韓國營養學會誌 24(4) : 378~392, 1991
Korean J Nutrition 24(4) : 378~392, 1991

Dietary Fatty Acids and Blood Cholesterol

K.C. Hayes*, Pramod Khosla, Andrzej Pronczuk & Saralyn Lindsey

Foster Biomedical Research Laboratory, Brandeis University, Waltham, M A

ABSTRACT

A series of studies in monkeys and hamsters, and reevaluation of published human data, indicate that dietary saturated fatty acids exert a dissimilar metabolic impact on cholesterol metabolism. Myristic acid(14 : 0) appears to have a major cholesterol-raising effect by means of decreasing LDL receptor activity and by increasing the direct production of LDL (from sources other than VLDL-catabolism). Palmitic acid (16 : 0) appears neutral in most cases (plasma cholesterol<200㎎/dl) or until the LDL receptor is down-regulated, as with high cholesterol intake or obesity. In such cases, the down-regulated LDL receptors coupled with an increased VLDL production (induced by 16 : 0 and 18 : 1) can divert VLDL remnants to LDL and expand the LDL pool. Furthermore, the cholesterolemic impact of any saturated fatty acid can be countered up to a saturable "threshold" level by dietary linoleic acid (18 : 2) which up-regulates the LDL receptor. Once above this "threshold", the major fatty acids (16 : 0, 18 : 0, 18 : 1, 18 : 2, 18 : 3) appear to exert an equal impact on the circulating cholesterol concentration.

Introduction

For almost 40 years we have realized that dietary fat alters the plasma cholesterol concentration, but the degree to which various fats or their composite fatty acids modulate these effects and the mechanism (s) involved are not well understood[1]. The situation has been complicated recently because the original findings of Keys[2] and Hegsted[3], especially regarding the effects of specific fatty acids, have been questioned[4][5]. Whereas the dietary P/S ratio and intake of total saturated fats were thought to constitute the main impact, attention is now focused increasingly on the contribution from monounsaturated fats[1][4][6].

The point of this discussion is not to dismiss dietary saturated fat as a key variable in the cholesterol response, but rather to point out often overlooked subtleties in the metabolism of the specific fatty acids incorporated in the fats we eat. This oversight has come to light now that research has focused on specific lipoproteins and their metabolism, as opposed to assessment of the total plasma cholesterol and triglycerides in previous years.

1. Fat and Energy As Stressors

The point to be emphasized is that fatty acids are metabolized in a highly dynamic pattern of metabolic interrelationships such that the impact on plasma cholesterol of any given fatty acid, e.g.

Table 1. Fatty acid composition of purified monkey and hamster diets

	Dietary[b] fatty acids (% of total)									
Diet[a]	12 : 0	14 : 0	16 : 0	18 : 0	20 : 0	16 : 1	18 : 1	18 : 2	18 : 3	Others[c]
1	–	0.2	11.9	2.2	–	0.2	25.1	59.9	0.6	–
2	47.5	22.2	12.9	4.1	–	–	10.8	2.5	–	–
3	–	1.1	23.1	13.5	–	2.8	46.7	11.9	0.9	–
4	7.2	9.9	32.9	15.1	–	2.2	28.9	3.0	0.8	–
5	–	4.7	15.1	3.7	–	6.7	19.2	22.6	0.3	27.7
6	15.8	12.0	15.4	4.4	–	6.6	14.5	3.4	0.2	27.7
7	–	2.2	24.6	18.8	–	3.7	46.4	4.3	–	–
8/13/19	47.8	18.8	10.7	3.3	–	–	9.4	8.5	0.9	–
9/20	23.8	9.6	8.6	3.0	0.2	0.2	37.0	16.0	1.2	–
10/21	13.4	5.8	25.1	3.6	0.2	–	37.2	13.3	0.8	–
11/14/22	0.2	1.0	40.3	4.1	0.3	–	37.0	15.4	1.0	–
12/15/23	0.4	0.7	23.4	3.9	0.3	–	41.1	27.2	2.7	–
16	1.5	1.3	6.3	2.5	–	0.1	13.7	72.8	0.2	–
17	2.3	1.4	40.7	4.8	–	–	39.1	9.8	0.4	–
18	1.6	1.3	5.2	0.3	–	0.1	74.1	14.4	0.4	–
24	2.3	7.5	21.9	6.5	0.4	0.9	35.8	20.4	4.3	–

	Total SFA[d]	Total MUFA[e]	Total PUFA[f]	P/S[g]
1	14.3	25.3	60.4	4.22
2	86.7	10.8	2.5	0.03
3	37.7	49.5	12.8	0.34
4	65.1	31.1	3.8	0.06
5	23.5	25.9	50.6	2.15
6	47.6	21.1	31.3	0.66
7	45.6	50.1	4.3	0.09
8/13/19	80.6	9.4	9.4	0.12
9/20	45.2	37.2	17.2	0.38
10/21	48.1	37.2	14.1	0.29
11/14/22	45.9	37.0	16.4	0.36
12/15/23	28.7	41.1	29.9	1.04
16	11.6	13.8	73	6.29
17	49.2	39.1	10.2	0.21
18	8.4	74.2	14.8	1.76
24	38.6	36.7	24.7	0.64

[a]Dietary Fats : 1, Corn Oil; 2, Coconut Oil; 3, Lard; 4, Butter; 5, 2/3 Fish Oil, 1/3 Corn Oil; 6, 2/3 Fish Oil, 1/3 Coconut Oil; 7,Tallow; 8, 13 & 19, 90% Coconut Oil/10% Soybean Oil; 9 & 20, 45% Coconut Oil/40% High-Oleic Safflower Oil/15% Soybean Oil; 10 & 21, 45% Palm Oil/22% Coconut Oil/20% High-Oleic Safflower Oil/13% Soybean Oil; 11, 14 & 22, 90% Palm Oil/10% Soybean Oil; 12, 15 & 23, 45% Palm Oil/40% Soybean Oil/15% High-Oleic Safflower Oil; 16, High-Oleic Safflower Oil; 17, Palm Oil; 18, High-Linoleic Safflower Oil; 24, 52% Butter/32% Canola Oil/16% Corn Oil.

[b]Diets 1-18 fed to monkeys, diets 19-24 fed to hamsters. The composition of the purified diets have been described elsewhere[5)11)12)16)]. Monkey diets were fed with fat contributing 31% energy except for #s 13-18, in which 40% energy was derived from fat. Hamster diets were fed with fat contributing 13% energy. Diets 8-24 were cholesterol free. Diets 1-7 had cholesterol added to them in order to equalize for the cholesterol present in Fish Oil(MAXEPA ®), which was used to formulate diets 5 & 6. For all formulated diets, the fatty acid composition was determined by GLC.

[c]20 : 4n-6, 20 : 5n-3, 22 : 5n-3 and 22 : 6n-3 fatty acids.

[d]Total saturated fatty acids (12 : 0, 14 : 0, 16 : 0, 18 : 0, 20 : 0).

[e]Total monounsaturated fatty acids (16 : 1, 18 : 1).

[f]Total polyunsaturated fatty acids (18 : 2, 18 : 3, 20 : 4n-6, 20 : 5n-3, 22 : 5n-3 and 22 : 6n-3).

[g] Ratio of PUFA/SFA.

myristic acid (14 : 0), can be ameliorated or exacerbated by the mix of accompanying nutrients (especially other fatty acids and cholesterol) that are consumed and metabolized along with it[5)20)]. This translates into the notion that "high-stress metabolism", such as trying to cope with the dietary burden of excess calories accompanied by an excessive cholesterol intake(>500 mg per day) and a low-fiber, highly-refined carbohydrate diet (which increases hepatic VLDL triglyceride secretion) exerts a negative impact on hepatic lipoprotein secretion and turnover. This imbalance enhances the chances of an LDL build-up and HDL depletion. On the other hand, the severe restriction of calories, inclusion of dietary complex carbohydrate and fiber, increased polyunsaturated fatty acids (PUFA) and a reduced level of cholesterol, can greatly minimize the 14 : 0 effect.

The apparent reason for this disparate effect is simple enough, i.e. lipoprotein metabolism is greatly altered(opposite almost) under the two situations outlined above. Unfortunately the mechanisms involved are more complicated than simply documenting the observed response. In the first instance, energy and cholesterol are being pumped into the lipoprotein transport sytem in excess, straining the normal metabolic ability to keep pace, in part, because all the necessary ing-

Table 2. Effect of short-term feeding of dietary fats on plasma lipids in monkeys[a]

	Dietary Fat Blend				
	1,2[b]	3	4	5,6[c]	7
	Monkeys raised on corn oil				
Plasma Cholesterol[d]	174 ± 9^{cl}	181 ± 12^{f}	193 ± 11^{g}	138 ± 10^{cfgh}	189 ± 14^{h}
LDL-Cholesterol[d]	86 ± 7^{cl}	93 ± 11^{f}	103 ± 7^{cg}	71 ± 9^{fgh}	94 ± 10^{h}
HDL-Cholesterol	77 ± 5^{c}	75 ± 5^{f}	74 ± 7^{g}	59 ± 4^{cfgh}	76 ± 6^{h}
LDL-C/HDL-C[d]	1.15 ± 0.11^{cl}	1.28 ± 0.15	1.50 ± 0.12^{c}	1.27 ± 0.15	1.27 ± 0.11
	Monkeys raised on coconut oil				
Plasma Cholesterol[d]	256 ± 20^{cfgh}	199 ± 20^{ci}	199 ± 18^{fi}	157 ± 16^{giik}	204 ± 16^{hk}
LDL-Cholesterol[d]	153 ± 17^{cfgh}	104 ± 15^{c}	111 ± 13^{fi}	90 ± 12^{gi}	106 ± 11^{c}
HDL-Cholesterol	86 ± 7^{cf}	78 ± 8^{g}	71 ± 6^{ch}	56 ± 3^{fghi}	82 ± 7^{i}
LDL-C/HDL-C[d]	1.88 ± 0.24^{cf}	1.33 ± 0.18^{c}	1.59 ± 0.17	1.57 ± 0.16	1.33 ± 0.13^{f}

24 monkeys (8 cebus, 8 rhesus and 8 squirrel) had been raised from birth for 8-12 years on cholesterol-free purified diets containing either corn oil (12 moekeys, 4 per species) or coconut oil. They were then fed either their basal diet or the test diet for 8 week periods.

[a]Adapted from (11). Values are mean± SEM;n=12.

[b]For the short term stusy (8 weeks), both the basal(corn oil or coconut oil) and test diets were supplemented with the indicated amounts of cholesterol (㎎/kcal) : corn oil or coconut oil, 0.11, butter 0.10, tallow, 0.06, lard 0.05.

[c]Monkeys raised on corn oil were fed 2/3 Fish Oil, 1/3 Corn Oil, (diet 5) whilst those raised on coconut oil were fed 2/3 Fish Oil, 1/3 Coconut Oil (diet 6). Both diets contained 0.11㎎/kcal cholesterol.

[d]Two-factor repeated-measures analysis of variance revealed significant interaction between original diet and short-term response to fats.

[cfghijk]Means in rows sharing a common superscript significantly different by repeated-measures ANOVA and Fishers' protected LSD paired analysis($p<0.05$).

[l]Indicates significant difference between basal coconut oil diets by Student t-test($t<0.05$).

redients(e.g. PUFA or fiber) are not present in sufficient amounts to facilitate removal. By contrast, in the second scenario, not only are the components of the diet in better balance (PUFA, fiber, low fat, etc.), but more importantly a low energy and low cholesterol intake are, in effect, causing the body to reverse energy flow with a net output of these components from body reserves. In the latter case the balance between metabolic hormones and body metabolic processes are at their highest efficiency and better prepared to cope with the energy flux.

The point is that the problem of hypercholesterolemia and atherogenesis only occurs (typically for the average person) in response to sustained periods(years) of energy excess and adipose tissue expansion[7)8)]. This, in turn, is affiliated with decreased low-density lipoprotein receptor(LDLr) activity and polygenic hypercholesterolemia[9)]. An increased body mass index is also associated with decreased HDL-cholesterol[7)8)]. During periods of reduced caloric intake and decreased cholesterol production, evidence now exists that cholesterol and fat are actually removed from the arteries[10)].

Table 3. Plasma lipids of 3 species of monkeys fed diets with 5 different fat blends[a]

		Dietary Fat Blend[b]				
Species	N	8	9	10	11	12
		Total cholesterol(㎎/dL)				
Rhesus	8	212± 15^{cd}	197± 10	201± 11^{cf}	184± 11^{cf}	183± 10^{dc}
Cebus	8	246± 17^{cdef}	191± 8^{dgi}	186± 13^{chj}	161± 11^{cgh}	151± 9^{fij}
Squirrel	5	245± 20^{c}	239± 38	233± 33	216± 22	193± 25^{c}
Combined sp.	21	232± 10^{cdef}	205± 11^{chj}	203± 10^{dgi}	183± 9^{cgh}	173± 9^{fij}
		LDL-cholesterol(㎎/dL)				
Rhesus	8	113± 13^{cdc}	88± 9^{d}	88± 5	82± 8^{c}	81± 6^{c}
Cebus	8	111± 16^{cdef}	82± 10^{cg}	70± 10	62± 5^{cd}	55± 5^{fg}
Squirrel	5	113± 11^{c}	116± 27^{d}	112± 21	100± 19	69± 18^{cd}
Combined sp.	21	112± 8^{cdef}	92± 8^{cgi}	87± 7^{dh}	79± 6^{cg}	68± 5^{fhi}
		HDL-cholesterol(㎎/dL)				
Rhesus	8	84± 5	92± 6	81± 4	86± 5	88± 5
Cebus	8	121± 10^{cdc}	101± 6^{df}	103± 16	79± 13^{c}	85± 9^{cf}
Squirrel	5	101± 11	106± 12	106± 13	97± 8	98± 9
Combined sp.	21	102± 6^{cd}	99± 4^{cf}	96± 6	96± 6^{cc}	89± 4^{df}
		LDL-C/HDL-C				
Rhesus	8	1.38± 0.20	1.00± 0.12	1.10± 0.08	0.98± 0.12	0.92± 0.06
Cebus	8	1.15± 0.22	0.85± 0.14	0.80± 0.18	0.69± 0.05^{c}	0.68± 0.07
Squirrel	5	1.13± 0.04	1.05± 0.14	1.07± 0.12	1.04± 0.14	0.67± 0.11
Combined sp.	21	1.23± 0.11^{cdc}	0.95± 0.08^{df}	0.98± 0.09^{g}	0.89± 0.07^{c}	0.77± 0.05^{cfg}

[a]Adapted from (5). Values are mean± SEM.

[b]See legend to Table 1 for description of fat blend and the fatty acid composition

^{cdefghij}Means sharing a common superscript in a given row are significantly different($P<0.05$).

2. Dietary Fat and Lipoproteins

How does the above atherogenic scenario translate into plasma lipoprotein profiles? To address this problem, we have conducted a series of experiments over the last few years utilizing purified diets of defined fatty acid composition(Table 1).

As just mentioned obesity is associated with expanded VLDL and LDL pools and decreased HDL[7)8)9)]. Specific aspects of the dietary fatty acid relationship impact lipoproteins and are modulated by an influential genetic component governing lipoprotein metabolism. These interactions are demonstrated in our recent comparative study of several fats in three species of monkeys [11)]. In that experiment only coconut oil (diet 2) and butter (diet 4) were uniformly hypercholesterolemic in all three species, whereas relatively saturated beef tallow(diet 7) and lard(diet 3) were scarcely different from corn oil(diet 1) in their ability to raise cholesterol(Tables 1 & 2). On the other hand, replacing 2/3 of the corn oil or coconut oil with fish oil(diets 5 & 6, respectively) induced an equally marked decline in cholesterol levels, even though the fatty acid composition of the fish oil blends contained more saturates and less polyenes than corn oil(Tables 1 & 2). The inference was that neither all saturates(12 : 0, 14 : 0, 16 : 0) nor all polyenes(n-6, n-3) were equally effective in modulating the plasma cholesterol response. Furthermore, the strong genetic influence was apparent in the relative non-responsiveness of the plasma cholesterol in rhesus monkeys(30% shift between corn oil and coconut oil) by comparsion to the sensitivity of cebus monkeys(85% shift between these dietary fats) (Table 2).

To examine the implication of these results we undertook two sets of studies, one in hamsters, the other continuing with monkeys. The monkey study[5)] was designed to explore two aspects of the problem. The first examined the general response in plasma cholesterol during a progressive shift in the dietary P/S ratio, and the second assessed specific comparisons between individual saturated fatty acids when the P/S ratio was held constant, specifically comparing the effect of 12 : 0+14 : 0 vs. 16 : 0. Again it was apparent that monkey species differed in the magnitude of their response, but more importantly 16 : 0(diets 10 and 11) were less cholesterolemic than 12 : 0+14 : 0(diets 8 and 9)in any of the three species of monkeys(Table 3).

In fact, no difference was noted in the exchange of 5% energy as 16 : 0 for an equal amount of 18 : 2(between 5% and 10% kcal as 18 : 2, diet 11 vs. 12, Table 1) in rhesus monkeys, and only a modest effect of this exchange was evident in the more responsive cebus monkey (Table 3). On the other hand, when dietary 18 : 2 represented only 2.7% of the total energy (Diet 8, Table 1) the impact of 12 : 0+14 : 0 was clearly evident in rhesus plasma cholesterol and had a major impact on cebus(Table 3). These dietary fatty acid exchanges and the different responses emphasize two aspects of the issue, i.e. 1) the highly diverse genetic contribution was evident between the diverse sensitivity of rhesus and cebus monkeys, and 2) the potential to elicit different responses depending upon the relative amount or "threshold" level of key fatty acids, especially 18 : 2 and 14 : 0(expressed as% dietary energy).

3. Mechanism of Fatty Acid Effect : Lipoprotein Kinetics

To explore the mechanism underlying the metabolic differences induced by specific fatty acids, we refed two of the diets(Diets 8 and 11, Table 1) to rhesus monkeys in a second study[12)], in

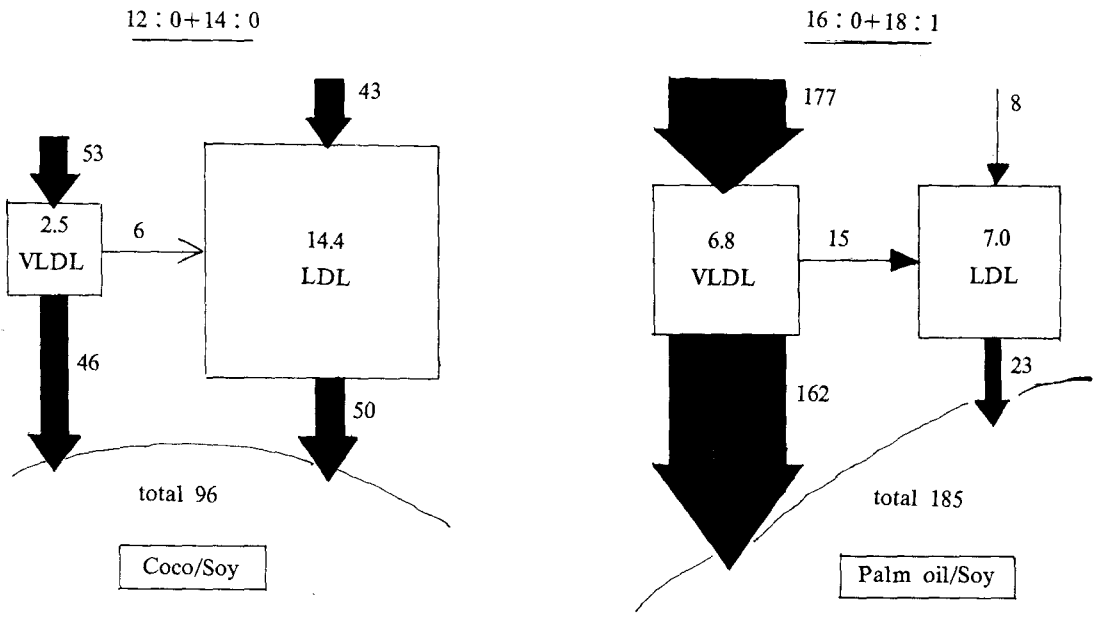

Fig. 1. Apo Bkinetics were determined in rhesus monkeys fed a coconut oil-soybean oil(12 : 0+14 : 0) diet or the same diet with plam oil-soybean oil(16 : 0+18 : 2). Vector arrows depict relative rates (㎎/㎏/hr)of secretion and clearance by clearance by the liver into and from the VLDL and LDL apoB pools(see text and reference 12 for detailed description).

order to compare the effects of 12 : 0+14 : 0 vs. 16 : 0+18 : 1. Apo B kinetics were evaluated following the simultaneous injection of ^{125}I-VLDL and ^{131}I-LDL. Since the rhesus plasma cholesterol was only moderately responsive to changes in fat saturation, we were surprised to find a major difference in the metabolism of apo B lipoproteins under these dietary circumstances. Specifically, 16 : 0+18 : 1 induced a 3-fold higher VLDL apo B transport rate, whereas 12 : 0+14 : 0 induced an increase(5-fold greater than 16 : 0+18 : 1) in the transport rate of LDL apo B derived from VLDL-independent sources (i.e. increased 'direct' production of LDL apo B) resulting in a 2-fold increase in the circulating LDL pool and slight decrease in HDL relative to 16 : 0+18 : 1. These changes, in turn, had a significant, negative impact on the LDL/HDL ratio. This detrimental shift towards LDL expansion, typical of 14 : 0-rich diets(see Tables 2 and 3), took place even though 16 : 0+18 : 1 caused a 2-fold greater flux of apo B through the lipoprotein pool. These relationships are summarized in Fig. 1.

As Fig. 1 suggests, 16 : 0+18 : 1 enhanced triglyceride synthesis and VLDL production, whereas 12 : 0+14 : 0 reduced VLDL output but increased 'direct LDL' production. In the process of VLDL catabolism HDL is generated [13], and VLDL remnants return to the liver via the LDL receptor[14] (Fig. 2). Thus clearance of the increased VLDL produced by 16 : 0+18 : 1 depends on adequate LDLr activity[1][14]. The direct production of LDL generally has been considered to be a minor component of hepatic lipoprotein sec-

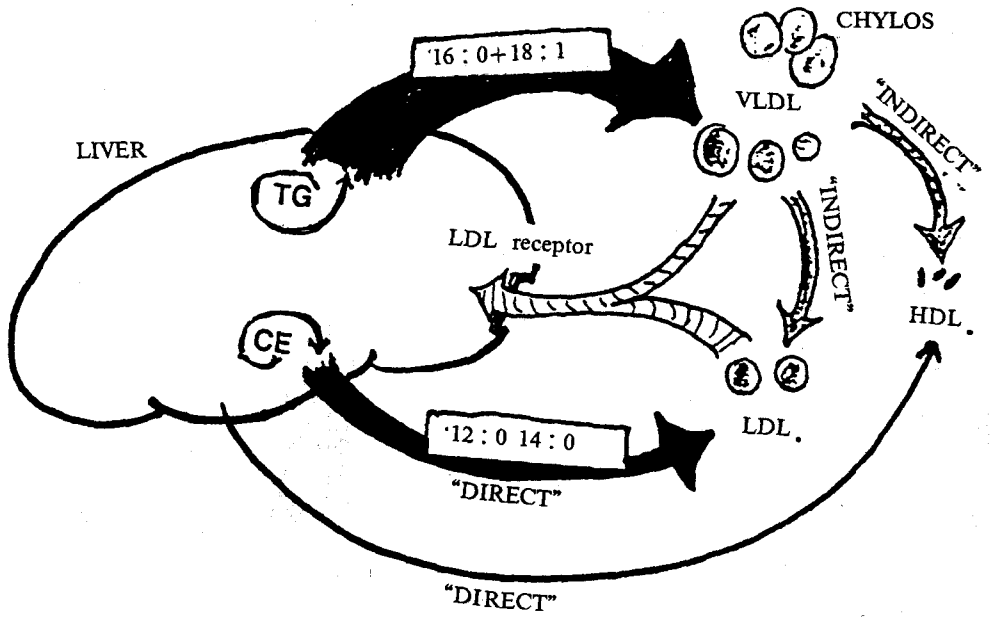

Fig. 2. Using the diet comparison described by Figure 1, the relative impact of dietary 12 : 0+14 : 0 vs. an equal caloric exchange with 16 : 0+18 : 1 on lipoprotein metabolism is depicted. While 16 : 0+18 : 1 enhance VLDL production, 12 : 0+14 : 0 favor the "diect" production of LDLand down-regulation of the LDL receptor. Under normal circumstances increased VLDL production and catabolism would increase the indirect generation of HDL with rapid clearance of VLDL remnants via the LDL receptor. Any circumstance that down-regulates the LDL receptor would potentially slow VLDL remnant clearance, resulting in increased LDL and decreased indirect generation of HDL.

retion on other occasions[1)14)], but these studies provide the first evidence that direct LDL production may be a major factor in the expansion of the LDL pool by specific saturated fatty acids, notably 12 : 0+14 : 0. As discussed elsewhere[5)12)], it is likely that 14 : 0 was responsible for most of the effect.

4. Hepatic mRNA Abundance

To further investigate this metabolic scenario at the molecular level, similar dietary fat blends (although representing only 13% energy from fat) were fed to Syrian hamsters, and certain hepatic mRNAs associated with cholesterol metabolism were measured. In the first study[15)] we found that 18 : 1 greatly increased apo Al (mRNA) and LDLr mRNA abundance compared to oils containing 20 : 5n3, and to a lesser extent, 18 : 2n6 (Fig. 3). A subsequent study[16)] also revealed that the highest apo Al(HDL) and LDLr mRNA abundance was associated with the 16 : 0+18 : 1-rich diets compared to several others, including 12 : 0 +14 : 0 and an American Fat Blend rich in 14 : 0 (Table 4).

Collectively, these observations in monkeys and hamsters point to a major metabolic disparity resulting from consumption of 14 : 0 vs. 16 : 0-rich fats. Not only is the cholesterolemia greater with

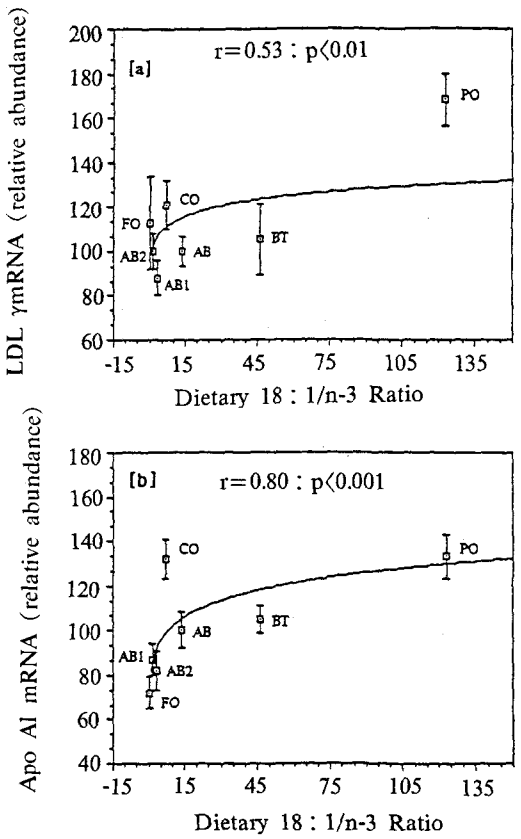

Fig. 3. Regression analysis(log) of the hepatic mRNA abundance for the LDL receptor and apoAI against independent dietary fatty acid variables, revealed the depressing effect of n3 fatty acids and stimulating effect of 18 : 1 on both LDL on both LDL receptor(top) and apoAI (below). The dietary fats represented are AB1 (5% American Fat Blend : fish oil, 4 : 1), AB2(5% American Fat Blend : fish oil 3 : 2), BT(5% beef tallow); PO(5% polm oil), CO(5 % canola oil). Fish oil was MAXEPA®.

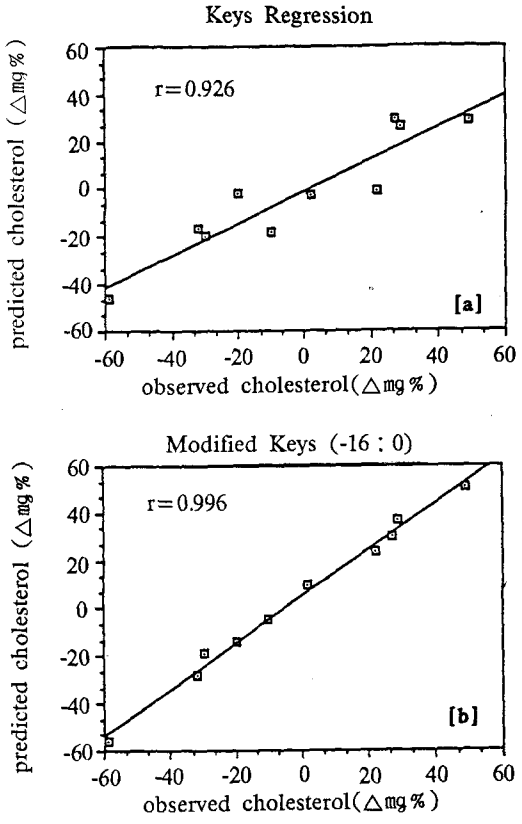

Fig. 4. Correlations between the predicted vs. the observed plasma cholesterol values are depicted. Predicted values were generated using the regular Keys regression equation (a) or a modified version (b) in which palmitic acid (16 : 0) was considered neutral. The data points (taken from Table 3) represent the mean changes for three species of monkeys with comparisons between all possible combinations for the 5 dietary fat blends examined.

14 : 0, but the distribution of cholesterol among lipoproteins appeared to differ such that dietary 14 : 0 tended to increase LDL (in both monkeys and hamsters) more than HDL, i.e., the opposite of the 16 : 0+18 : 1 effect. It is not entirely clear at this point whether 16 : 0 or 18 : 1 are equal or whether one is selectively more important than the other in the HDL response. Although the second monkey study[5](where 12 : 0+14 : 0 and 16 : 0 were exchanged) implied that 16 : 0 alone might induce these positive changes(Table 3) the mRNA data in hamsters suggested that 18 : 1 provided the greatest mRNA abundance for the LDLr and apo AI[15][16] (Fig. 3, Table 4), the latter reflecting HDL synthesis. Work is in progress to separate these potential differences in animal models and humans.

Table 4. Relative abundance of mRNA in namsters fed different fat blends[a]

Criterion	Dietary Fat Blend[b] 19	20	21	22	23	24
Apo Al mRNA(% of control)						
Liver	91± 7^{c,h}	112± 5^{d,g}	115± 6^{c,e}	118± 4^{f}	115± 6^{b}	100± 4^{c,d}
Gut	90± 10^{c,e}	119± 12^{c}	119± 11^{d}	113± 12	131± 10^{e}	100± 11
Apo E mRNA(% of control)						
Liver	104± 5	111± 5	121± 7^{c}	123± 7^{d,e}	110± 7^{c}	100± 8^{c,d}
Gut	108± 3	108± 3	102± 5	105± 6	113± 7	100± 12
Apo B mRNA(% of control)						
Liver	95± 8^{d}	125± 10^{c,d}	109± 6	112± 6	112± 6	100± 9^{c}
Gut	118± 5	125± 14	130± 8	125± 8	120± 12	100± 12
LDL_R mRNA(% of control)						
Liver	137± 16^{c,h,i}	141± 23^{d,j}	158± 20^{e,h,j,k}	154± 19^{f,i}	142± 14^{g,k}	100± 11^{c-g}
Gut	103± 15	127± 14	119± 7	117± 11	128± 17	100± 16

[a]Adapted from (16). Values are mean± SD (n=10 per diet)

[b]See legend to Table 1 for description of fat blend and the fatty acid composition

[cdefghijk]Mean values sharing a common superscript in a given row are significantly different by a one-factor ANOVA($P<0.05$).

It is noteworthy that one of the most complete descriptions of the plasma cholesterol response to individual dietary fatty acids covering a wide range in fat saturation in humans[3] (2 years, 36 diets) also distinguished between 14 : 0 and 16 : 0, identifying 14 : 0 as four times more cholesterolemic than 16 : 0. But a subsequent study by these same investigators using semisynthetic fats[17], led them to conclude that their original observations were incomplete and that 12 : 0, 14 : 0, and 16 : 0 were equally cholesterolemic.

Having obtained these monkey data, which agreed in principle with the Hegsted and Keys regression equations in humans(Fig. 4), especially if 16 : 0 was considered neutral, we were puzzled by the inference from current reports[4)6)18)] that 18 : 1 was as effective as 18 : 2 in human diets in terms of its cholesterol-lowering ability. At the same time 18 : 1 did not exert the HDL-depressing effect often seen with high levels of 18 : 2. An HDL-enhancing effect would be consistent with our data, i.e. 18 : 1 drives apo Al mRNA abundance whereas increasing polyenes (18 : 2, 20 : 5n3) decrease apoAl (Fig. 3). Several reports demonstrate that the circulating HDL shows a modest but persistent decline between 3% and 30% dietary kcals as 18 : 2, with a significant decline detectable when 18 : 2 reaches approximately 20% or more dietary energy[19]. However, the Keys and Hegsted regressions and our data in monkeys and hamsters would suggest that 18 : 1 is rather neutral in its ability to lower plasma cholesterol, at least when counteracting the cholesterol elevation induced by dietary saturated fatty acids(especially 14 : 0).

Our explanation for this discrepancy involves the concept of fatty acid "thresholds", i.e. the amount of any fatty acid (as a% dietary energy) above or below which its presence either begins or ceases to exert an impact on cholesterol meta-

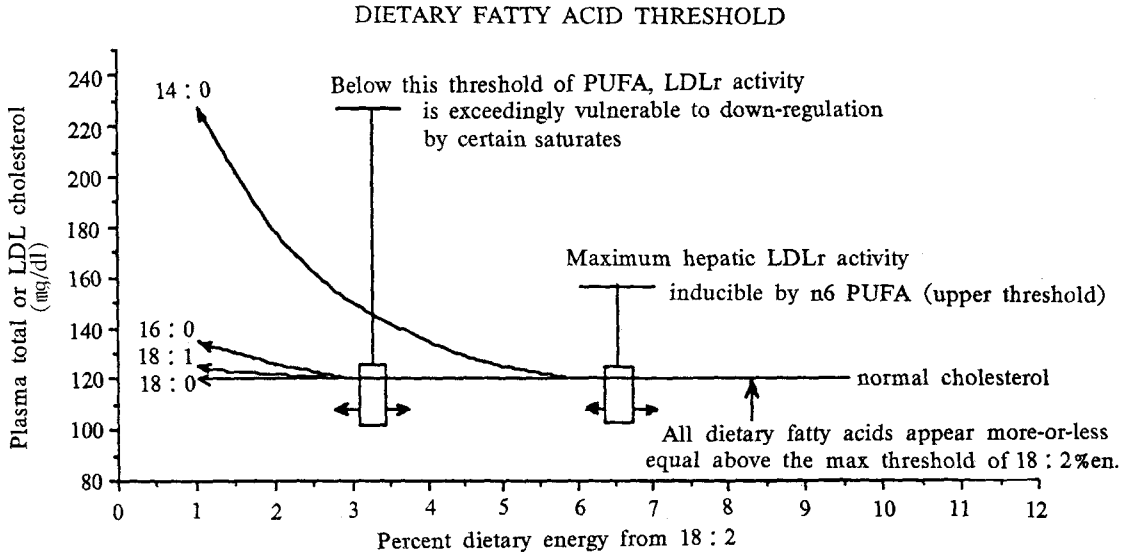

Fig. 5. In the above scenario the fatty acid "threshold" refers to the concentration of a dietary fatty acid (as% dietary energy) above or below which its presence of absence in the diet will modulate cholesterol metabolism as reflected in the total plasma cholesterol or LDL/HDL ratio. According to the above scheme the threshold for linoleic acid (18 : 2) would vary depending on the relative concentration of other fatty acids in the diet, particularly the amount and chain length of dietary saturates (14 : 0, 16 : 0, 18 : 0). Although generated on the basis of data from normocholesterolemic monkeys fed a common diet in which only the dietary fat composition varied, it is conceivable (probable) that any threshold will vary depending on other related factors impaction LDL receptor activity, such as the type of dietary protein, level of fat, type of carbohydrate, fiber content, dietary cholesterol load and the inherent LDL receptor status of the host.

bolism[20] (Fig. 5). Evaluation of the discrepancy between the Keys and Hegsted data and more recent studies[4][6][18] concerning the impact of 18 : 1 suggest that the latter studies exchanged 18 : 1 for 18 : 2 above the critical "threshold" for 18 : 2. This threshold relationship is readily discerned in the regression of the plasma cholesterol response against the dietary 18 : 2 en% for human or monkey data(Fig. 6). In other words, in recent human studies a relatively high % of dietary energy was fed as 18 : 2(above it's 6% en "threshold") in the relative absence of 14 : 0 in the saturated fat pool. By contrast, Keys and Hegsted typically examined the 18 : 1 for 18 : 2 exchange between 1-6% energy with normal to exaggerated levels of 14 : 0 often present in the diet, 14 : 0 tending to raise the total cholesterol substantially as discussed above. Since 14 : 0 reportedly decreases the LDLr activity[21][22] in addition to causing 'direct' LDL production[12], its absence in the diet would mean that minimal dietary 18 : 2 is needed to assure maximal LDLr activity, allowing a neutral fatty acid such as 18 : 1 to appear to be as efficient as 18 : 2 when the saturated fat load is insignificant(Fig. 5). Specifically, when the exchange is made above the 6% energy "threshold" for 18 : 2, this residual 6% en as 18 : 2 is more than enough to exert a maximal cholesterol lowering effect no matter what other fatty acids(except 14 : 0) are present. Thus substituting 18 : 1 "seems equivalent" to 18 : 2 because the plasma cholesterol (i.e. LDL) will not decline further ba-

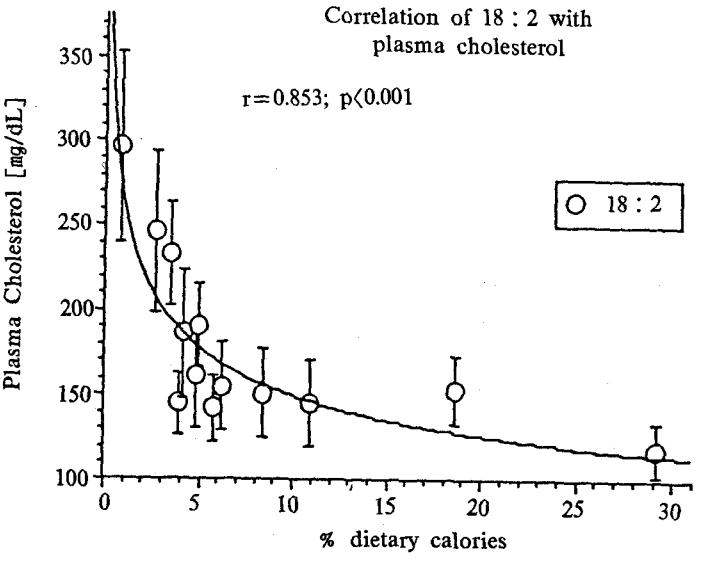

Fig. 6. The correlation of the % dietary energy from linoleic acid (18 : 2) plotted against the observed plasma cholesterol concentration in cebus monkeys reveals a threshold for 18 : 2 at 5-6% en. Values shown are the mean± SD. The data were obtained from a total of 16 monkeys with 4-10 monkeys rotated through 13 different cholesterol-free purified diets (Diets 1, 2, and 8-18, Table 1)for 6-12 week periods. For each diet, the 18 : 2 content (as a % of total acids, Table 1) was multiplied by the percent energy contributed by the dietary fat (31% or 40% energy) to calculate the % energy contributed by 18 : 2.

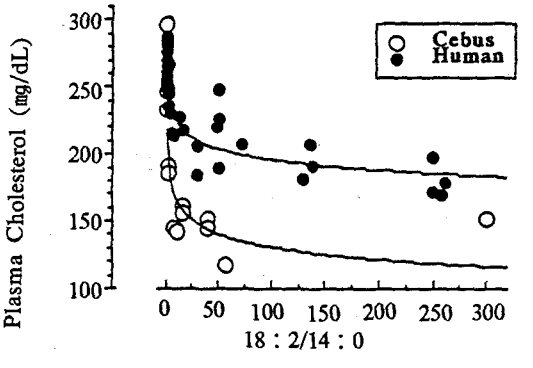

Fig. 7. The observed total cholesterol concentration in cebus monkeys and humans are regressed against the % dietary energy as the 18 : 2/14 : 0 ratio. This ratio provided the simplest, most predictive expression of the relationship and suggests that maximum lowering of cholesterol is achieved when the ratio is 10 or more. The monkey data represent 13 cholesterol-free diets (see legend to Fig. 6). The human data (representing 36 diets) are taken from Hegsted et. al[3]. In the latter study, diets aldo contributed 110-686 mg cholesterol per day.

sed on the mix of saturates and unsaturates present in the diet. According to this reasoning, at least two factors need to be considered in future studies of dietary fat saturation : 1) the nature of the "challenge" contributed by each saturated fatty acid, with the % energy from 14 : 0 being most critical and 2) the counterbalance or "threshold"% energy contributed by polyenes, primarily 18 : 2 in the typical diet.

If one examines this relationship carefully in the study best designed to designed to expose it[3], 14 : 0 appears to have 3-4x the cholesterol-elevating power that 18 : 2 has for reducing it. Hegsted [3] used % energy contributed by each fatty acid to express this relationship, which is probably the best procedure when dietary fat represents 30-40% of total energy. This relationship between key fatty acids is consistent with the analysis of our cebus data collected for 13 cholesterol-free diets(Hayes

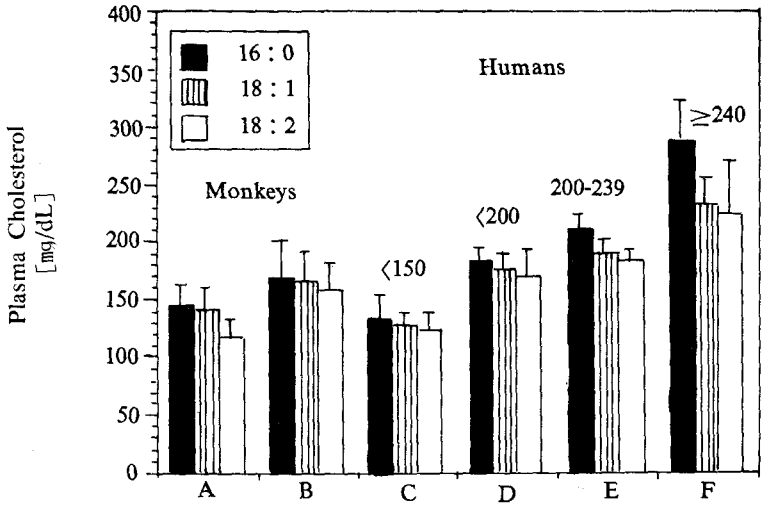

Fig. 8. Comparison of the effects of dietary 16 : 0, 18 : 1 and 18 : 2 on plasma cholesterol concentrations in primates. A and B represent data from cebus and rhesus monkeys, respectively (Khosla and Hayes, unpublished observations). C to F represent human data obtained from the literature : C, reference 25;D to F, reference 4. The human data represent both normocholesterolemic subjects[25] and hypercholesterolemic subjects[4]. The mean plasma cholesterol of the normocholesterolemic subjects at the time of study was 166± 19 mg/dL(n=12) and for the hypercholesterolemic subjects this value was 263± 50mg/dL (n=20). In plotting D to F, the 20 hypercholesterolemia subjects were grouped into the indicated categories (<200mg/dL, n=7;200-239mg/dL, n=7;≥240mg/dL, n=6) based on the plasma cholesterol concentrations measured after consumption of the 16 : 0-rich diet. Values are means± SD. Only in subjects with total cholesterol above 200mg/dL does 16 : 0 appear more cholesterolemic than 18 : 1 or 18 : 2.

and Khosla, unpublished observations). When the dietary 18 : 2/14 : 0 ratio was examined (Fig. 7), these two dietary fatty acids explaind 78% of the variation in total plasma cholesterol and 88% of the LDL/HDL ratio response, relationships that were scarcely improved by adding several other fatty acids into the regression. This relationship presumably reflects the impact on LDLr activity, i.e. the receptors would be "maximally upregulated" during high intake of 18 : 2 and minimal intake of 14 : 0 or "maximally shut down" during high intake of 14 : 0 and minimal consumption of 18 : 2. Because of the disparate power of these two fatty acids, presumably on LDLr acitvity and 'direct' production of LDL, minimal 14 : 0 requires considerable 18 : 2 to balance it. Thus 0.5% en as 14 : 0 may require as much as 3-4% en as 18 : 2, whereas 1-2% 14 : 0 may require 6-8% of 18 : 2 to counter its impact. In a practical sense 14 : 0 seldom represents more than 2.5% energy in the human diet, so the upper 18 : 2 "threshold" should never exceed about 12% energy in the worst-case scenario. This is conjecture at this point since a direct test across sufficient ratios has never been examined in any species, and other dietary factors, such as cholesterol, fiber, protein, etc., and the inherent host LDLr status, presumably affect the relationship.

We examined the 18 : 1 vs. 18 : 2 relationship along with 16 : 0 in monkeys(Khosla and Hayes, unpublished observations), feeding the atypical fatty acid profiles(Diets 16-18, Table 1) present

in the Mattson-Grundy diets[4]. The results were both revealing and supportive of the above hypothesis, exposing the importance of the host status (LDLr activity) as another potential variable in such studies(Fig. 8). As implied by our previous data[5] and the Hegsted regression equation[3], 18 : 1 and 16 : 0 were essentially neutral and similar, whereas the 18 : 2 -rich safflower oil diet(32% energy as 18 : 2) induced a significantly lower cholesterol level (due to a decrease in HDL) in the more sensitive cebus monkey, but not in rhesus. In essence, without 14 : 0 or cholesterol in the diet and with adequate 18 : 2 present (at or above its critical the threshold) the plasma cholesterol does not increase in normocholesterolemic individuals fed 16 : 0. Nor does 18 : 1 lower the cholesterol more than 16 : 0 or 18 : 0 under such circumstances. Furthermore, if one examines the literature carefully, in no case does 18 : 1 lower an elevated plasma cholesterol level as effectively as 18 : 2 if the exchange with 18 : 1 takes place below the critical "threshold" of 18 : 2 needed for the mix of saturates in the diet.

Puzzled by the discrepancy between the monkey data(Khosla and Hayes, unpublished observations) and the Mattson-Grundy human data[4], we reexamined the latter with the idea that the host status may have influenced the plasma lipid response, especially since the human population studied was hypercholesterolemic. When the plasma cholesterol response was separated into high, medium, or low responders to the "saturated fat" (i.e. palm oil), the low responders(cholesterol values less than 200㎎/dl during palm oil) also revealed similar total cholesterol responses during 18 : 1 and 18 : 2 consumption (Fig. 8). Only as the total plasma cholesterol increased above 200 ㎎/dl did 16 : 0 appear more cholesterolemic than 18 : 1 or 18 : 2. The point we would make, referring again to Fig. 2 and the role that the LDLr plays in controlling the size of the LDL pool and, ultimately, the total cholesterol pool, is that down-regulation of the LDL receptor associated with polygenic hypercholesterolemia[9] would deter clearance of the VLDL remnant by the liver. This would increase VLDL conversion to LDL and further expand the LDL pool. The above scenario also suggerts that the influence of fat saturation on cholesterol metabolism would be biased by the presence of cholesterol in the diet because absorbed cholesterol would tend to down-regulate the LDL receptor, thereby affecting the extent to which a given saturated fatty acid would appear cholesterolemic. For example, under conditions the increased VLDL production[12] associated with 16 : 0+18 : 1 would lead to cholesterolemia. Numerous examples of this dietary cholesterol effect are reported, including recent examples in hamsters[23] and monkeys[24].

The implication of these various studies suggests the need to focus our attention an individual dietary fatty acid relationships rather than dietary fats or aggregates of saturates and polyenes.

Acknowledgments

The studies described herein were supported in part by Best Foods (Union, NJ), Mead Johnson Nutrition Division (Evansville, IN), National Livestock and Meat Board (Chicago, IL), National Institute of Health-DK #35375 (Bethesda, MD), and the Palm Oil Research Institute of Malaysia (Kuala Lumpur, MALAYSIA). We are grateful to Drs. Zouhair Stephan, Deborah Diersen-Schade and George Patton for their contribution to these studies.

Literature cited

1) Grundy, SM and Denke MA. Dietary influences on serum lipids and lipoproteins. *J Lipid Res 31* : 1149-1172, 1990

2) Keys A, Anderson JT and Grande F. Prediction of serum cholesterol responses of man to changes

in fats in the diet. *Lancet 2* : 959-966, 1957

3) Hegsted DM, McGandy RB, Myers ML and Stare FJ. Quantitative effects of dietary fat on serum cholesterol in man. *Am J Clin Nutr 17* : 281-295, 1965
4) Mattson FH and Grundy SM. Comparison of effects of dietary saturated, monounsaturated, and polyunsaturated fatty acids on plasma lipids and lipoproteins in man. *J Lipid Res 26* : 194-202, 1985
5) Hayes KC, Pronczuk A, Lindsey S and Diersen-Schade D. Dietary saturated fatty acids (12 : 0, 14 : 0, 16 : 0) differ in their impact on plasma cholesterol and lipoproteins in nonhuman primates. *Am J Clin Nutr 53* : 491-498, 1991
6) Mensink RP and Katan MB. Effect of a diet enriched with monounsaturated or polyunsaturated fatty acids on levels of low-density and high-density lipoprotein cholesterol in health women and men. *N Engl J Med 321* : 436-441, 1989
7) Berns MAM, de Vries, JHM and Katan MB. Increases in body fatness as a major determinant of changes in serum total cholesterol and HDL in young men over a ten year period. *Am J Epi 130* : 1109-1122, 1989
8) Denke MA, Sampos CT and Grundy SM. Excess body weight : an unrecognized cause of high blood cholesterol. *Circulation 82* : Suppl. III, Abstract, 288, 1990
9) Grundy SM and Vega GL. Influence of mevinolin on metabolism of low density lipoproteins in primary moderate hypercholesterolemia. *J Lipid Res 26* : 1464-1475, 1985
10) Blankenhorn DH, Nessim SA, Johnson RL, Sanmarco ME, Azen SP and Cashin-Hemphill L. Beneficial effects of combined cholestipol-niacin therapy on coronary atherosclerosis and coronary venous bypass grafts. *JAMA 257* : 3233-3240, 1987
11) Pronczuk A, Stephan ZF, Patton G and Hayes KC. Species variation in the atherogenic profile of monkeys : Relationship between dietary fats, lipoproteins, and platelet aggregation. *Lipids 26* : 213-222, 1991
12) Khosla P and Hayes KC. Dietary fat saturation in rhesus monkeys affects LDL concentrations by modulating the independent production of LDL apolipoprotein B. *Biochim Biophys Acta 1083* : 46-56, 1991
13) Tall AR Plasma high density lipoproteins. Metabolism and relation to atherogenesis. J *Clin Invest 86* : 379-384, 1990
14) Havel, R J. The formation of LDL : mechanisms and regulation. *J Lipid Res 25* : 1570-1576, 1984
15) Hayes KC, Lindsey S, Pronczuk A and Dobbs S.Dietary 18 : 1/18 : 2 ratio correlates highly with hepatic FC and mRNAs for apo AI, apo E and the LDL receptor. *Circulation 78 : Suppl. 14. Abstract 0383. pII-96, 1988*
16) Lindsey S, Benattar J, Pronczuk A and Hayes KC. Dietary palmitic acid (16 : 0) enhances HDL cholesterol and LDL receptor mRNA abundance in hamsters. *Proc Soc Exp Biol Med 195* : 261-269, 1990
17) McGandy RB, Hegsted DM and Meyers ML. Use of semisynthetic fats in determining the effects of specific dietary fatty acids on serum lipids in man. *Am J Clin Nutr 23* : 1288-1298, 1970
18) Chan JK, Bruce VM and McDonald BE. Dietary α-linolenic acid is as effective as oleic and linoleic acid in lowering blood cholesterol in normolipidemic men. *Am J Clin Nutr 53* : 1230-1234, 1991
19) Shepherd J, Packard CJ, Patsch JR, Gotto AM Jr and Taunton OD. Effects of dietary polyunsaturated and saturated fat on the properties of high density lipoproteins and the metabolism of apoliporotein A-1. *J Clin Invest 61* : 1582-1592, 1978
20) Hayes KC. Dietary saturated fatty acids and low density or high density lipoprotein cholesterol(letter to the editor). *N Engl J Med 322* : 402-404, 1989
21) Spady DK and JM. Dietschy. Interaction of dietary cholesterol and triglycerides in the regulation of hepatic low density lipoprotein transport in the hamster. *J Clin Invest. 81* : 300-309, 1988
22) Nicolosi RJ, Stucchi AF, Kowala MC. Hennessy LK, Hegsted DM and Schaefer EJ. Effect of dietary fat saturation and cholesterol on LDL composition and metabolism. In vivo studies of receptor and nonreceptor-mediated catabolism of LDL in

cebus monkeys. *Arteriosclerosis 10*: 119-128, 1990

23) Ohtani H, Hayashi K, Hirata Y, Dojo S, Nakashima K, Nishio E, Kurushima H, Saeki M and Kajiyama G. Effects of dietary cholesterol and fatty acids on plasma cholesterol level and hepatic lipoprotein metabolism. *J Lipid Res 31*: 1413-1422, 1990

24) Rudel LL, Haines JL and Sawyer JK. Effects on plasma lipoproteins of monounsaturated, saturated, and polyunsaturated fatty acids in the diet of African green monkeys. *J Lipid Res 31*: 1873-1882, 1990

25) Becker N, Illingworth DR, Alaupovic P, Connor WE and Sunberg EE. Effects of saturated, monounsaturated, and w-6 polyunsaturated fatty acids on plasma lipids, lipoproteins, and apoproteins in humans. *Am J Clin Nutr 37*: 355-360, 1983