Effect of *trans* fatty acids on calcium influx into human arterial endothelial cells[1–3]

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ABSTRACT

Background: A recent task force of The American Society for Clinical Nutrition and American Society for Nutritional Sciences recommended in a position paper on *trans* fatty acids that models be developed to assess the effects of changes in fat intake on disease risk.

Objective: The objective was to investigate, using human arterial endothelial cells as a model, the influence of *trans* fatty acids and magnesium on cell membrane composition and on calcium influx into arterial cells, a hallmark of atherosclerosis.

Design: Endothelial cells were cultured for 3 d in media with high (adequate) or low (inadequate) amounts of magnesium plus various concentrations of *trans*,*trans* linoelaidic; *cis*,*cis* linoleic; *trans* elaidic; oleic; or stearic acids. The cells were then harvested and the fatty acid composition and the amount of $^{45}Ca^{2+}$ incorporated into the cell was determined.

Results: The percentage of fatty acids incorporated into the endothelial cells was proportional to the amount added to the culture medium. Adequate magnesium was crucial in preventing calcium influx into endothelial cells. Without an adequate amount of magnesium in the culture medium, linoelaidic and elaidic acids, even at low concentrations, increased the incorporation of $^{45}Ca^{2+}$ into the cells, whereas stearic acid and oleic acid did not ($P < 0.05$).

Conclusion: Our model indicated that a diet inadequate in magnesium combined with *trans* fat may increase the risk of calcification of endothelial cells. *Am J Clin Nutr* 1999;70:832–8.

KEY WORDS *trans* Fatty acids, calcium infiltration, endothelial cells, atherosclerosis, umbilical cord, magnesium

INTRODUCTION

The Task Force on *Trans* Fatty Acids of the American Society for Clinical Nutrition and the American Society for Nutritional Sciences (1) stated "Concerns about possible adverse effects of *trans* fatty acids on health center on the question of whether the intake of *trans* fatty acids is associated with the development and/or acceleration of disease, thereby increasing morbidity and mortality." Epidemiologic (2–7) and clinical (8–13) studies have addressed this issue. Additional overviews and commentaries have emphasized the importance of continued research of this issue (14–19).

The percentage of *trans* fat in a food item is not provided on the label, so one can only surmise the percentage from the published literature (20). Margarine and the shortenings that are used in baked products contain from 6.8% to 41.0% and cooking oils and coating fat on candies contain from 1.4% to 79.0% *trans* fatty acids (21, 22). The major *trans* isomeric fatty acid in hydrogenated fat is *trans* elaidic acid (*t*-18:1n−9). *t*,*t*-Linoelaidic acid (*t*,*t*-18:2) is present in partially hydrogenated vegetable oils and *cis*,*cis* linoleic acid (*c*,*c*-18:2n−6) is present in unhydrogenated vegetable oil. *trans* Fatty acids consumed as part of the US diet are metabolized and are deposited in human tissue, as are other dietary fatty acids. We found 4–14% *trans* fatty acids in the heart and 4.6–9.3% in arterial tissue (23). Human milk contains from 4.7% (24) to as much as 17.0% (25) *trans* fatty acids.

Many cellular functions are affected when the composition of unsaturated fatty acids in the cell membrane (26, 27) or the polar head group of the cell membrane (28) is modified. *trans* Unsaturated fatty acids inhibit phosphatidylcholine–sterol *O*-acyltransferase and alter its positional specificity (29). Engelhard et al (30) found that the addition of a *trans* fatty acid to a culture medium resulted in a large increase in the fluidity of choline-supplemented membranes. Tongai et al (31) showed that erythrocyte plasma membrane from magnesium-deficient rats was more fluid than that of control rats. We found that a medium deficient in magnesium changed the polyunsaturated fatty acid content of the cell membrane (32), changed the percentage of cellular phospholipid in the cell membrane, and increased free cytosolic calcium concentrations in the cell compared with cells cultured in a sufficient amount of magnesium (33). Because stenosis is due to atherosclerosis of the coronary arteries (34), characterized by lipid and calcium deposits in the arterial wall (35–37), we chose to study the effects of *trans* fatty acids on arterial cell membrane composition and on calcium influx into cultured arterial endothelial cells. In previous studies (38, 39), we found that a decrease in the magnesium concentration of culture medium from 0.95 mmol/L [normal content in Eagle's minimum essential medium (MEM)] to < 0.57 mmol/L affected cell

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growth and functions. We chose magnesium concentrations of 0.95 and 0.57 mmol/L and a 3-d incubation period to test the effect of *trans* fatty acids on cell fatty acid composition and on $^{45}Ca^{2+}$ influx into endothelial cells.

MATERIALS AND METHODS

$^{45}Ca^{2+}$ was purchased from Moravek (Brea, CA) and New England Nuclear (Boston); the fatty acids were purchased from NuCheck Prep Inc (Elysian, MN). The endothelial cells were isolated from the arteries of human umbilical cords obtained by cesarean delivery (Carle Foundation Hospital, Urbana, IL) and were cultured to 60% confluence in Eagle's MEM containing 10% calf bovine serum. These cells were then cultured in MEM containing 5% calf bovine serum and various combinations and concentrations of *t*,*t*-18:2, *c*,*c*-18:2n−6, *t*-18:1n−9, oleic acid (18:1n−9), or stearic acid (18:0) and either a high (0.95 mmol/L) or low (0.57 mmol/L) amount of magnesium. After 3 d of incubation, the cells were harvested and lipids were extracted with 10 mL chloroform:methanol (2:1, by vol). The lipids then were saponified and acidified and the fatty acids converted to methyl esters. The composition of fatty acids in each sample was determined in a model 428 gas chromatograph equipped with an all-glass injection splitter and flame ionization detector (Hewlett-Packard, Palo Alto, CA). Retention time, peak areas, and relative peak area percentages were determined electronically with a Hewlett-Packard model 3390A Reporting Integrator. Relative retention times were compared with authentic standards to identify methyl esters of fatty acids (32). Butylated hydroxytoluene was added as an antioxidant at a concentration of 0.005% (wt:vol) to all solvents, and all procedures were carried out under nitrogen. We also determined the fatty acid composition of both the freshly isolated arterial cells from the umbilical cords and those cultured in MEM, which were used as experimental models.

Because calcification of arterial cells is a common hallmark of atherosclerosis (34–37), we tested the influx of calcium ($^{45}Ca^{2+}$) into the cells. After the cells had been cultured for 3 d in MEM containing the various combinations and concentrations of fatty acids and either 0.95 or 0.57 mmol Mg/L, the cells were pulsed with $^{45}Ca^{2+}$ (37 MBq/L, or 1 μCi) in 1 mL MEM for 1 h. The monolayers then were washed and digested. The amount of radioactivity incorporated into the cells was counted in a Beckman (Fullerton, CA) scintillation counter (39).

To test the efflux of $^{45}Ca^{2+}$, we exposed the fatty acid–treated cells to $^{45}Ca^{2+}$ (74 MBq/L, or 2 μCi/mL) for 1 h. We then washed $^{45}Ca^{2+}$-pulsed cells with phosphate-buffered saline and cultured them for 1 h in 1 mL fresh MEM. Aliquots (100 mL) were collected at 5, 15, and 60 min, and the amount of $^{45}Ca^{2+}$ in the MEM was measured at each time interval.

The data were analyzed by analysis of variance and with a Scheffe test (40). A P value <0.05 was considered significant. All data are presented as means ± SEs.

RESULTS

The percentage of *c*,*c*-18:2n−6 in the endothelial cells increased from 1.2% in the cells freshly isolated from the umbilical cord to 2.8% in the cells cultured in MEM containing 0.95 mmol Mg/L (**Table 1**). The percentages of 18:0 and 18:1n−9 increased whereas the percentages of palmitoleic (16:1n−9) and arachidonic (20:4n−6) acids decreased significantly after being cultured for 3 d in MEM. Endothelial cells cultured for 3 d in 0.95 mmol Mg/L containing 12 μmol *t*-18:1n−9/L incorporated 0.9% *t*-18:1n−9 and more 18:2n−6 than did cells cultured in MEM. Cells cultured in a medium containing 18:0 contained more 18:0 and less $\triangle^7$-octadecenoic acid (18:1n−7) than did cells cultured in *t*-18:1n−9. There was no significant difference in fatty acid composition between endothelial cells cultured with 12 μmol *t*-18:1n−9/L and endothelial cells cultured with 12 μmol 18:0/L.

The percentage of *t*,*t*-18:2 and *c*,*c*-18:2n−6 incorporated into endothelial cells depended on the ratio of *t*,*t*-18:2 to *c*,*c*-18:2n−6 added to the media (**Table 2**). After endothelial cells were cultured for 3 d in MEM containing 100 μmol *c*,*c*-18:2n−6/L and 0.95 mmol Mg/L, the *c*,*c*-18:2n−6 content in the endothelial

TABLE 1
Percentage of fatty acids and the double bond index (DBI) in endothelial cells from freshly isolated umbilical cords and in cells cultured for 3 d in Eagle's minimum essential medium (MEM) containing either stearic (18:0) or *trans* elaidic (*t*-18:1n−9) acid[1]

	Freshly isolated	Cultured in MEM	Cultured with 12 μmol 18:0/L	Cultured with 12 μmol *t*-18:1n−9/L
Fatty acid (%)				
14:0	1.7 ± 0.1^{a,b}	1.6 ± 0.0^{a}	2.0 ± 0.1^{a,b}	1.8 ± 0.0^{b}
16:0	26.7 ± 0.9	27.7 ± 2.1	26.8 ± 0.5	28.3 ± 1.0
16:1n−9	3.3 ± 0.1^{a}	0.1 ± 0.0^{b}	0.2 ± 0.0^{b}	0.3 ± 0.0^{b}
18:0	17.0 ± 0.3^{a}	28.4 ± 2.5^{b,c}	33.3 ± 1.1^{b}	26.2 ± 0.5^{c}
18:1n−9	15.4 ± 0.4^{a}	23.2 ± 2.6^{b}	24.8 ± 2.3^{b}	27.1 ± 0.9^{b}
18:1n−7	3.9 ± 0.2^{a,b}	2.4 ± 0.5^{a,b}	3.4 ± 0.1^{a}	4.9 ± 0.0^{b}
t-18:1	—	0.1 ± 0.0^{a}	0.2 ± 0.0^{a,b}	0.9 ± 0.1^{b}
18:2n−6	1.2 ± 0.1^{a}	2.8 ± 0.1^{b}	3.6 ± 0.2^{b,c}	4.6 ± 0.1^{c}
20:3n−6	1.6 ± 0.1^{a,b}	2.1 ± 0.1^{a}	1.6 ± 0.1^{a,b}	1.5 ± 0.1^{b}
20:4n−6	12.8 ± 0.5^{a}	3.6 ± 0.3^{b}	3.0 ± 0.1^{b}	2.7 ± 0.2^{b}
22:4n−6	1.8 ± 0.2^{a}	0.8 ± 0.0^{b}	0.5 ± 0.1^{b}	0.4 ± 0.1^{b}
22:5n−6	2.9 ± 0.1^{a}	0.2 ± 0.0^{b}	0.3 ± 0.0^{b}	0.2 ± 0.0^{b}
22:5n−3	3.0 ± 0.1	—	—	—
22:6n−3	4.1 ± 0.2^{a}	0.2 ± 0.0^{b}	0.3 ± 0.0^{b}	1.0 ± 0.1^{b}
DBI	142.3	54.7	57.7	65.4

[1] $\bar{x}$ ± SE. Values in the same row with different superscript letters are significantly different, $P < 0.05$.

TABLE 2
Percentage of fatty acids and the double bond index (DBI) in endothelial cells cultured for 3 d in Eagle's minimum essential medium containing various concentrations of *trans,trans* linoelaidic (*t,t*-18:2) and *cis,cis* linoleic (*c,c*-18:2n−6) acids[1]

	100 μmol *t,t*-18:2 + 0 μmol *c,c*-18:n−6	75 μmol *t,t*-18:2 + 25 μmol *c,c*-18:n−6	50 μmol *t,t*-18:2 + 50 μmol *c,c*-18:n−6	25 μmol *t,t*-18:2 + 75 μmol *c,c*-18:n−6	0 μmol *t,t*-18:2 + 100 μmol *c,c*-18:n−6
Fatty acid (%)					
14:0	1.03 ± 0.3[a]	1.10 ± 0.1[a]	1.37 ± 0.2[a]	1.30 ± 0.2[a]	2.13 ± 0.2[b]
14:1	0.30 ± 0[a]	0.33 ± 0.1[a,b]	0.43 ± 0.1[b]	0.40 ± 0.1[a,b]	0.47 ± 0.1[a,b]
16:0	19.1 ± 0.3[a]	17.5 ± 0.4[b]	18.1 ± 1.1[a,b]	18.5 ± 1.2[a,b]	24.5 ± 0.7[c]
16:1n−7	0.78 ± 0.2	0.80 ± 0.2	0.75 ± 0.1	0.55 ± 0.1	0.63 ± 0.1
16:1n−5	1.55 ± 0.2[a]	0.80 ± 0.1[b]	1.40 ± 0.3[a,b]	3.23 ± 1.1[a]	0.60 ± 0.1[b]
18:0	12.1 ± 1.3[a,b]	11.8 ± 0.5[a]	13.5 ± 2.0[a,c]	14.7 ± 0.6[b,c]	17.4 ± 1.6[c]
18:1n−9	9.50 ± 1.7[a,b]	10.3 ± 0.6[b]	8.40 ± 0.7[a,b]	9.70 ± 1.2[a,b]	7.98 ± 0.6[a]
18:1n−7	1.45 ± 0.2[a]	1.68 ± 0.1[b]	1.45 ± 0.2[b]	1.63 ± 1.1[b]	0.80 ± 0.2[c]
t,t-18:2	29.5 ± 2.3[a]	24.0 ± 0.7[a]	17.1 ± 1.1[b]	9.10 ± 1.0[c]	—
18:2n−6	5.35 ± 0.2[a]	10.3 ± 0.4[b]	13.4 ± 0.9[c]	15.8 ± 0.8[d]	15.5 ± 1.0[c,d]
18:3n−6	—	—	0.40 ± 0.0	—	1.20 ± 0.1
20:2n−6	0.20 ± 0.0[a,b,c]	0.45 ± 0.1[a]	0.95 ± 0.1[b]	1.53 ± 0.1[c]	1.88 ± 0.4[b,c]
20:3n−6	0.93 ± 0.1[a]	1.35 ± 0.2[a,b]	1.40 ± 0.1[b]	2.35 ± 0.2[c]	6.75 ± 0.4[d]
20:4n−6	6.30 ± 0.2	7.60 ± 0.6	7.40 ± 0.7	7.80 ± 0.7	6.80 ± 0.6
22:2n−6	1.75 ± 0.3[a,c]	0.87 ± 0.2[d]	1.08 ± 0.3[a,d]	0.92 ± 0.2[b,d]	1.95 ± 0.1[c]
22:4n−6	2.30 ± 0.2[a]	3.13 ± 0.2[b]	3.60 ± 0.3[b]	3.95 ± 0.5[b]	3.08 ± 0.2[b]
22:5n−6	1.35 ± 0.2	1.60 ± 0.1	1.68 ± 0.2	1.50 ± 0.2	1.38 ± 0.4
22:6n−3	0.80 ± 0.0	1.28 ± 0.2	0.98 ± 0.1	0.73 ± 0.1	1.10 ± 0.7
Total n−6	18.0 ± 0.4[a]	25.1 ± 1.3[b]	29.6 ± 2.0[b,c]	33.8 ± 2.5[c,d]	38.5 ± 2.0[d]
Saturated	31.2 ± 1.6[a,c]	29.3 ± 0.6[a]	31.7 ± 3.0[a,c]	33.2 ± 1.0[c]	41.9 ± 1.7[b]
DBI					
With *t,t*-18:2	135.9	147.8	142.1	136.1	126.0
Without *t,t*-18:2	76.9	99.8	107.9	117.9	126.0

[1] $\bar{x}$ ± SE. Values in the same row with different superscript letters are significantly different, $P < 0.05$.

cells increased from 5.35% to 15.5%. Cells cultured in MEM plus 75 μmol *c,c*-18:2n−6/L and 25 μmol *t,t*-18:2/L incorporated 15.8% *c,c*-18:2n−6 and 9.1% *t,t*-18:2, respectively. When the endothelial cells were cultured in MEM plus 50 μmol *c,c*-18:2n−6/L and 50 μmol *t,t*-18:2/L, the isolated cells contained 13.4% *c,c*-18:2n−6 and 17.1% *t,t*-18:2. Consequently, as the concentration of *t,t*-18:2 in the MEM increased, more *t,t*-18:2 was incorporated into the endothelial cells.

$^{45}Ca^{2+}$ influx increased significantly in endothelial cells cultured at a high ratio of *t,t*-18:2 to *c,c*-18:2n−6 (**Table 3**). Endothelial cells cultured for 3 d in 0.95 mmol Mg/L with various percentages of *t,t*-18:2 and *c,c*-18:2n−6 were tested for calcium influx at 2 different time intervals. With no *t,t*-18:2 or *c,c*-18:2n−6 added to the MEM, an influx of $^{45}Ca^{2+}$ was noted at 3.67 cpm/μg protein. When the cells were cultured with 100 μmol *t,t*-18:2/L, $^{45}Ca^{++}$ influx increased significantly compared with that from the cells treated with 100 μmol *c,c*-18:2n−6/L. When the cells were incubated with decreasing concentrations of *t,t*-18:2, from 97.5 to 80 μmol/L, a significant increase in $^{45}Ca^{2+}$ influx was also noted. However, when the concentration of *t,t*-18:2 decreased to ≤75 μmol/L, no significant difference in $^{45}Ca^{2+}$ influx was noted.

The increased influx of $^{45}Ca^{2+}$ into endothelial cells cultured in MEM with a high amount of magnesium (0.95 mmol Mg/L) and 12 μmol *t,t*-18:2, *t*-18:1n−9, or 18:0/L was not significant when compared with the cells cultured without fatty acids (**Table 4**). However, $^{45}Ca^{2+}$ influx increased significantly when endothelial cells were cultured in medium with a low concentration of magnesium (0.57 mmol/L) plus either 12 μmol *t,t*-18:2 or *t*-18:1n−9/L, but did not increase significantly with 18:0. $^{45}Ca^{2+}$ influx also did not increase significantly in endothelial cells cultured in medium with 0.57 mmol Mg/L combined with either 18:1n−9 or 18:0. However, a significant increase was noted when endothelial cells were cultured in MEM with *t*-18:1n−9 (**Table 5**). The efflux of $^{45}Ca^{2+}$ from endothelial cells was not influenced by the presence of *t,t*-18:2 (**Table 6**).

DISCUSSION

Our tissue culture data indicate that *trans* fatty acids combined with a low amount of magnesium in MEM modify

TABLE 3
$^{45}Ca^{2+}$ influx into endothelial cells cultured with various concentrations of *tran,trans* linoelaidic acid (*t,t*-18:2) and *cis,cis* linoleic acid (*c,c*-18:2n−6)[1]

Fatty acid (μmol/L)	$^{45}Ca^{2+}$ influx
	cmp/μg protein
0 *t,t*-18:2 + 100 *c,c*-18:2n−6	4.76 ± 0.2[a]
100 *t,t*-18:2 + 0 *c,c*-18:2n−6	6.78 ± 0.1[b]
75 *t,t*-18:2 + 25 *c,c*-18:2n−6	5.08 ± 0.1[a]
50 *t,t*-18:2 + 50 *c,c*-18:2n−6	4.74 ± 0.2[a]
25 *t,t*-18:2 + 75 *c,c*-18:2n−6	4.86 ± 0.2[a]
0 *t,t*-18:2 + 0 *c,c*-18:2n−6	3.67 ± 0.2[a]
97.5 *t,t*-18:2 + 2.5 *c,c*-18:2n−6	5.98 ± 0.3[b]
85 *t,t*-18:2 + 15 *c,c*-18:2n−6	5.29 ± 0.4[b]
80 *t,t*-18:2 + 20 *c,c*-18:2n−6	4.59 ± 0.3[b]

[1] $\bar{x}$ ± SE of 6 separate experiments. After human arterial endothelial cells were cultured with *t,t*-18:2 and *c,c*-18:2n−6 for 3 d, the cells were pulsed with $^{45}Ca^{2+}$ for 1 h. The monolayers then were washed, digested, and analyzed for radioactivity and protein content. Values in the same column with different superscript letters are significantly different, $P < 0.05$.

TABLE 4
$^{45}Ca^{2+}$ influx into endothelial cells cultured with *tran,trans* linoelaidic acid (*t,t*-18:2), *trans* elaidic acid (*t*-18:1n−9), or stearic acid (18:0) with high and low amounts of magnesium[1]

Fatty acid	$^{45}Ca^{2+}$ influx	
	0.95 mmol Mg/L	0.57 mmol Mg/L
	cpm/μg protein	
0 μmol/L	1.82 ± 0.1	2.07 ± 0.2
12 μmol *t,t*-18:2/L	2.01 ± 0.1	2.57 ± 0.3[2]
12 μmol *t*-18:1n−9/L	1.73 ± 0.1	2.41 ± 0.2[2]
12 μmol 18:0/L	1.74 ± 0.3	1.78 ± 0.2

[1] $\bar{x}$ ± SE of 6 separate experiments. After human arterial endothelial cells were cultured with *t,t*-18:2, *t*-18:1n−9, or 18:0 for 3 d, the cells were pulsed with $^{45}Ca^{2+}$ for 1 h. The monolayers then were washed, digested, and analyzed for radioactivity and protein content.
[2] Significantly different from 0.95 mmol Mg/L, $P < 0.05$.

endothelial cell membranes, permitting increased $^{45}Ca^{2+}$ influx into endothelial cells. As indicated in the Introduction, cellular functions and responses are affected when the unsaturated fatty acid content of the cell membrane is modified (26). Magnesium plays a vital role in this process (38). It acts as a cofactor (41) in the activity of the Δ^5- and Δ^6-desaturase enzymes, which play a role in decreasing the content of unsaturated fatty acids in the cell membrane. For example, when there was a magnesium deficiency, the fatty acid composition of liver microsomes indicated a slower rate of conversion of 18:2n−6 to 20:4n−6, which is consistent with the decrease in Δ^6-desaturase activity observed in the liver microsomes of magnesium-deficient rats (42). The decrease in Δ^6-desaturase activity was attributed to the lower concentration of enzyme molecules as a result of the decreased rate of protein synthesis associated with the magnesium deficiency (43). We found that *trans* 18:1 fatty acids in partially hydrogenated soybean oil have a more inhibitory effect than do saturated acids on essential fatty acid metabolism, even when there is an abundance of essential fatty acids in hydrogenated fat (42). The relevance of the magnesium content of the tissue culture medium to the atherogenic effect of *trans* fatty acids may rest on the pronounced effects of magnesium on the physical state of membrane bilayer lipids. Rayssiguier et al (44) believe that defective membrane function could be the primary reason for the underlying cellular disturbance that occurs in magnesium deficiency. Our data indicate that *t,t*-18:2 and *t*-18:1n−9 contribute to the cellular disturbance of the membrane.

A previous study indicated that cells cultured in MEM with 18:0 contained significantly more 18:1n−9 than did cells cultured with 18:2n−6, or 39.3% and 10.0%, respectively. The increased concentration of 18:1n−9 in cells cultured in 18:0 was necessary to maintain the physical state of the membrane (45). The physical state of the membrane can be conveniently determined by calculating the double bond index (DBI). The DBI is based on the sum of the percentages of unsaturated fatty acids multiplied by the number of double bonds in each unsaturated fatty acid (46). Hypothetically, if one considers *t,t*-18:2 to be an unsaturated fatty acid and if both *trans* and *cis* fatty acids are incorporated into a cell membrane, the DBI of the lipid extracted from the endothelial cells cultured with *t,t*-18:2 would be higher than that from the endothelial cells cultured with *c,c*-18:2n−6, or 135.92 and 126.01, respectively. However, Mahfouz et al (47) showed that *trans* fatty acids have physical properties similar to those of 18:0 and are inserted into acyl lipids in a manner more similar to that of a saturated acid than to an unsaturated acid. The acyltransferase (ie, 1-acylglycerophosphocholine *O*-acyltransferase) in rat liver microsomes discriminates between the normal *cis* isomers and the unnatural *trans* isomers with respect to their esterification in phospholipids and triacylglycerol, and a chain containing *trans* double bonds is transferred as if it were a saturated carbon chain (48). If the DBI is calculated on the basis of *trans* fatty acids acting similarly to 18:0 in the membrane, the DBI of the data in Table 2 would vary from 76.9 to 126.0. It is evident that the influence of *trans* fatty acids on the physical state of the membranes of endothelial cells cultured in medium with a low amount of magnesium requires further study.

Although the endothelial cells freshly isolated from the umbilical cord contained only 1.2% 18:2n−6, they contained 12.8% 20:4n−6 and more n−6 and n−9 unsaturated fatty acids than did the endothelial cells cultured for 3 d. The DBIs were 142.8 and 54.7, respectively, indicating a significant change in membrane fluidity between freshly isolated and cultured cells. Scott et al (49) found only 2.4% 18:2n−6 in the phospholipid fraction of the lipids extracted from the coronary arteries of infants aged <24 h. This is comparable with the percentage we found (1.2% *c,c*-18:2n−6) in the endothelial cells freshly isolated from the arteries of umbilical cords. Scott et al also found that 18:2n−6 increased from 2.4% to 4.8% and that 20:4n−6 increased from 11.0% to 19.0% in infants and adolescents, respectively.

In 1980, Seelig (50), in a book devoted to the role of magnesium deficiency in cardiovascular pathogenesis, listed >1000 references to ischemic heart disease in infants, primarily due to arteriosclerosis in the coronary arteries. A MEDLINE (National Library of Medicine, Bethesda, MD) search revealed that 195 additional articles dealing with arteriosclerosis in infants have been published since 1980. Seelig et al (51–53) have called attention to the low magnesium content of the US diet, and many other studies have suggested that magnesium intakes are inadequate in the US population (54–56).

Stary et al (35) found fatty streaks (type II lesion) in the aorta of 99% of infants and adolescents tested. According to Stary et al, type II lesions could develop into atherosclerotic type III lesions. Such a change may be governed by plasma factors, eg, excessive amounts of hematin (57) and homocysteine (58). A recent study

TABLE 5
$^{45}Ca^{2+}$ influx into endothelial cells cultured with *trans* elaidic acid (*t*-18:1n−9), oleic acid (18:1n−9), stearic acid (18:0), or *cis,cis* linoleic acid (*c,c*-18:2n−6) with high and low amounts of magnesium[1]

Fatty acid	$^{45}Ca^{2+}$ influx	
	0.95 mmol Mg/L	0.57 mmol Mg/L
	cpm/μg protein	
0 μmol/L	16.92 ± 1.9	13.31 ± 2.5
12 μmol *t*-18:1n−9/L	20.57 ± 6.1	25.40 ± 3.0[2]
12 μmol 18:0/L	15.67 ± 1.6	14.01 ± 2.9
12 μmol 18:1n−9/L	15.94 ± 3.3	14.83 ± 2.3
12 μmol *c,c*-18:2n−6/L	17.82 ± 2.1	19.81 ± 2.0

[1] $\bar{x}$ ± SE of 8 separate experiments. After human arterial endothelial cells were cultured with *t*-18:1n–9, 18:0, 18:1n–9, or *c,c*-18:2n–6 for 3 d, the cells were pulsed with $^{45}Ca^{2+}$ for 1 h. The monolayers then were washed, digested, and analyzed for radioactivity and protein content.
[2] Significantly different from 0.95 mmol Mg/L, $P < 0.05$.

TABLE 6

$^{45}Ca^{2+}$ efflux from endothelial cells cultured with various concentrations of *tran,trans* linoelaidic acid (*t,t*-18:2) and *cis,cis* linoleic acid (*c,c*-18:2n−6)[1]

	$^{45}Ca^{2+}$ efflux		
Fatty acid (μmol/L)	5 min	10 min	60 min
		cpm/μg protein	
0 *t,t*-18:2 + 100 *c,c*-18:2n−6	39.6 ± 9.0	41.1 ± 7.0	18.8 ± 2.1
100 *t,t*-18:2 + 0 *c,c*-18:2n−6	34.4 ± 2.1	35.5 ± 1.0	23.3 ± 2.5
75 *t,t*-18:2 + 25 *c,c*-18:2n−6	29.9 ± 3.4	31.5 ± 2.4	17.8 ± 1.8
50 *t,t*-18:2 + 50 *c,c*-18:2n−6	29.3 ± 4.1	28.8 ± 2.3	17.1 ± 1.2
25 *t,t*-18:2 + 75 *c,c*-18:2n−6	25.0 ± 2.6	31.6 ± 3.1	17.3 ± 1.7

[1] $\bar{x}$ ± SE of 6 separate experiments. After human arterial endothelial cells were cultured with *t,t*-18:2 and *c,c*-18:2n–6 for 3 d, the cells were pulsed with $^{45}Ca^{2+}$ for 1 h. The monolayers then were washed, digested, and analyzed for radioactivity and protein content. There were no significant differences.

indicated that the concentration of homocysteine in plasma is associated with an increased risk of vascular disease (59). A significantly higher concentration ($P < 0.01$) of homocysteine was found in the plasma of young blacks and young Hispanics than in young non-Hispanic whites. In another recent study, Strong et al (60) found type II lesions in the abdominal aorta of 100% of adolescents tested; white adolescents had 1.4% calcification of the aorta. By young adulthood, the calcification had increased to 16.7% in blacks and to 12.6% in whites. Therefore, a high plasma homocysteine concentration, which results from an insufficient dietary intake of vitamin B complex vitamins, may also be a factor that increases the calcification of endothelial cells.

The atherosclerosis in infants noted by Seelig (50) may develop in coronary arteries containing only 2% 18:2n−6 in infants fed human milk high in *trans* fatty acids (25) and low in magnesium. Human milk has a high biological value but has a lower magnesium content than does cow milk: 40 and 130 kg/L, respectively (61). A MEDLINE search found 2365 studies on *trans* fatty acids since 1966. Some of these studies (62) were performed with the liquid-formula diet used by Ahrens et al (63, 64). This liquid formula, which provided 2500 kcal (10 460 kJ)/d, lacked sufficient magnesium and contained only 96 mg/d, or <25% of the recommended dietary allowance (65). The magnesium content of the diets in previous studies of hydrogenated fats may be responsible for the observed differences.

Our study defines conditions under which *trans* fatty acids may be a risk factor in the development of atherosclerosis: *1*) if there is a low 18:2n−6 concentration in the phospholipid fraction of the endothelial cell membrane, as we found in the arteries of umbilical cords and as Scott et al (49) found in the coronary arteries of infants aged <24 h; *2*) if *trans* fatty acids are present in the diet; and *3)* if there is insufficient magnesium in plasma. Infants receiving ≈850 kcal (3556 kJ)/d from human milk may only consume 40 mg Mg/d. This inadequate amount of magnesium plus the high content of *trans* fatty acids in human milk may enhance the development of type II lesions into type III lesions (35, 66). When there was an adequate amount of magnesium and *c,c*-18:2n−6 in our MEM, a higher than normal calcium influx into endothelial cells did not occur unless an excessive amount of *trans* fatty acids was added.

Studies in rats showed that *trans* fatty acids from hydrogenated fat did not transfer from the mothers to their young through the placenta (67) but rather from the mother's milk. We found only traces of *trans* fatty acids in human umbilical cords. When hydrogenated fat was removed from the diet of rats, the concentration of *trans* fatty acids decreased rapidly from the fat tissue of both the mother and her nursing young (68). *trans* Fatty acids in human milk originate from *trans* fatty acids in hydrogenated fats. Lactating women would be well advised to increase their dietary magnesium intake and avoid ingesting hydrogenated fat containing a high concentration of *trans* fatty acids. Our study showed that a diet deficient in magnesium and containing a high amount of *t*-18:1n−9 and *t,t*-18:2 increases the calcification of endothelial cells. Such calcification is a common hallmark of atherosclerosis.

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