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A rationale for plasma cholesterol modulation by dietary fatty acids: Modeling the human response in animals

K.C. Hayes, Andrzej Pronczuk, and Pramod Khosla

Foster Biomedical Research Laboratory, Brandeis University, Waltham, MA USA

At low levels of dietary cholesterol intake (<200 mg/day human equivalent), the plasma cholesterol response in different species (man and animals) is governed by two key dietary fatty acids: myristic acid (14:0) and linoleic acid (18:2). Thus, in human subjects and animals with presumably normal lipoprotein metabolism, 14:0-rich fats routinely raise the plasma cholesterol in a linear relationship, whereas 18:2-rich fats lower it in a curvilinear fashion, i.e., there is a "threshold" intake of 18:2 above which a further decline in plasma cholesterol is less pronounced. Palmitic (16:0) and oleic (18:1) acids appear to be neutral under these circumstances. In situations involving impaired lipoprotein metabolism (e.g., diminished low density lipoprotein [LDL] receptor activity), or in the presence of high levels of dietary cholesterol (probably >500 mg/day), the plasma cholesterol response is no longer described accurately by dietary 14:0 and 18:2 alone. In such situations 16:0 appears to contribute to plasma cholesterol elevation. The hypercholesterolemic potential of 16:0, possibly reflecting a synergism between dietary cholesterol and 16:0, is thought to reside, in part, in the ability of 16:0 to increase the transport of very low density lipoprotein (VLDL) apoB. Increased production of VLDL, coupled with impaired LDL receptor activity, results in an expansion of the LDL pool when the ability to clear VLDL remnants is impaired. Evidence is also available to suggest that the position of saturated fatty acids on the TG molecule affects its hypercholesterolemic ability. An argument is made for selecting animal models for investigation of the fat saturation effect based initially on the total plasma cholesterol (TC) response, with subsequent emphasis being placed on lipoproteins and the actual control mechanism(s) once the generic similarity in the TC response with that in humans has been established. (J. Nutr. Biochem. 6:188–194, 1995.)

Historical perspective

Studies on diet-related cholesterolemia over the past 40 years have clearly established that saturated fats raise plasma cholesterol whereas polyunsaturated fats lower it.[1–4] These findings led to mass introduction of polyenes in the marketplace (since the 1950s) which doubled the typical polyene consumption between 1940 and 1985 in the United States from 2.5 to 5.5% energy.[5] The rise in polyunsaturated fat intake was followed by a peak and decline in serum cholesterol and coronary heart disease.[1] However, despite the vast body of data, much controversy persists concerning the impact of specific dietary fatty acids on plasma cholesterol, and more importantly, concerning their underlying mechanism of action on low density lipoprotein (LDL) and high density lipoprotein (HDL) dynamics.

Dietary fatty acid modulation of lipoproteins is important because the LDL/HDL ratio appears critical to the atherogenic process.[6] In theory it is conceivable that a proper balance in the fats (specifically the fatty acids) consumed would greatly improve the circulating lipoprotein profile. Historically we were taught that saturated fats containing 12:0, 14:0, and 16:0 (lauric, myristic, and palmitic acid, respectively) raised the plasma cholesterol and LDL-C, whereas those containing less than 12:0 as well as 18:0 (stearic acid) had no effect (see Refs. 1–4 for recent reviews on the subject). Furthermore, monounsaturated fats rich in oleic acid (18:1) had no effect on plasma cholesterol when exchanged for carbohydrate but exerted a cholesterol-lowering effect (both total and LDL) when exchanged for saturated fatty acids. Similarly, the major polyunsaturated

Address reprint requests to Dr. K.C. Hayes at the Foster Biomedical Research Laboratory, Brandeis University, Waltham, MA 02254.
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fatty acid, linoleic acid (18:2), was found to be cholesterol-lowering (mainly LDL) when exchanged for dietary saturated fatty acids or, in the case of hypercholesterolemia, when simply added to the diet without removing other fats.[7,8] However, linoleic acid (18:2) can also lower HDL-C at high intakes (>20% energy).[9]

Refocus on fatty acids, not fats

In an early experiment with monkeys[10] it was observed that tallow and lard (as saturated fats) were scarcely more hypercholesterolemic than corn oil and generally less so than coconut oil or butter, even though both contained appreciable amounts of saturates. Initially, to ascertain the effects of specific fatty acids per se, cholesterol-free diets were formulated with blends of oils in which total saturates, monos, and polys were held constant. Under such circumstances the exchange of dietary 16:0 for 12:0 + 14:0 caused a decrease in plasma cholesterol.[11] This result suggested that palmitic acid was not hypercholesterolemic but might be neutral under certain conditions (i.e., no exogenous cholesterol in normocholesterolemic individuals), and that the widely held belief that most saturates (12:0, 14:0, 16:0) were equally hypercholesterolemic may not hold true in all dietary situations. In a collaborative study, the same result and the hypothesis were confirmed by data from normocholesterolemic humans, i.e., 16:0 was less hypercholesterolemic than 12:0 + 14:0, even with 200 mg of cholesterol in the diet.[12]

In an attempt to understand the underlying mechanism(s), lipoprotein kinetic studies were carried out in rhesus monkeys fed diets rich in either 12:0 + 14:0 or 16:0 + 18:1.[13] Analysis of apoB-specific activity data revealed that although total apoB and very low density lipoprotein (VLDL) apoB transport rates were 2 and 3 fold greater, respectively, in monkeys fed the 16:0 + 18:1–rich diet, the plasma LDL apoB concentration was actually reduced. The lower LDL apoB was associated with a smaller mass and proportion of LDL apoB derived independent of VLDL catabolism, i.e., via so-called "direct" LDL secretion. The LDL receptor did not appear to be affected by the fatty acid exchanges. These results indicated that the saturation of specific dietary fatty acids modulated the amount of LDL apoB that was derived from VLDL or came "directly" from the liver, apparently without affecting LDL receptors. Other studies[14,15] support the idea that both increasing chain length and saturation of dietary fatty acids increase VLDL output. In addition, experiments in cebus monkeys fed cholesterol-free diets[16] suggested that extreme distortions in fat saturation per se can affect LDL receptor activity and the size of the circulating LDL pool, although this was not the case in cynomolgus monkeys[17] or guinea pigs[18] fed less extremes in fat saturation. Furthermore, the results may or may not be altered by the simultaneous intake of cholesterol.[16–20]

Palmitic acid "conditionally" cholesterolemic

In contrast to the above findings on the neutrality of 16:0, studies in both hypercholesterolemic human subjects,[9] non-human primates,[19] and hamsters[20] fed cholesterol-containing diets had suggested 16:0 was hypercholesterolemic in comparison to 18:1 (as well as 18:2). The rhesus data[13] raised the possibility that in these former studies,[9,19,20] the presence of impaired LDL receptor activity might have prevented the clearance of VLDL remnants and thereby diverted them to LDL, causing the LDL pool to expand. In addition, the increased VLDL output from cholesterol feeding[21] and from 16:0 itself,[13] or altered LDL particles that bind poorly to LDL receptors as in polygenic hypercholesterolemia,[22] could have contributed to the expansion of the LDL pool. In the absence of dietary cholesterol and with normal LDL receptor activity, 16:0 and 18:1 might exert equal effects on receptor-mediated LDL clearance and not modulate total plasma cholesterol (TC).

To examine this possibility initially, normocholesterolemic cebus and rhesus monkeys were fed diets similar to those fed in the human study in which the dietary fat was derived from a single oil rich in 16:0, 18:1, or 18:2 without cholesterol.[23] Animals were then coinjected with radiolabeled native and methylated LDL. Both receptor-independent and receptor-mediated LDL clearance were found to be similar for all three diets, indicating that 16:0, 18:1, and 18:2 exerted equivalent effects on LDL metabolism when diets contained adequate 18:2 and no 14:0 or cholesterol. The total cholesterol was lower in cebus monkeys fed the high 18:2 intake, but this decrease was attributable to depressed HDL (putatively apoA1 production). In fact, the 16:0-rich diet produced the lowest LDL/HDL ratio (significantly better than the 18:2-rich diet) which supported a previous finding in hamsters[24] and more recent data in humans that the 16:0[25] or 12:0 + 14:0[26] in saturated fat contributes substantially to a rise in HDL, even as stearic acid (18:0) may depress HDL.[26] Based on the observations in monkeys,[23] 16:0 was exchanged for 18:1 (up to 7% energy) in normocholesterolemic humans by comparing palm olein with olive oil[27] or canola oil.[25] As with the monkey study,[23] the 16:0 − 18:1 exchange caused no differences in LDL or HDL or total cholesterol, whereas when dietary 12:0 + 14:0 replaced 16:0, a significant rise in LDL and total cholesterol was observed,[12] as noted in an earlier monkey study.[11]

The issue of the 16:0 vs. 18:1 equivalency in humans requires further resolution because a number of ill-defined factors (both dietary and metabolic) appear to influence the impact of these two fatty acids. Aside from LDL receptor activity and its depression during hepatic cholesterol accumulation, the structure of the 16:0-rich triglyceride (i.e., the source of saturated fat) may be important, since piglets fed palm oil with 16:0 in the sn1,3 positions had lower plasma cholesterol values than piglets fed an equivalent mass of 16:0 in the sn2 position from a synthetic lard.[28] A similar situation implicating the molecular structure of triglycerides exists in the gerbil[1] (unpublished data).

Thus, the issue of 16:0 neutrally is complicated by the fact that the hypercholesterolemic effect of 16:0 (relative to 18:1) has generally been reported either in situations where one might expect to find depressed LDL receptor activity and an elevated 18:2 "threshold" (see below), i.e., a lipoprotein metabolism generally "under duress," or when a significant portion of the 16:0 residues in the sn2 position.

Under these circumstances 16:0 appears hypercholesterolemic relative to 18:1[29] for putative reasons pertaining to the LDL receptor setpoint, the 18:2 threshold, and VLDL production rates (as discussed above and below).

Differences in LDL transport and LDL receptor activity may exert a major influence on how dietary fatty acids affect lipoprotein metabolism.[13,30] In normolipemic animals and humans (TC <200 mg/dL) fed cholesterol-free diets only 14:0-rich triglycerides seem to elevate TC.[31,32] In polygenic hypercholesterolemic individuals (i.e., >225 to 250 mg/dL) the LDL receptor activity is probably reduced,[22] and adding more dietary cholesterol (>400 mg/day) in the presence of an appropriate fat type apparently keeps this activity depressed, as discussed elsewhere.[31,32] Accordingly, 16:0 could add to the LDL pool in humans if the LDL receptors are depressed. Since 18:2 can independently lower an elevated TC value in humans[7] and can alleviate depressed LDL receptor activity,[16,31,32] this fatty acid would be expected to improve plasma LDL clearance. Whether saturates exert an opposite effect on LDL activity independent of the 18:2 threshold in humans is not clear. Our animal data indicate that the potential of 18:1 is limited in this situation. Like the Keys–Hegsted regressions, our data suggest that in reasonable (practical) diets 18:1 is not equal to 18:2 in its cholesterol-lowering ability during hypercholesterolemia, i.e., in individuals with nonstressed lipoprotein profiles 18:1 is neutral unless it replaces saturates (we would argue "14:0-rich triglycerides" rather than "saturates") in most people with TC <225 mg/dL.

Other saturated fatty acids

As for other saturated fatty acids, recent data from this laboratory have demonstrated that 12:0 and 16:0 are interchangeable and comparably neutral in both gerbils and monkeys fed cholesterol-free diets,[33] in keeping with recent reports in humans.[34,50] Stearic acid in natural fats continues to demonstrate a neutral effect in the normal intake range[35,36] and may even depress both LDL and HDL when consumed at atypically high intakes in the form of cocoa butter or shea butter.[26]

The 18:2 threshold

Another important finding from our animal studies[31,32] that awaits confirmation in appropriately designed human experiments is the observation that the plasma cholesterol response to dietary POLYs (i.e., 18:2 intake) is nonlinear (*Figure 1*). In fact, both in monkey[31] and, more strikingly, in gerbil experiments,[32] the ability of 18:2 to alter plasma cholesterol is most demonstrable between 1 and 5% energy from 18:2. Above that intake, the 18:2-induced decrease in total cholesterol is generally less pronounced. For want of a better term, we have referred to the breakpoint in the plasma cholesterol response curve as the "18:2 threshold." The exact threshold appears to vary between species, individuals, and probably between human populations for metabolic reasons that are not clear at this time. That means that certain individuals respond to 18:2 up to 3 to 4% energy, whereas others may continue to show a substantial decrease in plasma cholesterol up to 7 to 8% energy before the impact of dietary 18:2 abates. This presumably relates to differences in EFA status at the time of dietary intervention. The individuality and shifting nature of the threshold level in humans may obscure obviousness in human studies, especially since most diets supply 18:2 at intakes (3 to 10% energy) where the response is most linear.

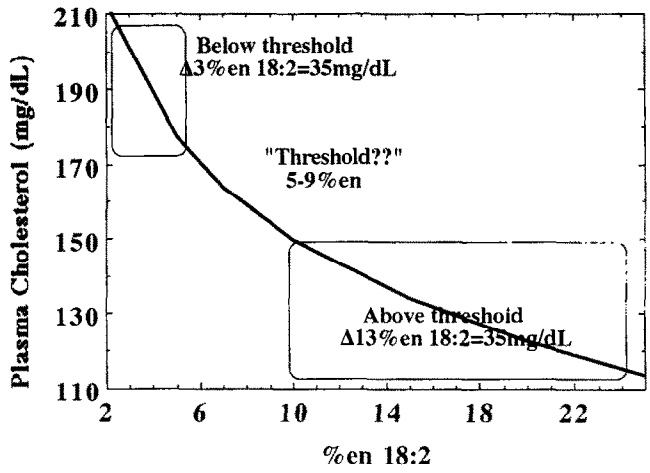

Figure 1 Nonlinear response of plasma cholesterol to dietary 18:2. This schema depicts the plasma TC response to dietary 18:2 in cebus monkeys. Note that the response is nonlinear, i.e., to induce a 35 mg/dL shift in TC requires only 3% energy change in 18:2 intake below "threshold" but a 13% energy shift in 18:2 above threshold. The absolute threshold value is relative because it seems to vary between individuals or populations and can be modified by various dietary factors, e.g., dietary cholesterol intake. Actual metabolic parameters that dictate threshold level are unknown, but LDL receptor activity may be involved, especially in humans.

Based on published human data (Western diets) the average threshold for 18:2 appears to be approximately 5% energy. However, because of the differences just stated, a meta-analysis threshold of 5% energy is not very meaningful when considering individual responses or putative differences in threshold between populations. Individual 18:2 thresholds may reflect, in part, the initial plasma cholesterol at the time of intervention because these parameters appear to be related, i.e., the higher the initial plasma cholesterol value, the more 18:2 seems to be required to achieve threshold. One possibility is that the initial total cholesterol value (at least in humans where most cholesterol circulates as LDL) represents a function of the LDL receptor activity. The increasing benefit from increments of 18:2 would reflect up-regulation of receptors leading to lower LDL-C. That may be too simple a model because the gerbil responds to changes in POLYs or SATs by altering HDL-C much more than LDL-C. Nonetheless, the curvilinear, logarithmic response to 18:2 and the 18:2 threshold concept greatly alters our perception of plasma cholesterol modulation by dietary fatty acids, especially since the 18:2 effect is distinct from the saturated fatty acid elevation of cholesterol, i.e., decreasing intake of 18:2 (below threshold) raises plasma cholesterol independent of the elevating impact attributed to certain saturated fatty acids.[32]

The public health implication of this observation is that we should maximize the benefit from 18:2 intake (i.e., attain individual and population 18:2 thresholds) if maximal

dietary lowering of plasma cholesterol is the objective. Furthermore, people with the highest cholesterol values stand to benefit the most from extra 18:2 because their "thresholds" are presumably higher and subject to greater correction by POLYs. The empirical data that initially revealed this point were ironically demonstrated almost 40 years ago by Bronte-Stewart[7] and then forgotten, only to reappear 35 years later using a similar design.[8]

The curvilinear response to 18:2 is conceptually important for at least two reasons. First, it is obvious that the plasma cholesterol concentration does not decline indefinitely (linearly) in response to 18:2, which indicates that a basal or minimal TC level exists for a given individual or population. Furthermore, the "bottom" or low-point for the cholesterol level that can be achieved by fat modification appears somewhat related to the initial cholesterol level at the time of intervention. Also, individuals with high cholesterol levels tend to respond more to 18:2 than those with low values, even though their ultimate "bottom" values may differ. Second, a curvilinear response or threshold helps explain the observation that 18:1,[9,37,38] or even 16:0,[11,23,25] can be equivalent to 18:2 once the threshold for 18:2 has been surpassed. When the maximum 18:2 benefit for cholesterol reduction has been attained, any surplus 18:2 can be replaced by dietary 18:1, or even 16:0, without compromising the metabolic requirement for 18:2 if certain other ill-defined, metabolic constraints are met. On the other hand, below the 18:2 threshold, neither 18:1 nor 16:0 is as effective as 18:2.[35,39]

Although both Keys[40] and Hegsted[41] developed predictive regression equations for serum cholesterol that defined a linear relationship for either saturated or unsaturated fatty acid consumption, they were unaware that over the full range of potential 18:2 intakes (1 to 30% energy) the resulting serum cholesterol response may be nonlinear. Brown[42] and Vergroesen[43] were among the first to make this observation, although the range of 18:2 intake from which they drew their conclusion was limited. The Keys and Hegsted data also suggested that monoenes were relatively neutral, neither raising nor lowering cholesterol. However, recent reports[2,9,19,37] tout the cholesterol-lowering superiority of monounsaturated fat (rich in 18:1) compared with polyunsaturates, in effect lowering LDL without lowering HDL. We have come to realize that total fat substitution with a monounsaturated oil (which seldom occurs in practical diets because other dietary fats are present) does two important things, i.e., it removes all the cholesterol-raising 14:0-rich triglycerides from the diet and supplies more than enough 18:2 (about 4% en) to maximize the putative LDL receptor efficiency (by satisfying the 18:2 threshold) in the absence of 14:0. As pointed out above, 18:1 is not as effective as 18:2 in countering either a 14:0 or dietary cholesterol–induced elevation in TC, where one can assume the 18:2 threshold (requirement for 18:2) is increased. In other words, below the critical 18:2 threshold, 18:1 does not appear to work as well as 18:2 to lower plasma cholesterol.[31,32,35,39]

Multiple regression equations

Using accumulated data from the feeding of as many 13 to 38 cholesterol-free fat blends to cebus, gerbils, and hamsters,[31,32] regression equations were generated to predict the impact of the various fatty acids on the total plasma cholesterol response. In order to make legitimate comparisons with humans, we carried out analyses similar to those used in the human data set.[41] The dietary content of 14:0 and 18:2 was able to explain almost 92% of the observed variation in plasma cholesterol in cebus monkeys, with 16:0 and 18:1 appearing to be neutral. Since the neutrality of 16:0 and 18:1 did not fit current dogma, we reexamined the Hegsted data,[41] the only published report where the entire dietary fatty acid profile (not just SATs vs. POLYs) was published along with the cholesterol response in a large number of dietary manipulations (36 diets) fed to the same individuals. Just as Hegsted originally reported for all 36 diets, the best multiple regression predicted that 14:0 was four times more cholesterolemic than 16:0, with 18:2 being the only fatty acid that lowered plasma cholesterol. However based on our hypothesis[13,23,31] that 16:0 would be neutral in situations where LDL receptor activity was not compromised (e.g., by dietary cholesterol), the Hegsted data were reanalyzed for individuals during low ($\leq$300 mg) or high (>400 mg) cholesterol intakes. During the consumption of the 17 human diets in which cholesterol intake was $\leq$300 mg, 85% of the observed variation in serum cholesterol could be explained solely on the basis of 14:0 and 18:2 intake. However, in the 19 human diets where more than 400 mg of cholesterol was fed, 16:0 appeared slightly cholesterolemic. Regression analysis was also applied to data from hamsters (13 diets) and gerbils (38 diets). Again 75% (hamsters) to 89% (gerbils) of the observed variation in plasma cholesterol could be explained by the dietary 14:0 and 18:2 content[32] with cholesterol-free diets. Also the regression coefficients for the simplest regression equation derived for gerbils, cebus, and humans appeared remarkably similar, with hamsters being somewhat less sensitive than the other three species:

$$\text{Cebus TC} = 187 + 10\ (\pm 1.6)\ E_{14:0} - 42\ (\pm 11)\ \log E_{18:2}, \quad r^2 = 0.91 \text{ (based on 18 diets)}$$
$$\text{Gerbil TC} = 126 + 8\ (\pm 0.8)\ E_{14:0} - 40\ (\pm 4)\ \log E_{18:2}, \quad r^2 = 0.89 \text{ (based on 38 diets)}$$
$$\text{Human TC} = 229 + 8\ (\pm 1.6)\ E_{14:0} - 36\ (\pm 8)\ \log E_{18:2}, \quad r^2 = 0.85 \text{ (based on 19 diets)}$$
$$\text{Hamster TC} = 161 + 5\ (\pm 1.6)\ E_{14:0} - 25\ (\pm 11)\ \log E_{18:2}, \quad r^2 = 0.75 \text{ (based on 13 diets)}$$

In the above equations TC denotes the total plasma cholesterol (mg/dL), $E_{14:0}$ and $E_{18:2}$ denote the % energy from 14:0 and 18:2, respectively, and the constant term represents the average plasma cholesterol concentration of the host population for all dietary fats tested. The cebus equation is an update of an earlier equation[31] using two addi-

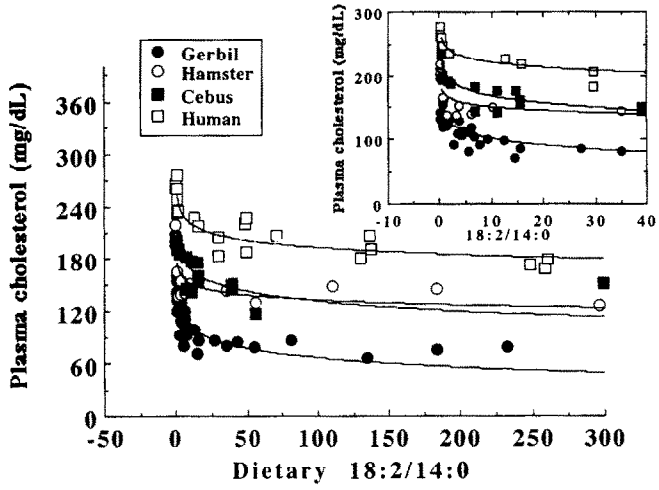

Figure 2 Relationship between plasma cholesterol and the dietary 18:2/14:0 ratio. This figure depicts the importance of the ratio of % energy consumed as 18:2 and 14:0 in natural triglycerides in predicting the plasma cholesterol response in different species. The nonlinear curves in all four cases demonstrate the cross-species applicability of the 18:2 threshold concept. The curves do not superimpose because the initial plasma cholesterol "setpoint" (i.e., the intercept term in the equations described in the text) differs between species. The insert amplifys the cholesterol response when the 18:2/14:0 ratio is ≤40.

tional diets.[33] The gerbil equations are based on diets described elsewhere.[32] The 19 human diets represent a subset of the data of Hegsted et al.[41] as discussed previously[31] and the hamster data represent unpublished observations. The importance of the dietary 14:0 and 18:2 content in predicting the plasma cholesterol response in all four species is illustrated in *Figure 2*.

In the presence of dietary cholesterol, dietary 16:0 emerged as contributing to the cholesterolemia in gerbils,[32] as was the case in the human subjects consuming >400 mg cholesterol (see above). A likely explanation for the 16:0 cholesterolemia has been discussed above and elsewhere.[31] However, as previously demonstrated in cebus monkeys[29] the biggest impact was attributed to dietary cholesterol per se, with the contribution from 16:0 being secondary. Similarly, in humans the major factor affecting the potential impact of dietary 16:0 may be the inherent hepatic and lipoprotein cholesterol metabolism at the time of intervention, which reflects a myriad of factors, both dietary and metabolic, including such examples as dietary cholesterol, total fat intake, insulin status, and body mass index.

Cross-species modeling

The commonly applied Keys–Hegsted regression equations, used to predict the human TC response to fat, compare SATs versus POLYs for the sake of simplicity. Thus, they fail to acknowledge the impact of individual fatty acids on TC and even more importantly on the LDL/HDL ratio. On the other hand, it seems equally misleading to utilize an animal model to focus on a single lipoprotein response, such as LDL, while ignoring the dynamics of HDL or VLDL, especially if the latter two lipoproteins are major carriers of cholesterol in that species (unlike humans). In such cases, the TC response would appear to be a more appropriate end point for preliminary cross-species comparisons because the response in TC to fatty acid changes is relatively uniform among fat-sensitive species, even though LDL or HDL fluctuations are often dissimilar and the host metabolic status may differ, as in weanling gerbils versus obese old men. The currently used hamster model[30] for fatty acid perturbation of LDL during cholesterol-induced cholesterolemia (12:0-14:0-16:0 all equally cholesterolemic) seems limited by the fact that it does not apply to the normocholesterolemic hamster. For example, when LDL receptors are not partially down-regulated by dietary cholesterol, only 14:0-rich triglycerides induce appreciable cholesterolemia.[32] The model may not apply to normocholesterolemic people either, since 16:0 does not appear to be as cholesterolemic as 14:0 + 12:0 (nor consistently even different from 18:1) in such individuals,[12,25–27] in contrast to results obtained in hamsters when LDL receptors are down-regulated and most fats (fatty acids) appear similar.[20,30,32,36]

Because 16:0 can actually be equivalent to 18:1, and even 18:2, when modulating the TC and LDL response in monkeys[11,23] and humans when TC is <200 mg/dL,[25,27,44] another issue to be reconciled with the current hamster model before extrapolating it to humans is the level of dietary cholesterol in humans required to mimic the SAT fat effect observed in cholesterol-fed hamsters, i.e., At what cholesterol intake do 12:0, 14:0, 16:0 appear equivalent in humans? Reportedly[20,30] 0.12% dietary cholesterol is needed to down-regulate LDL receptors by 50% in a chow-fed hamster, but that represents at least 1,000 mg/day in humans (or 2 to 3× the level in Western Diets) based on relative caloric concentration and intake (2,200 kcal/day) or 4,500 mg/day for a 70 kg human (based on relative weights). This an extraordinary load, especially since the relative importance (absorption and metabolism) of dietary cholesterol in humans is reportedly less than that in hamsters. Thus, humans might require up to 2,000 to 5,000 mg/day to approximate the experimental conditions in the hamster model. Accordingly, it seems more appropriate to argue that the majority of the world's human population (those <225 mg/dL) respond to fatty acids more like normocholesterolemic cebus, gerbils, (and hamsters) than hamsters supplemented with 0.12% cholesterol. At some point of hypercholesterolemia (>240 mg/dL?) a person consuming a Western-type diet may fit the cholesterol-fed hamster model. Under normal metabolic circumstances (in gerbils, cebus, humans, and even hamsters) only 14:0-containing TGs appear cholesterolemic, with 16:0-rich fats contributing to an increase only when lipoprotein metabolism appears compromised, e.g., in hypercholesterolemic individuals with TC >225 mg/dL[31] or gerbils fed 0.08% cholesterol.[32]

Finally, another modeling constraint that awaits further documentation is the use of structured TGs to draw conclusions about specific fatty acids. It has been argued that TG structure, i.e., fatty acid positional isomers or relationships of specific pairs of fatty acids on a TG molecule, has an important bearing on lipid metabolism.[28,45–47] For example, when McGandy and Hegsted[48] increased 12:0 or 14:0

intake by interesterifying trilaurin or timyristin with other natural fats, the resulting cholesterolemia attributed to 14:0 was only one half that of 14:0 consumed as natural butterfat or coconut oil and was only slightly more than that for 16:0. A similar result was reported by Zock et al.[49] in humans fed a 14:0-enriched TG modified by interesterification, i.e., 14:0 was only 1.5 times as cholesterolemic as 16:0, as opposed to the four fold difference observed by Hegsted in humans[41] and the marked difference between these two fatty acids identified in cebus and gerbil models fed natural fats without cholesterol.[31,32] Preliminary work in our laboratory has also found that 14:0 per se can be much less cholesterolemic than predicted if fed in forms other than in TG molecules from natural fats (unpublished data). The point is that attempting to illustrate the relative potency of a specific fatty acid in a form other than natural TGs is fraut with problems of relevance to natural, edible fats. Feeding high levels of structured TG molecules probably is not a meaningful test of a specific fatty acid's true biological impact on cholesterol metabolism, or any other aspect of metabolism for that matter.

In summary, the impact of dietary fat on cholesterolemia appears to be a function of the specific fatty acids present in the triglyceride molecule. Furthermore, the placement of the fatty acid in question on the glycerol molecule as well as the accompanying fatty acids appear to influence the impact of the fat and its fatty acids on lipid metabolism.

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