18:3n-6 elongation step (see Fig. 12). In addition, this suggests that dietary 20:4n-6 would reduce 18:3n-3 conversion by inhibiting delta-6 desaturase. The implication is that phospholipid acyltransferases may have a more important role in regulation of 18:2n-6 and 18:3n-3 conversion than realized and that there is much that we do not understand about how humans regulate 18:2n-6 and 18:3n-3 conversion.

Results from these deuterated 18:2n-6 and 18:3n-3 studies are used to provide estimates for the synthesis of n-6 and n-3 LCPUFA metabolites from linoleic and linolenic acid (Table 1). These estimates are an average based on the amount of 18:2n-6 and 18:3n-3 in a typical US diet. The average estimated amounts of 20:4n-6 and 22:6n-3 synthesized are relatively low but not trivial. The conversion of 18:3n-3 to 22:6n-3 would easily provide the >5 mg/d of 22:6n-3 reported to be required by the adult human brain. The almost 300 mg/d of synthesized 20:5n-3 + 22:6n-3 is equal to about 75% of the 400 mg/d recommended in the 2003 WHO report. The approximately 100 mg/d of synthesized 22:6n-3 is equal to about 25% of the dietary intake recommended for patients with CHD disease. The estimates in Table 1 are based only on plasma lipid data and they do not include any 20:4n-6, 20:5n-3 and 22:6n-3 that was removed and incorporated into tissue lipids. For diets that contain a high levels of preformed 20:5n-3 and 22:6n-3, for example a typical Japanese diet, 20:4n-6 and 22:6n-3 synthesis is likely much lower because product inhibition would greatly reduce both 18:2n-6 and 18:3n-3 conversion.

Results from numerous other studies that have used both carbon-13 and deuterium labeled 18:3n-3 to estimate conversion to n-3 LCPUFA metabolites in both human subjects and animals have been previously summarized. These isotope tracer studies have used a variety of different protocols and the results illustrate a wide variability between studies for 18:3n-3 conversion to 22:6n-3. Some of the between study variability can be attributed to the different methodologies used to calculate percent conversion estimates and to the differences is experimental design but it is clear that between subject variability for 18:3n-3 conversion is high. In contrast to the results from our multi-labeled 18:2n-6 and 18:3n-3 studies, other tracer studies have not included an internal control and/or labeled 18:2n-6. In most other human isotope tracer studies, it is not possible to evaluate the influence of dietary fatty acids because the diets of the subjects were not rigorously controlled. Thus, results from the various human isotope tracer studies are valid but it difficult to identify specific factors that might be responsible for the variability between results from the different studies because they were not designed to allow a direct comparison of 18:2n-6 and 18:3n-3 conversion and diets were not well controlled.

These isotope tracer studies have answered the following questions: (1) humans can convert 18:3n-3 to 22:6n-3,

### Table 1
Estimated Synthesis of n-6 and n-3 Long Chain Polyunsaturated Fatty Acid Metabolites by Human Subjects

<table>
<thead>
<tr>
<th>Diet Study</th>
<th>20:3n-6, % (range)</th>
<th>20:4n-6, % (range)</th>
<th>20:3n-6, mg/d (range)</th>
<th>20:4n-6 mg/d (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hi &amp; Lo 18:2n-6</td>
<td>0.7 - 1.2</td>
<td>0.3 - 0.7</td>
<td>119 - 204</td>
<td>51 - 119</td>
</tr>
<tr>
<td>Hi &amp; Lo 22:6n-3</td>
<td>0.8 - 2.5</td>
<td>1.0 - 3.0</td>
<td>136 - 425</td>
<td>170 - 510</td>
</tr>
<tr>
<td>Hi &amp; Lo 20:4n-6</td>
<td>1.0 - 1.8</td>
<td>2.0 - 2.8</td>
<td>179 - 298</td>
<td>340 - 484</td>
</tr>
<tr>
<td>Average (n = 19)</td>
<td>1.3</td>
<td>1.25</td>
<td>221</td>
<td>279</td>
</tr>
<tr>
<td>Diet Study</td>
<td>20:5n-3, % (range)</td>
<td>22:6n-3, % (range)</td>
<td>20:5n-3, mg/d (range)</td>
<td>22:6n-3 mg/d (range)</td>
</tr>
<tr>
<td>Hi &amp; Lo 18:2n-6</td>
<td>3.4 - 8.0</td>
<td>3.6 - 4.0</td>
<td>68 - 160</td>
<td>72 - 80</td>
</tr>
<tr>
<td>Hi &amp; Lo 22:6n-3</td>
<td>6.4 - 20</td>
<td>1.8 - 11</td>
<td>128 - 400</td>
<td>36 - 220</td>
</tr>
<tr>
<td>Average (n = 13)</td>
<td>9.5</td>
<td>5.1</td>
<td>189</td>
<td>102</td>
</tr>
</tbody>
</table>

Calculated mg/d values are based on typical US diet intakes of 17 g/d 18:2n-6 and 2 g/d 18:3n-3

percent conversion of 18:3n-3 to 22:6n-3 appears to be higher than 18:2n-6 conversion to 20:4n-6 and (3) dietary n-3 and n-6 fatty acids have a significant effect on conversion due to substrate and product inhibition. The most vexing unanswered question is “what is responsible for the large between subject and between study variability for 18:3n-3 and 18:2n-6 conversion to their respective LCPUFA metabolites?” Results from a plethora of various respected and competent research groups have been inconsistent and have lead to a variety of different conclusion and, sometimes, controversial recommendations. In some cases, vested commercial interests have tainted the objectivity used to interpret results, which has contributed to the confusion about this issue. It is likely that there are undiscovered mechanisms or factors that regulate and control formation and incorporation of endogenous n-6 and n-3 LCPUFA metabolites into tissue lipids. The bottom line is that there is still much that we do not understand about what controls the regulation and conversion of dietary n-6 and n-3 fatty acids.

References


