Change in dietary saturated fat intake is correlated with change in mass of large low-density-lipoprotein particles in men[1–3]

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ABSTRACT We tested whether nutrient intakes estimated from 4-d diet records were associated with plasma lipoprotein subclasses in 103 men who were randomly assigned to a low-fat (24% fat) and a high-fat (46% fat) diet for 6 wk each in a crossover design. Postheparin plasma lipoprotein lipase (LPL) and hepatic lipase (HL) activities were also determined in a subset of 43 men. Changes in intake (ie, high fat minus low fat) of total saturated fatty acids, as well as myristic (14:0) and palmitic (16:0) acids, were positively correlated ($P < 0.01$) with increases in mass of large LDL particles [measured by analytic ultracentrifugation as mass of lipoproteins of flotation rate (S_f^o) 7–12] and with LDL peak particle diameter and flotation rate, but not with changes in LDL-cholesterol concentration. Changes in total saturated fatty acids as well as myristic and palmitic acids were also inversely associated with changes in HL activity ($P < 0.05$). With the high-fat diet only, variation in dietary total saturated fatty acid intake was inversely correlated ($P < 0.01$) with concentrations of small, dense LDL of S_f^o 0–5. This correlation was significant specifically for myristic acid ($P < 0.001$). Stearic acid (18:0), monounsaturates, and polyunsaturates showed no significant associations with lipoprotein concentrations. These data indicate that a high saturated fat intake (especially 14:0 and 16:0) is associated with increased concentrations of larger, cholesterol-enriched LDL and this occurs in association with decreased HL activity. *Am J Clin Nutr* 1998;67:828–36.

KEY WORDS Diet, saturated fat, low-density lipoprotein subfractions, high-density lipoprotein, lipoprotein lipase, hepatic lipase, high-fat diet, low-fat diet, men

INTRODUCTION

Cross-cultural and metabolic ward studies provide evidence that dietary nutrients influence plasma lipids and lipoproteins (1, 2). Saturated fat feeding has been reported to increase LDL cholesterol and HDL cholesterol (1, 2). On the other hand, monounsaturates and polyunsaturates do not increase LDL cholesterol when added to a low-fat diet, but do increase HDL cholesterol, the latter effect being less marked than for saturated fat (1, 3). Dietary saturates, monounsaturates, and polyunsaturates all reportedly decrease plasma triacylglycerol concentrations, relative to carbohydrates, to about the same extent (3). However, many early cross-sectional studies in free-living populations (4–7) failed to show such relations, perhaps because of the inability to assess the usual nutrient intakes of individuals accurately or the inability to perform detailed measurements of lipoprotein components. In addition, most studies of the effects of dietary fat on plasma lipoproteins have not reported the intakes of individual dietary fatty acids. More recent cross-sectional reports using multiple-day diet records and lipoprotein subfraction concentration measurements describe significant correlations between intakes of dietary fat and carbohydrate with concentrations of LDL and HDL subclasses (8–11). Also, experimental evidence (12) shows associations of LDL-subclass distributions with changes in dietary fat and carbohydrate intake.

Among the numerous metabolic influences on plasma lipoproteins that may mediate dietary effects are lipoprotein lipase (LPL) and hepatic lipase (HL). Previous reports showed in humans (13–15) and monkeys (16, 17) that an increase in dietary fat is associated with increases in both LPL and HL. LPL hydrolyzes triacylglycerol in chylomicrons and VLDL (18). LPL activity was shown to correlate negatively with VLDL and positively with HDL (13, 19). HL has been associated with the metabolism of VLDL and intermediate-density lipoproteins (IDLs) and in the conversion of HDL_2 to HDL_3 (20–22). An inverse correlation was found between HL and HDL concentrations (19, 23). Both LPL and HL have been implicated in lipoprotein metabolism leading to the formation of LDL. LPL deficiency results in reductions in LDL cholesterol and low HL is associated with larger, more buoyant LDL particles (20–22, 24).

The objective of the present study was to use detailed nutritional analyses and refined lipoprotein measurements to assess the relations of plasma lipids, lipoproteins, and lipoprotein-subclass

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mass concentrations to nutrient intakes in 103 nonobese men consuming standardized low-fat and high-fat diets. In addition, this report describes the relation of nutrient intakes to postheparin LPL and HL in a subset of 43 subjects consuming these diets.

SUBJECTS AND METHODS

Subjects

The subjects participated in an outpatient crossover study of low- and high-fat diets, the details of which were reported previously (12). We recruited healthy, nonsmoking men > 20 y of age through newspaper and radio announcements, fliers, and direct mail. Subjects were selected if they had been free of chronic disease during the past 5 y and were not taking medication likely to interfere with lipid metabolism. In addition, they were required to have plasma total cholesterol < 6.74 mmol/L (260 mg/dL), triacylglycerol < 5.65 mmol/L (500 mg/dL), resting blood pressure < 160/105 mm Hg, and body weight < 130% of ideal (25).

The Committee for the Protection of Human Subjects at Ernest Orlando Lawrence Berkeley National Laboratory, University of California, Berkeley, approved the study protocol and each participant signed a consent form and participated in a medical interview. One hundred five men completed the study (12). Their mean (± SD) age and body mass index (BMI; kg/m^2) were 48.9 ± 11.1 y (range: 28.0–79.0 y) and 25.5 ± 3.0 (range: 17.4–35.1), respectively. Two subjects were eliminated from the present analyses (one who did not complete food records and another who did not participate in the diet protocols).

Dietary protocol

As described previously (12), the subjects were randomly assigned to outpatient treatment with diets of either low or high fat content (described below) for 6 wk each. The subjects then switched to the alternate diet for an additional 6 wk. The participants were not provided with food, but were instructed on the experimental diets by registered dietitians and were given 2-wk cycle menus showing the number and size of servings. The subjects abstained from alcohol throughout the study and were counseled to keep weight and exercise patterns constant between the two diets. There were no significant diet-induced changes in mean body weight between the low-fat and high-fat diets (12).

Dietary information on the subjects following each experimental diet was collected at the end of the sixth week of each diet by registered dietitians using 4-d (Thursday to Sunday) food records of measured and weighed food intake (26). Nutrient intakes were calculated by using the Minnesota Nutrition Data System (NDS) software (version 2.1), developed by the Nutrition Coordinating Center, University of Minnesota, Minneapolis (27, 28). The subjects recorded any dietary deviations from the menu daily as another measure of compliance with the experimental diets. If the daily dietary deviations averaged > 5% of total energy, the subject was considered noncompliant and his data were not included in the analyses. Only one subject was eliminated for noncompliance.

Because the subjects were free-living and consuming prescribed diets, there was no external verification of food consumed other than that reported in the diet record. Grocery store receipts were obtained to verify that the study food was purchased. Because food consumed on only 4 of 14 d of the diet was recorded, these analyses assume that the intakes seen in the 4-d diet record reflect of the rest of the dietary period.

The reported dietary intake of selected nutrients ($\bar{x}$ ± SD) for the sample of 103 men following the experimental diets is shown in **Table 1**. Nutrients are expressed as a percentage of total energy except for dietary cholesterol (mg/kJ) and dietary fiber (g/kJ). The low-fat diet contained 24% of energy as fat (6% saturated, 12% monounsaturated, and 4% polyunsaturated) and 59% as carbohydrate, with equal amounts of simple and complex carbohydrates. The high-fat diet contained 46% of energy as fat (18% saturated, 13% monounsaturated, and 12% polyunsaturated) and 39% as carbohydrate. Palmitic acid (16:0) was the primary dietary saturated fatty acid in both diets, followed by stearic (18:0) and myristic (14:0) acids, which are representative of the major saturated fatty acids in most human diets (3). In the high-fat diet, the largest increase in saturated fat was palmitic acid. Although prescribed dietary proportions of total protein (16%), cholesterol (0.030–0.036 mg/kJ), the ratio of polyunsaturated fat to saturated fat (P:S, 0.7), and dietary fiber (0.96–1.20 g/kJ) were not significantly different in the two diets, differences in reported intakes of these nutrients were observed.

The nutrients that make up the present analyses are the following: total protein, total carbohydrate, total fat, total saturated fatty acids, myristic acid, palmitic acid, stearic acid, total monounsaturated fatty acids, oleic acid (18:1), total polyunsaturated fatty acids, linoleic acid (18:2), cholesterol, and dietary fiber. Other individual fatty acids supplied a negligible percentage of total energy intake and therefore were not included in the analyses. Mean nutrient intake as estimated from the reported 4-d food records indicated good group compliance with the experimental diets (12). However, the individual variability in dietary compliance enabled us to examine associations of nutrient intake with lipoproteins and lipase activities for both the low-fat and high-fat diets. For example, the distribution of reported dietary intake of saturated fat, monounsaturated fat, and polyunsaturated fat is shown in **Figure 1** in the 103 men following the low- and high-fat diets. The change in dietary fatty acids (high-fat diet minus low-fat diet) is shown in **Figure 2**. The distributions of reported intakes of myristic, palmitic, and stearic acids for the low- and high-fat diets were similar to that of total dietary saturated fatty acid (data not shown).

Laboratory procedures

The subjects reported to our clinic in the morning after the sixth week of each experimental diet, having abstained for 12–14 h from all food and vigorous activity. Blood samples for lipid analyses were first collected in tubes containing disodium EDTA (1.4 g/L). Blood samples for HL and LPL analyses were then obtained 10 min after intravenous injection of heparin (75 U heparin/kg). Blood and plasma were kept at 4 °C until processed. Postheparin plasma was stored at −70 °C for lipase analyses.

Plasma total cholesterol and triacylglycerol were determined in our laboratory by enzymatic procedures on a Gilford Impact 400E analyzer (Ciba Corning Diagnostics Corp, Oberlin, OH). These measurements and measurement error were consistently within limits set by the CDC standardization program. HDL cholesterol was measured after heparin-manganese precipitation of plasma (29). LDL cholesterol was calculated from the formula of Friedewald et al (30), unless triacylglycerol concentrations were > 4.51 mmol/L (400 mg/dL), in which case, LDL cholesterol was measured by direct beta quantitation in the ultracentrifugal plasma fraction with density (d) > 1006 g/L. Apolipoprotein (apo) A-I and apo B concentrations in plasma were determined by maximal radial immunodiffusion (31, 32).

TABLE 1
Reported (4-d food record) mean daily nutrient intake for 103 middle-aged men consuming low-fat and high-fat diets[1]

Nutrient	Low-fat diet	High-fat diet	Change
Protein (% of energy)	16.6 ± 1.9	16.3 ± 0.9	−0.2 ± 1.8
Carbohydrate (% of energy)	59.0 ± 2.9	38.8 ± 2.3	−20.2 ± 3.7[2]
Fat (% of energy)	24.2 ± 3.0	45.5 ± 2.3	21.3 ± 3.4[2]
Saturated fat (% of energy)	5.9 ± 1.0	18.4 ± 1.2	12.5 ± 1.6[2]
4:0 (butyric)	0.0 ± 0.0	0.7 ± 0.1	0.7 ± 0.1[2]
6:0 (caproic)	0.0 ± 0.0	0.4 ± 0.1	0.4 ± 0.1[2]
8:0 (caprylic)	0.0 ± 0.0	0.2 ± 0.1	0.2 ± 0.1[2]
10:0 (capric)	0.0 ± 0.0	0.5 ± 0.1	0.5 ± 0.1[2]
12:0 (lauric)	0.1 ± 0.0	0.5 ± 0.3	0.5 ± 0.2[2]
14:0 (myristic)	0.3 ± 0.1	2.3 ± 0.3	2.1 ± 0.3[2]
16:0 (palmitic)	3.7 ± 0.5	9.0 ± 0.5	5.4 ± 0.7[2]
18:0 (stearic)	1.5 ± 0.3	4.0 ± 0.3	2.3 ± 0.4[2]
Monounsaturated fat (% of energy)	11.8 ± 1.7	12.5 ± 1.0	0.7 ± 1.9[2]
16:1 (palmitoleic)	0.4 ± 0.1	0.8 ± 0.1	0.5 ± 0.1[2]
18:1 (oleic)	11.7 ± 1.7	11.7 ± 1.0	0.0 ± 1.9
20:1 (eicosenoic)	0.1 ± 0.0	0.0 ± 0.0	0.0 ± 0.0[2]
Polyunsaturated fat (% of energy)	4.2 ± 0.9	11.8 ± 1.6	7.5 ± 1.9[2]
18:2 (linoleic)	3.9 ± 0.8	10.8 ± 1.6	7.2 ± 1.9[2]
18:3 (linolenic)	0.3 ± 0.1	0.6 ± 0.1	0.4 ± 0.1[2]
20:4 (arachidonic)	0.1 ± 0.0	0.0 ± 0.0	0.0 ± 0.0[2]
Cholesterol (mg/kJ)	0.033 ± 0.007	0.037 ± 0.005	0.004 ± 0.007[2]
P:S	0.7 ± 0.1	0.6 ± 0.1	−0.1 ± 0.2[2]
Fiber (g/kJ)	1.17 ± 0.14	1.10 ± 0.14	−0.12 ± 0.65[3]

[1] $\bar{x}$ ± SD. P:S, ratio of polyunsaturated to saturated fat.
[2,3] Significant difference: [2] $P < 0.001$, [3] $P < 0.05$.

Lipoproteins were analyzed by analytic ultracentrifugation, which measures mass of lipoproteins as a function of Svedberg flotation rate [S_f^o d < 1063 g/L; and $F^0_{1.2}$ d < 1210 g/L]. Mass concentrations were determined for VLDL (S_f^o 20–400), IDL (S_f^o 12–20), and for four major LDL subclasses: LDL-I (S_f^o 7–12), LDL-II (S_f^o 5–7), LDL-III (S_f^o 3–5), and LDL-IV (S_f^o 0–3) (33). For LDL, this procedure provides a measurement of peak flotation rate (S_f^o) as well as density (g/L) of the peak LDL for each subject (34). In addition, mass was determined for concentrations of two major HDL subclasses: HDL_2 ($F^0_{1.2}$ 3.5–9) and HDL_3 ($F^0_{1.2}$ 0–3.5) (34).

Nondenaturing polyacrylamide gradient gel electrophoresis, which separates LDL particles by size and shape, was used to identify the major LDL peak particle diameters, measured in nm (33). Electrophoresis of whole plasma was performed by using Pharmacia PAA 2% to 16% gradient gels (Uppsala, Sweden), as described previously (33, 35). Stained gels were scanned with a Transidyne RFT Scanning Densitometer (Transidyne Corp, Ann Arbor, MI), and LDL peak particle diameters were calculated from calibration curves by using standards of known size (33).

Lipase activities were determined by the method of selective inhibition with protamine sulfate as described previously (13, 36). All determinations were in triplicate and a control sample was included with each batch of test samples. Between-assay and within-assay CVs for a control sample were 8.1% and 2.8%, respectively. Lipase activities were expressed in mmol fatty acid $\cdot L^{-1} \cdot h^{-1}$.

Statistical analyses

The strengths of the relations between amounts of nutrients and plasma lipoprotein concentrations and amounts of nutrients and LPL and HL were measured by Spearman's correlation coefficients (r_s). These procedures were repeated for the low-fat and high-fat diets and for changes (high-fat minus low-fat values). Spearman's correlation coefficients provide a nonparametric test for significant association, have high efficiency when the data are in fact normal and are robust to outliers. For all of the nutrient-lipid correlations, a P value < 0.01 was considered significant. Because HL and LPL analyses were carried out in only 43 subjects, correlations with a P value < 0.05 were considered significant for these variables. The computer program StatView 4.0 (Abacus Concepts, Inc, Berkeley, CA) was used for the analyses.

RESULTS

The subjects in the present study were healthy, nonsmoking men with normal lipid and lipoprotein concentrations at screening ($\bar{x}$ ± SD, mmol/L): triacylglycerol, 1.37 ± 0.69; total cholesterol, 5.39 ± 0.76; LDL cholesterol, 3.52 ± 0.69; and HDL cholesterol, 1.23 ± 0.23. Plasma concentrations of lipids, lipoproteins, and major lipoprotein subfractions in all subjects consuming the two diets were reported elsewhere (12, 37) and summarized in **Table 2**. Activities of LPL and HL during the two diets were also published previously (15) and are shown in Table 2.

Correlations of dietary fat with plasma lipoproteins

During the low-fat and high-fat diets, dietary protein, carbohydrate, cholesterol, and fiber did not correlate with plasma lipoproteins (data not shown). Changes in these dietary variables also did not correlate with changes in plasma lipoproteins (data not shown). The correlations of dietary total fat and total saturated fat with plasma lipoproteins during the low-fat and high-fat diets, as well as correlations of the changes in these variables are shown in **Table 3**. During the low-fat diet, dietary total fat was correlated negatively with HDL_3 mass. Although not shown in Table 3, HDL-cholesterol concentrations were positively associ-

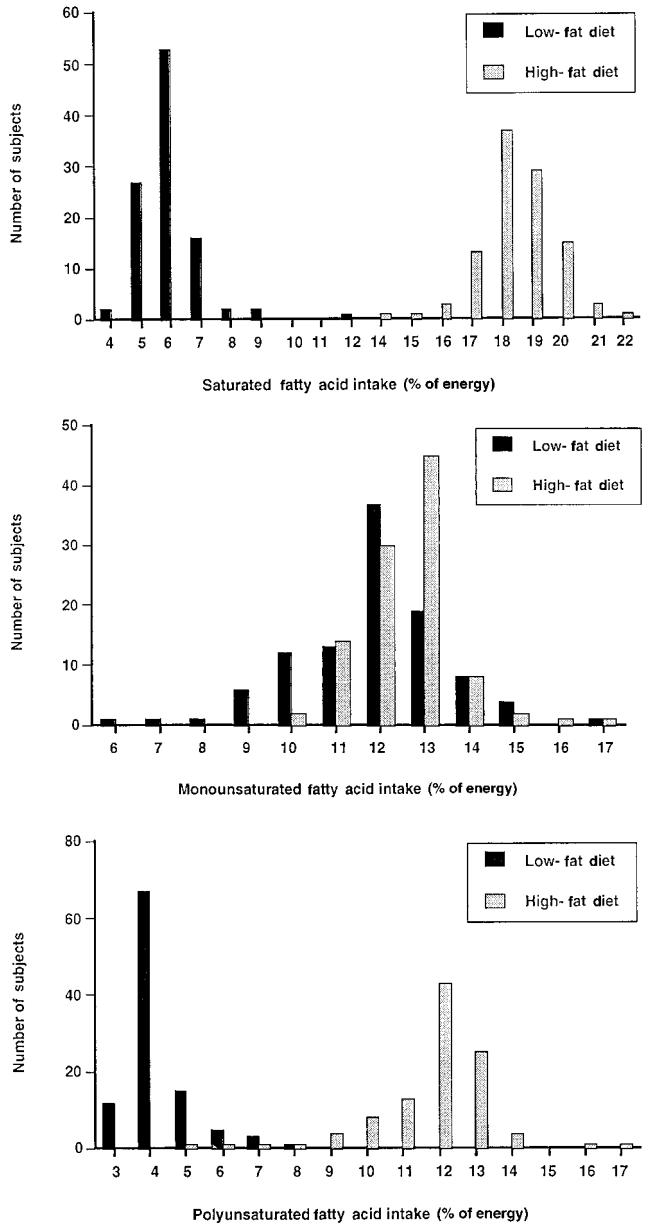

FIGURE 1. Distribution of dietary saturated, monounsaturated, and polyunsaturated fatty acid intake as a percentage of total energy in 103 men consuming low-fat and high-fat diets.

ated with changes in total saturated fatty acids (r_s = 0.20, P < 0.05) and inversely associated with changes in total carbohydrate (r_s = 0.19, P = 0.06). During the high-fat diet, saturated fat was correlated negatively with mass of smaller LDL particles (S_f^o 0–5). Changes in total fat and saturated fat were associated positively with change in large LDL mass (S_f^o 7–12). Change in saturated fat was also associated positively with LDL diameter and flotation rate, indicating increased size of LDL particles. Total monounsaturated fat (and oleic acid) and total polyunsaturated fat (and linoleic acid) did not significantly correlate with plasma lipoproteins during the low-fat and high-fat diets, nor were there associations with dietary change (data not shown). P:S also did not significantly correlate with plasma lipoproteins (data not shown).

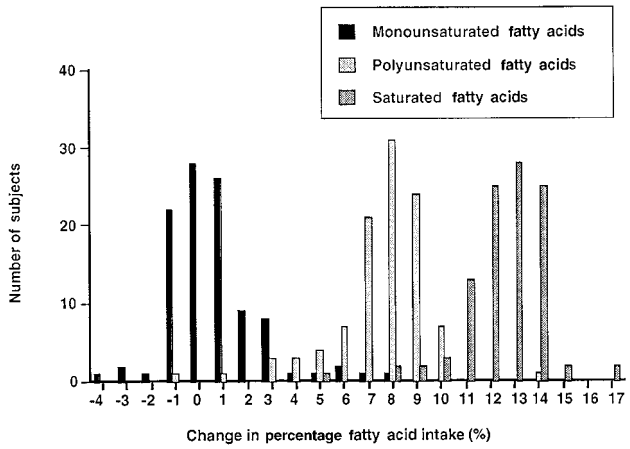

FIGURE 2. Distribution of change in fatty acid intake as a percentage of total energy in 103 men consuming high-fat and low-fat diets (high-fat minus low-fat).

Correlations of individual saturated fatty acids with plasma lipoproteins

Significant correlations of individual saturated fatty acids with plasma lipoproteins during the low-fat and high-fat diets were found for myristic and palmitic acids. During the high-fat diet, myristic acid correlated negatively (P < 0.001) with mass of LDL-III (r_s = −0.38) and LDL-IV (r_s = −0.33) and positively (P < 0.01) with LDL flotation rate (r_s = 0.27). Change in myristic acid correlated positively (P < 0.01) with LDL-I (r_s = 0.28), and LDL diameter (r_s = 0.31) and flotation rate (r_s = 0.32). Change in palmitic acid correlated positively (P < 0.01) with LDL-I (r_s = 0.29) and LDL diameter (r_s = 0.29). Stearic acid was not significantly correlated with plasma lipoproteins during either the low-fat or high-fat diet or during dietary change.

Correlations of dietary nutrients with postheparin hepatic and lipoprotein lipase activities

During both the low-fat and high-fat diets, dietary protein, carbohydrate, monounsaturated fat, polyunsaturated fat, cholesterol, P:S, and fiber, did not correlate with LPL or HL activity (data not shown). There also were no significant correlations between changes in these dietary variables with LPL or HL activity (data not shown). The correlations between dietary total saturated fat, myristic, palmitic, and stearic acids with LPL and HL during the low-fat and high-fat diets are shown in **Table 4**. During the high-fat diet, total saturated fat, as well as myristic and palmitic acids, were correlated inversely with HL activity. Changes in dietary saturated fat and myristic acid were also associated inversely with HL activity. There were no significant correlations between saturated fatty acids and LPL activity during either the low-fat or high-fat diets, nor were the changes in these variables correlated.

DISCUSSION

We describe here associations of dietary nutrient intake with plasma lipoproteins and lipoprotein subclasses in healthy men. The results indicate significant associations of dietary saturated fat intake with plasma LDL-particle distributions. Change in dietary saturated fat was associated positively with mass of larger LDL particles and with peak LDL particle diameter and LDL flotation rate. These results suggest, therefore, that feeding

TABLE 2
Plasma lipoprotein concentrations in all subjects[1]

	Low-fat diet	High-fat diet
Triacylglycerol (mmol/L)	1.59 ± 0.09	1.12 ± 0.05[2]
LDL cholesterol (mmol/L)	3.26 ± 0.08	3.70 ± 0.09[2]
HDL cholesterol (mmol/L)	1.08 ± 0.02	1.27 ± 0.03[2]
Apolipoprotein A-I (mmol/L)	40.87 ± 0.53	44.84 ± 0.60[2]
Apolipoprotein B (mmol/L)	1.98 ± 0.04	2.00 ± 0.05
Lipoprotein mass (g/L)		
VLDL	127.30 ± 8.84	75.91 ± 6.10[2]
IDL	33.49 ± 1.66	32.86 ± 1.64
LDL		
LDL-I (S_f^o 7–12)	92.44 ± 3.91	131.83 ± 4.56[2]
LDL-II (S_f^o 5–7)	106.70 ± 3.48	122.57 ± 3.81[2]
LDL-III (S_f^o 3–5)	81.26 ± 3.98	59.82 ± 3.76[2]
LDL-IV (S_f^o 0–3)	17.99 ± 1.52	10.95 ± 1.02[2]
HDL_2	24.64 ± 2.41	36.94 ± 3.39[2]
HDL_3	181.98 ± 3.06	190.73 ± 3.26[3]
LDL peak particle		
Diameter (nm)[4]	25.86 ± 0.08	26.48 ± 0.07[2]
Flotation rate (S_f^o)	5.30 ± 0.10	6.11 ± 0.10[2]
Lipoprotein lipase (mmol fatty acids · L^{-1} · h^{-1}) (n = 43)	4.09 ± 0.40	4.86 ± 0.48[5]
Hepatic lipase (mmol fatty acids · L^{-1} · h^{-1}) (n = 43)	15.36 ± 0.81	16.65 ± 0.84[2]

[1] $\bar{x}$ ± SEM; n = 103 unless otherwise noted. S_f^o, Svedberg flotation rate.
[2,3,5] Significantly different from low-fat diet: [2] $P < 0.0001$, [3] $P < 0.01$, [5] $P < 0.05$.
[4] Determined by gradient-gel electrophoresis.

saturated fat is associated with increased mass of larger LDL. This association was found with the long-chain saturated fatty acids myristic and palmitic acids, but not with stearic acid.

The present results accord with cross-sectional correlations that show a positive association between diets high in saturated fat and elevations in larger LDL particles (8, 9). The increase in concentrations of larger LDLs is also consistent with results from studies in monkeys indicating that diets high in saturated fat increase LDL particle size (38).

In this study, the association between small LDL and saturated fat was significant with the high-fat diet but not with the low-fat diet. This may be accounted for by somewhat greater variability in dietary adherence to the high-fat diet than the low-fat diet. Alternatively, because there is evidence that LDL subclasses are affected by genetic factors (39–42) as well as nongenetic influences (12, 37, 43, 44), an interaction between a high-fat diet and other determinants of the LDL-particle size distribution may have contributed to the significant associations reported here.

Although increased concentrations of the largest, most buoyant LDL particles have been found in subgroups of patients with coronary artery disease (CAD) (45, 46), it is currently unknown whether increased concentrations of large LDL particles in a healthy population are associated with increased CAD risk. Studies of the relation between LDL subclasses and CAD have, in contrast, established that a predominance of small, dense LDL particles (LDL subclass pattern B) is associated with increased risk of myocardial infarction (47, 48) and angiographically documented CAD (48–50). Some studies have also shown that small LDL particles are potentially more atherogenic than larger LDL because of increased susceptibility to oxidation (51, 52) and increased promotion of intracellular cholesterol ester accumulation (53). In addition, reductions in small LDL particles, not in larger LDL particles, have been associated with decreased CAD progression (54, 55).

An increase in dietary saturated fat has been associated with the progression of CAD independent of LDL-cholesterol concentrations (56), and in cross-cultural studies, higher intakes of dietary saturated fat are associated with higher prevalence rates of CAD (57). This association of increased dietary saturated fat

TABLE 3
Spearman's correlations of percentage total dietary fat and saturated fat with plasma lipids, lipoproteins, and LDL peak particle diameter and flotation rate in 103 men consuming low-fat and high-fat diets[1]

	Total fat			Saturated fat		
	Low-fat diet	High-fat diet	Change	Low-fat diet	High-fat diet	Change
Triacylglyerol	−0.06	−0.12	0.01	0.04	−0.10	−0.09
LDL cholesterol	−0.10	−0.07	0.11	−0.01	−0.14	0.08
HDL cholesterol	−0.11	0.08	0.18	−0.10	0.13	0.20
Apolipoprotein A-I	−0.11	0.00	0.09	−0.04	0.03	0.12
Apolipoprotein B	−0.05	−0.05	0.09	−0.01	−0.18	0.02
Lipoprotein mass						
VLDL	−0.03	−0.12	−0.01	0.04	−0.12	−0.09
IDL	0.06	−0.08	0.11	0.12	−0.10	−0.05
LDL						
LDL-I (S_f^o 7–12)	0.01	0.11	0.29[2]	0.12	0.08	0.30[2]
LDL-II (S_f^o 5–7)	−0.16	−0.06	0.00	−0.02	−0.18	−0.08
LDL-III (S_f^o 3–5)	−0.02	−0.18	−0.17	0.04	−0.31[2]	−0.19
LDL-IV (S_f^o 0–3)	−0.02	−0.12	−0.08	0.04	−0.26[2]	−0.02
HDL_2	−0.13	0.08	0.10	−0.11	0.11	0.18
HDL_3	−0.27[2]	−0.17	−0.08	−0.14	−0.22	−0.14
LDL peak particle						
Diameter[3]	−0.02	0.10	0.17	−0.02	0.16	0.31[2]
Flotation rate	−0.03	0.19	0.10	−0.05	0.22	0.28[2]

[1] S_f^o, Svedberg flotation rate.
[2] $P < 0.01$.
[3] Determined by gradient-gel electrophoresis.

TABLE 4
Spearman's correlations of percentage dietary saturated fatty acid (SFA) intake compared with lipoprotein and hepatic lipase activities for low-fat and high-fat diets and change (high-fat minus low-fat diet) in 43 men

	Total SFA			Myristic acid			Palmitic acid			Stearic acid		
	Low-fat diet	High-fat diet	Change	Low-fat diet	High-fat diet	Change	Low-fat diet	High-fat diet	Change	Low-fat diet	High-fat diet	Change
Lipoprotein lipase	−0.14	0.11	0.17	−0.04	0.16	0.17	−0.15	0.15	0.13	−0.14	−0.04	−0.01
Hepatic lipase	0.24	−0.33[1]	−0.26	0.11	−0.36[1]	−0.33[1]	0.22	−0.32[1]	−0.23	0.20	−0.12	−0.08

[1] $P < 0.05$.

with CAD, however, may be limited to a subset of normolipidemic individuals whose lipoprotein changes differ from those reported in the general population (12, 37). For example, we showed previously that in the minority of subjects studied here with LDL-subclass pattern B following the high-fat diet, concentrations of both small and large LDL particles were higher than for the low-fat diet (37). In contrast, in most subjects with predominantly large LDL (pattern A) following the high-fat diet, concentrations of large LDL particles were higher but small LDL particles concentrations were lower than during the low-fat diet. Therefore, genetic and environmental factors may contribute to such interindividual variation in dietary response and promote variable increases in large LDL particles and more atherogenic small LDL particles with a high-saturated-fat diet (58).

The present study extends information on the relation between changes in diet, lipoproteins, and the lipolytic enzymes (13–15). An increase in dietary saturated fat (specifically 14:0) was associated with increases in large LDL particles (S_f^o 7–12) and with decreases in HL, suggesting that diet-induced changes in HL may contribute to the regulation of large, buoyant LDL particles. This inference is consistent with other reports showing that buoyant LDL particles accumulate in patients with HL deficiency (21, 59) and after inhibition of HL activity in the cynomolgus monkey (60). In addition, more recent reports (61, 62) have shown an inverse relation between buoyant LDL particles and HL.

The distribution of lipoprotein mass among LDL particles is a result of a variety of metabolic events including interconversions that accompany the loss of triacylglycerol during lipolysis (63). In the present study, an increase in large LDL particles was associated with a decrease in plasma triacylglycerol ($r_s = -0.33$, $P < 0.001$) (DM Dreon and RM Krauss, unpublished observations, 1997), consistent with the known inverse relation between triacylglycerol concentration and LDL particle size (63–65). Thus, the catabolism of triacylglycerol-rich lipoproteins is closely linked to LDL subclasses such that decreased triacylglycerol concentrations may promote the production of larger LDL particles (66). Alternatively, plasma concentrations of larger LDL particles may reflect nutritional influences on LDL receptors that regulate different forms of LDL (67).

In the present study, correlation analyses revealed significant positive relations of change in intake of the long-chain saturated fatty acids myristic and palmitic acids with change in plasma concentrations of large LDL particles. These findings are consistent with studies showing that, of the long-chain saturated fatty acids, myristic and palmitic are the most hypercholesterolemic (1–3, 68–73). Also, it was reported that lauric acid (12:0), another long-chain saturated fatty acid, is hypercholesterolemic (70, 71), but the negligible amount of lauric acid in our experimental diets did not allow us to evaluate the magnitude of its cholesterolemic effects. In contrast with the significant association of myristic and palmitic acids with large LDL particles, there were no significant correlations of stearic acid with LDL. This finding is consistent with the results of studies (68, 69, 73–76) showing that, in men, stearic acid is not hypercholesterolemic compared with the other long-chain saturated fatty acids.

In the present study, monounsaturated and polyunsaturated fatty acids did not show associations with plasma lipoprotein concentrations, a finding that differs from reports showing that unsaturated fatty acids are hypocholesterolemic (1, 71). Our unexpected finding may be explained by several factors: *1*) the amounts of monounsaturated and polyunsaturated fatty acids in the diets were lower than those used by other investigators reporting hypocholesterolemic effects of unsaturated fatty acids (1, 77–81), *2*) the P:S was held constant, and *3*) the variance in the range of intakes of unsaturated fats was not large enough to detect associations with lipoprotein concentrations.

Lipoproteins other than LDL were less strongly correlated with dietary variables. Although of marginal significance, the correlations we observed of change in HDL cholesterol with total saturated fatty acid ($r_s = 0.20$, $P < 0.05$) and total carbohydrate ($r_s = -0.19$) were similar to those shown in a meta-analysis with greater numbers of subjects in controlled feeding environments (1). Although changes in triacylglycerol were also reported to occur with changes in both dietary fat and carbohydrate (1), the small number of subjects studied here may have limited the power to detect significant correlations.

In summary, the present study showed that changes in dietary saturated fat are associated with changes in LDL subclasses in healthy men. An increase in saturated fat, and in particular, myristic acid, was associated with increases in larger LDL particles (and decreases in smaller LDL particles). LDL particle diameter and peak flotation rate were also positively associated with saturated fat, indicating shifts in LDL-particle distribution toward larger, cholesterol-enriched LDL. This study also showed that increases in dietary saturated fat were associated with decreases in HL activity. This finding, together with our previous cross-sectional analyses that revealed significant inverse relations of HL activity with LDL peak flotation rate (15), suggests an inverse association of HL activity with concentrations of buoyant LDL particles. Although there is a possibility that a subset of large LDL particles may be atherogenic (46), earlier results (37) point to a differential benefit of low-saturated-fat diets on LDL concentrations in individuals who have an atherogenic lipoprotein profile denoted by a predominance of small LDL particles.

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Letters to the Editor

Bioavailability of iron glycine

Dear Sir:

The title and conclusions of the article, "Bioavailability of iron glycine as a fortificant in infant foods," by Fox et al (1) are not supported by the data presented. Their data show that the absorption of iron from a glycine chelate is as well regulated by the body as is iron absorption from $FeSO_4$. Their data do not address bioavailability because regulation of iron absorption when there are sufficient iron stores in the body is the predominant feature of the research.

The first paragraph of the Results section states that the initial mean hemoglobin concentration of the subjects in study 1 was 114.0 ± 1.4 g/L and in study 2 was 118.0 ± 1.9 g/L. Rather than being iron deficient, the children involved in this test were iron sufficient from the beginning. Data from a 7-mo study involving 185 children with a broad range of iron status indicated that no significant change in hemoglobin status can be expected when initial hemoglobin concentrations are >110 g/L (2).

The claim of degradation of the chelate in the presence of phytates is hypothetical because no attempts were made to determine the molecular natures of the compounds being absorbed. The findings of other experiments indicate different conclusions. Isotope data of Bovell-Benjamin et al (unpublished observations, 1998) confirmed that iron glycinate does not mix with the inorganic iron pool, indicating that the iron glycinate must be absorbed differently than is $FeSO_4$. If the iron glycinate were being broken apart during digestion, there would be no differentiation of the iron pool.

In their discussion, Fox et al cited other investigations in support of their hypotheses. These citations are brief and do not represent all of the conclusions of the authors being cited. The researchers cited by Fox et al (3) actually stated that in their study of weanling rats, mean hemoglobin concentrations increased significantly ($P < 0.001$) with iron glycinate but not with $FeSO_4$. Liver concentrations were also higher with iron glycinate, but the increase was not significant because the animals were growing rapidly. The authors concluded, "Ferrous sulfate is often used as a standard with which to compare the bioavailability of different dietary sources of Fe, and it is unusual to find a compound that has Fe of higher bioavailability, but clearly, the Fe glycine complex was more readily utilized than ferrous sulfate" (3).

The fact that Fox et al included large amounts of ascorbic acid (0.83 mg ascorbic acid/mg Fe) with the $FeSO_4$ doses, but not with the iron glycinate chelate, suggests that they were actually intending to compare the absorption of ferrous ascorbate (and not $FeSO_4$) with that of iron glycinate. Fox et al cited the results of Olivares et al (4) as further proof of the lower bioavailability of the chelate than of $FeSO_4$. Olivares et al also claimed that the absorption of iron glycinate is no different from that of ferrous ascorbate. Olivares et al reported that $FeSO_4$ absorption in milk is only 4–5% compared with 15.4% (when normalized) for iron glycinate. They also reported that $FeSO_4$ absorption can double when ascorbic acid is added. Olivares et al concluded, "Iron bisglycine has a bioavailability comparable to that of $FeSO_4$, plus ascorbic acid in milk." When ascorbic acid was not present with $FeSO_4$, they found that iron glycinate had a bioavailability 2–2.5-fold higher than that of $FeSO_4$.

Finally, Fox et al conjecture that if their hypothesis that iron glycinate disassociates in a manner similar to that of $FeSO_4$ is correct, then iron glycinate will have the same poor organoleptic properties as $FeSO_4$. On the contrary, Olivares et al (4) state that iron glycinate (as the amino acid chelate) has low prooxidant properties and is stable when exposed to ambient air and temperatures. They further state that iron glycinate has a shelf life of >6 mo when mixed with milk and stored at room temperature.

In conclusion, it can be deduced from the data presented by Fox et al that absorption of iron from chelated iron glycinate is as well regulated by the body as is iron from $FeSO_4$ (or ferrous ascorbate) in situations in which there is not a great metabolic need for iron uptake, as indicated by a hemoglobin concentration >110 g/L. No data from a comparison of the bioavailability of iron glycinate and $FeSO_4$ (or ferrous ascorbate) are presented by Fox et al because sufficient iron stores existed at the onset of the study, ensuring that iron uptake from all sources would be tightly regulated by normal physiology to prevent the overabsorption of iron and its subsequent toxicity.

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Reply to HD Ashmead

Dear Sir:

The results of our study entirely support Ashmead's proposal that absorption from iron glycine chelate is regulated to the same extent as that from $FeSO_4$ (or ferrous ascorbate). However, we disagree with his statement that we were not measuring bioavailability.

The conclusions drawn from our study are based on the measurement of hemoglobin incorporation of an oral dose of stable isotope–labeled $FeSO_4$ and stable isotope–labeled iron glycine chelate. This technique assumes that 90% of the absorbed iron is used for hemoglobin (1) and is a valid method for comparing the absorption and bioavailability of 2 different chemical forms of iron within the same individual (when 2 different stable isotopes are used to label the compounds). The use of subjects who are iron deficient would increase the sensitivity of iron absorption measurements. Although the infants in our study had hemoglobin concentrations >110 g/L, their neonatal iron stores would have been depleted by 9 mo of age and thus they would have had a high iron requirement due to rapid growth and, hence, a high efficiency of iron absorption. However, even in the absence of iron deficiency, the method used in our study would still be a valid technique to compare the bioavailability of different chemical forms of iron. As for Ashmead's comments on our earlier work, it is difficult to extrapolate iron absorption data from animal studies to humans because rats are known to have a higher fractional absorption of iron and are less sensitive to differences in iron bioavailability than are humans.

Many studies investigating the bioavailability or absorption of iron use a reference dose to normalize results between individuals. $FeSO_4$ in combination with ascorbic acid is the most commonly used reference dose and many researchers have used molar ratios of ascorbate to iron >1:1 [Cook et al (2), 2:1; Bezwoda et al (3), 10:1; and Hallberg et al (4), 10:1]. We used a molar ratio of 1:1 [iron as $Fe_2(SO_4)_3$] and most of the ascorbate involved in the reduction of ferric iron to ferrous iron would have been oxidized to dehydroascorbic acid or dioxogulonic acid. The resulting solution would therefore be mainly $FeSO_4$ and not ferrous ascorbate.

Our findings confirm that the iron glycine chelate is indeed a highly bioavailable form of iron because hemoglobin incorporation was comparable with that of freshly prepared $FeSO_4$ in the presence of ascorbic acid. Ashmead's comment that our reference to the work of Olivares et al (5) was further proof of the iron glycine chelate having a lower bioavailability is incorrect; we cited this reference in support of our observation that the absorption of iron glycine chelate and $FeSO_4$ are similarly affected by dietary modifiers. There was no mention made in our paper that the iron glycine chelate had a lower bioavailability than $FeSO_4$ or ferrous ascorbate. The absorption of iron from the glycine chelate was reduced by the presence of a known inhibitor of iron absorption, phytic acid. From this observation we concluded that some or all of the iron from the chelate had dissociated at some point and mixed with the intraluminal pool of ingested nonheme iron, where some of it was rendered unavailable through ligand formation with phytic acid.

Olivares et al (5), who used the same chelate we did (prepared by Albion Laboratories), also found that the absorption of iron glycine chelate was reduced by inhibitors found in milk and that a known enhancer (ascorbic acid) increased iron absorption from the chelate. These observations further substantiate our conclusion that iron is dissociated from the chelate in the gastrointestinal tract, where it can participate in chemical reactions with other dietary constituents. Exactly where, when, and how much of this dissociation takes place is open to further investigation. What can be postulated is that if the poor organoleptic properties associated with $FeSO_4$ under certain food conditions are not observed with the chelate, then dissociation of the iron glycine complex must be occurring within the gastrointestinal tract after ingestion. Ashmead cites unpublished work that apparently refutes our findings. Clearly, we cannot comment on this at present.

If absorption of the iron glycine chelate is being regulated by the body, as proposed by Ashmead in his letter, we must ask by what mechanism? If the iron chelate is absorbed intact by an amino acid transport mechanism, regulation would be governed by the presence of glycine and not iron. Thus, there would be no regulation of iron absorption per se. The other possibility is that the chelate dissociates and iron enters the common nonheme pool, the absorption of which is controlled by host-related factors such as iron stores, which is the mechanism indicated by our data and that of Olivares et al (5).

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Mild cobalamin deficiency in older Dutch subjects

Dear Sir:

The report by van Asselt et al (1) is of great interest and I could not agree more with most of their conclusions. Permit me to add

several of our observations in support and extension of theirs. We too have been impressed that half or more of the low cobalamin concentrations in the elderly (and others) cannot be explained by malabsorption (2–4). Because factors other than cobalamin status may affect serum cobalamin concentrations (4), it is important to keep in mind that 25% or more of the low cobalamin concentrations are not accompanied by any metabolic abnormalities and may not represent actual deficiency. Nevertheless, the causes responsible for the low concentrations, especially for the 75% that are associated with metabolic evidence of deficiency, need to be identified. Like van Asselt et al, we have found poor dietary intake of cobalamin to be virtually nonexistent in the elderly (5). Our data also support their observation of an ameliorative effect of cobalamin supplement use on cobalamin status, although it is noteworthy that many of our patients remained mildly deficient despite supplement use (5). Ironically, supplement use also appeared to be highest in subjects who had higher cobalamin intakes from food and, thus, presumably a lesser need for supplementation.

However, a strong word of caution is in order about any automatic equation between atrophic gastritis and food– cobalamin malabsorption. The 2 are not synonymous (3). Half of the patients with severe food– cobalamin malabsorption whom we biopsied and subjected to gastric analysis had neither atrophic gastritis nor achlorhydria (6). Thus, although nearly all patients with atrophic gastritis may have food– cobalamin malabsorption, many without atrophic gastritis may also have food– cobalamin malabsorption. Van Asselt et al might have found a higher prevalence of malabsorption and perhaps even a stronger association with Helicobacter pylori infection had they actually tested absorption directly.

It is too early in our still incomplete understanding of food-cobalamin malabsorption to allow ourselves the liberty of resorting to indirect markers when studying it. In recent years, various authors have proposed not only gastric and duodenal histology but serum gastrin concentrations, holotranscobalamin II concentrations, and other such substitutes for direct testing of food– cobalamin malabsorption. None of these substitutes were ever proven to be satisfactorily specific or sensitive, and at least one of the claims of equivalence has been retracted. I fear that unwarranted methodologic shortcuts will only add confusion to the subject.

As for Dr. Russell's accompanying editorial (7), early answers have begun to appear to his question concerning consequences of elevated methylmalonic acid concentrations (or more precisely, of mild, preclinical cobalamin deficiency). Over the years, we have consistently found electroencephalographic, evoked potential, and P300 potential abnormalities in half or more of our patients with metabolically defined, mild, preclinical cobalamin deficiency (8–10). In most cases, these abnormalities were reversed with cobalamin therapy. Moreover, mild but reversible clinical abnormalities, including neuropathy and memory loss were part of the picture in several patients. The extent of this subtle neurologic dysfunction and its contribution to the risks of mild cobalamin deficiency is an important area for further study.

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Use of daily compared with weekly iron supplementation: apples and pears

Dear Sir:

About 10 y ago the World Health Organization (1) published recommendations on the design of large-scale iron supplementation programs with the aim of reducing the prevalence of iron deficiency anemia in populations of developing countries. One decade later, however, little has changed in the situation of iron deficiency anemia. Supplementation programs, when they exist at all, are largely ineffective for a variety of reasons, the most important being insufficient supply of iron tablets, low coverage of the target population, and poor compliance with tablet intake (2). A regimen that offers the possibility of lower cost, better compliance, and effectively raised hemoglobin concentrations in 2 or 3 mo is therefore surely worth consideration.

From the viewpoint of a clinician, Hallberg (3) appealed for the continued application in supplementation programs of the well-established, although inefficient, daily administration of iron and urged that supplementation on a weekly basis not be considered. His reason for this argument was that daily supplementation would provide a more rapid response in the treatment of anemia because the total amount of iron absorbed from a

given dose would be ≈6 times larger from a dose divided into daily administrations than from a corresponding weekly dose.

Community-based studies in children and nonpregnant women from China (4), Bolivia (5), Indonesia (6–8), and Vietnam (9), however, did not show a marked difference in hemoglobin response to weekly or daily iron supplementation. The existence of mild-to-moderate rather than severe anemia, a long duration of supplementation, and high doses of iron were mentioned by Hallberg as reasons daily supplementation did not have a better effect on hemoglobin status than weekly supplementation in the these studies. First, the anemia prevalence of the studied populations was ≈20–50%, which is typical for populations in developing countries that are generally considered in need of supplementation (1). Although the prevalence was high, most subjects had mild-to-moderate forms of anemia. Nevertheless, a high prevalence of even mild degrees of anemia (hemoglobin: 90–110 g/L) has profound consequences for human development (1). Second, the duration and the dose of the daily iron supplementation were in line with recommendations for iron supplementation programs made by the World Health Organization (1). The duration of an intervention in a long-term prevention program should not be judged by comparison with a short-term therapeutic response, even though the studies also showed an adequate therapeutic response over the period of observation.

Hallberg also argued that hemoglobin concentrations would rise faster if high amounts of iron were given on a daily basis. This argument is valid and important for therapy in individuals with severe anemia in a hospital setting. However, speed of recovery has less importance in programs aimed at broad coverage, for which factors such as distribution, cost, and compliance are of high importance.

To date, only one study has been published on the effect of weekly supplementation in pregnant women (10). Hallberg laments the lack of a control group in this study and even doubts the ethical correctness of trials without a placebo group. This remarkable statement again uncovers conceptual differences between clinical theory and practical reality. The recommendation to include a placebo group may be relevant for clinical efficacy trials but is unethical in operational research in countries such as Indonesia where every pregnant woman is entitled to receive iron supplementation according to law. Hallberg also points to the low increase in hemoglobin concentrations in both the daily and weekly intervention groups (10). Although this is true, this study was carried out under program circumstances in which tablet intake was not supervised and under these conditions the effects of weekly and daily supplementation were not significantly different.

Innovation is required to solve the repeated problem of low effectiveness of daily iron supplementation programs under practical conditions. Studies published to date indicate that weekly supplementation may be a much cheaper option (11) for improving the iron status of children and nonpregnant women because it reduces anemia prevalence similarly to daily supplementation when used for the currently recommended duration. Because of the lack of completed studies, however, conclusive recommendations for pregnant women must await additional efficacy and effectiveness studies.

This is not the first and will not be the last discussion resulting from the misleading assumption that clinical experience with treatment can be uncritically applied to practical, population-based interventions. It is important to draw a clear line between appropriate therapy of moderate-to-severe anemia in individuals and cost-effective population-based programs. Let us recognize that apples are apples, and pears are pears. Both are valuable fruit and are tasty when eaten at the right moment of ripeness. But don't try to make an apple pie from pears!

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Reply to W Schultink and R Gross

Dear Sir:

It is obvious that Schultink and Gross misunderstood the essence

of my critique of their recommendation to give iron weekly in combating iron deficiency (1). The concept that weekly administration of iron would be as effective as daily administration was based on the hypothesis that there is a mucosal block in the absorption of therapeutic doses of iron that makes a continuous (daily) supply of iron unnecessary, redundant, and irrational. As shown in my critical review (1), however, there is no such mucosal block during iron therapy in humans and thus no foundation for the concept of giving iron weekly. Actually, there is good quantitative evidence from 2 research groups that ≈6 times more iron is absorbed from daily doses than from weekly doses of the same total amount of iron (2, 3). At first glance it was then surprising that the therapeutic response—the increase in hemoglobin concentration—was almost the same after weekly compared with daily administration of iron in several studies. A reasonable explanation for these paradoxical findings, as pointed out in my analysis of these studies, is that the doses of iron given were high, the treatment periods were long, and the subjects included in the studies had only mild anemia. Because hemoglobin concentrations can reach only individual optimal concentrations, no valid comparisons of the efficacy of the 2 treatment models can be made; the therapeutic response can be expected to be the same under the conditions used. It would be expected, for example, that lower daily iron doses or much shorter treatment periods would result in the same increase in hemoglobin. Moreover, under such conditions even better compliance would be expected at a lower cost than that for administering high doses of iron weekly.

The arguments for weekly administration of iron used by Schultink and Gross are the "low effectiveness of daily iron supplementation programs under practical conditions" and that "innovation is required." It is certainly true that great efforts must be made to improve iron supplementation programs, especially in developing countries. For example, the delivery system of tablets must be improved so that tablets reach the target subjects; it is also desirable to improve the pharmaceutical properties of the iron tablets to increase their efficacy and reduce side effects. It is time to take new initiatives in this area (4). A further important area for research, initiated for example by the World Health Organization (WHO) and the United Nations Children's Fund (UNICEF), would be the development of better methods to motivate subjects to take tablets, for example, during pregnancy. I certainly share the disappointment of Schultink and Gross that so little has been done to combat iron deficiency by the WHO, UNICEF, and the countries involved. It is important to emphasize, however, that a change to the less-efficient weekly administration of iron would not solve the key problems of good efficacy, adequate tablet distribution, and high motivation.

Schultink and Gross do not seem to understand that before starting national or regional supplementation programs or any diagnostic, prophylactic, or therapeutic program, it is important to critically examine the effectiveness of such programs, including costs. This is not just a clinical approach that is "uncritically applied to practical, population-based interventions." The use of carefully controlled studies, with inclusion of groups given placebo, is a standard not only in clinical trials but also in field studies in developing countries. This fundamental approach has been discussed in detail in different publications from the WHO over the years on the basis of work of experts from countries where iron deficiency anemia is both common and severe and is not based solely on the work of so-called "clinicians." In all these reports and recommendations it is clearly stated that subjects should first be divided into groups according to severity of anemia (because the therapeutic response is related to the severity of anemia) and then be randomly allocated to different groups that will be given, for example, different doses of iron. It is also clearly emphasized that one of the groups must be a placebo group so that both effects and side effects can be evaluated (5–7). For ethical reasons, those with more severe iron deficiency anemia are excluded. The cutoff for severe iron deficiency anemia is different in different countries. In some of the Indian studies (8) and in the WHO reports (5, 6) the limit was set at a hemoglobin concentration of 80 g/L. In other studies cutoffs as high as ≤120 g/L were used (9). The details of the experimental design may also vary in different studies, for example, if the role of folate deficiency is also tested or if the effects of hookworm infestation are excluded.

The statement that controlled studies of pregnant women are not allowed in Indonesia is surprising. Even so, controlled studies can be carried out in any of the many other countries where daily and weekly administration of iron have been compared. The strict attitude of the ethics committees in Indonesia toward assignment of pregnant women to placebo groups makes it hard to believe that iron supplementation with a dose associated with one-sixth of the absorption of daily doses could ever have been approved.

The development of good iron supplementation programs is important considering our increasing awareness of the importance of an optimal supply of iron in infants, children, and adolescents. It is important that future work in developing countries be based on adequate, carefully controlled studies. The metaphor that such studies are apples and the ones carried out by Schultink and Gross are pears does not necessarily form a good basis for a fruitful discussion of an important topic.

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No evidence for dietary protein and dietary salt as main factors of calcium excretion in healthy children and adolescents

Dear Sir:

Itoh et al (1) state new evidence in their recent paper in this Journal that protein intake is a main factor of calcium excretion. They report detailed findings on the relation between protein, sodium, and calcium intakes and calcium excretion. Moreover, they provide estimates of the size of the effect. In what follows, I will explain some weaknesses in their data interpretation that may have resulted in overestimation of the effects. In addition, I have evidence of a different finding in healthy children and adolescents, and hence conclude that in these populations there is no evidence that dietary salt and dietary protein are main factors of calcium excretion.

Itoh et al state that errors in the measurement of daily dietary protein intake are relatively high. It is well known that measurement error in factor variables causes biased effect estimates in standard multiple regression and that the bias depends on the accuracy of the measurement—see review by Reeves et al (2). Therefore, the authors used urinary urea excretion and urinary sulfate excretion to assess the effect of the components of protein intake. Urinary measurements of urea, sulfate, calcium, and sodium in Itoh et al's study were likely to have correlated measurement errors because the measurements were made in the same urine sample. Consequently, a crucial assumption of standard multiple regression analysis was not valid. The inappropriate use of multiple regression analysis led to the unjustified conclusion that dietary salt and protein intakes have a major effect on calcium excretion. Two other studies also report evidence that dietary salt is a main factor regulating calcium excretion; however, both of these studies suffer from the same misuse of multiple regression and consequently their findings are not valid (3, 4).

In a preliminary interim analysis of data from the Dortmund Longitudinal Study (5) in healthy German children and adolescents aged 2.8–18.3 y (1985–1997: n = 187 boys and 850 observations and n = 180 girls and 807 observations), Lausen (6) showed that interindividual variation, reciprocal relative growth velocity [(Δcm/y)/cm], and dietary protein intake (g/d) are the most important factors regulating urinary calcium excretion (mg/d). Other significant factors are magnesium intake (mg/d), sodium intake (mg/d), calcium intake (mg/d), and phosphorus intake (mg/d). The longitudinal data show that individual excretion explained 69% of the variance in the data ($R^2 = 0.69$).

In summary, evidence for dietary protein and dietary salt as main factors of calcium excretion when intakes of protein, salt, and calcium are within usual ranges is not convincing.

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Reply to B Lausen

Dear Sir:

We appreciate greatly the sound criticism of Lausen on the weakness of the data interpretation in our study. Recently, we also realized the bias in the analysis of the relation of the measured variables in the same 24-h urine specimens. As Lausen correctly pointed out, the positive correlations observed among the measurements of daily calcium excretion were possibly due to errors commonly associated with urinary specimens, especially errors that occur in 24-h urine collections, although we carefully excluded subjects who appeared to have had problems with urine collection. Thus, we reanalyzed the data from our previous study using daily excretions of various urinary constituents corrected for daily urinary creatinine excretion (1). The results of multiple regression analyses still showed significant and positive correlations between calcium-creatinine, urea-creatinine, calcium-creatinine, and sodium-creatinine ratios after adjustment for sex, age, and calcium intake in both age groups: 20–49- and 50–79-y-olds.

Of course, we recognize that multiple regression analysis does not completely eradicate the bias. However, multiple regression analyses showed that the daily dietary intake of protein estimated from dietary records was significantly and positively correlated with daily calcium excretion in the same population (1).

The reciprocal association between urinary calcium excretion and dietary protein intake observed by Lausen in healthy children and adolescents is an interesting and important finding. The association is reasonable because protein is an important constituent of bone, and young individuals, contrary to adults, require large amounts of protein for bone growth and are in strong positive

nitrogen balance. It is highly possible that low protein intakes prevent effective utilization of dietary calcium for bone formation, resulting in an increase in urinary calcium excretion.

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Erratum

Mendoza C, Viteri FE, Lönnerdal B, Young KA, Raboy V, Brown KH. Effect of genetically modified, low–phytic acid maize on absorption of iron from tortillas. Am J Clin Nutr 1998;68:1123–7.

Table 4 was inadvertently omitted from the article during production.

TABLE 4
Iron absorption from study diets and ferrous ascorbate[1]

	100% WTM tortillas	50% WTM + 50% LPM tortillas	100% LPM tortillas	Reference dose
Iron absorbtion (% of dose)	1.93 (1.27, 2.97)[a]	1.65 (1.13, 2.40)[a]	2.88 (1.93, 4.29)[b]	14.14 (10.88, 18.40)
Relative iron absorbtion (%)[2]	5.48 (4.56, 9.26)[a]	4.66 (3.22, 6.23)[a]	8.15 (5.48, 11.33)[b]	—

[1] Geometric $\bar{x}$; 95% CI in parentheses. n = 13. WTM, wild-type maize; LPM, low–phytic acid maize. Means within a row with different superscript letters are significantly different, $P < 0.05$ (repeated-measures ANOVA on unadjusted values and on the relative values followed by post hoc Tukey's analysis).

[2] Percentage after adjustment to 40% of reference dose absorption.

Erratum

Åkesson A, Bjellerup P, Berglund M, Bremme K, Vahter M. Serum transferrin receptor: a specific marker of iron deficiency in pregnancy. Am J Clin Nutr 1998;68:1241–6.

Parentheses were inadvertently omitted from the equations used to calculate sensitivity, specificity, positive predictive value, and negative predictive value. On page 1242, the first paragraph of the "Statistical analyses" section should read as follows:

> Sensitivity was defined as TP/(TP + FN) × 100 and specificity as TN/(TN + FP) × 100, where TP is true positive, FN is false negative, TN is true negative, and FP is false positive. Positive predictive value was defined as TP/(TP + FP) × 100 and negative predictive value as TN/(TN + FN) × 100.

Erratum

Dreon DM, Fernstrom HA, Campos H, Blanche P, Williams PT, Krauss RM. Change in dietary saturated fat intake is correlated with change in mass of large low-density-lipoprotein particles in men. Am J Clin Nutr 1998;67:828–36.

In Table 2, on page 832, all lipoprotein mass values should be given as mg/L as follows:

TABLE 2
Plasma lipoprotein concentrations in all subjects[1]

	Low-fat diet	High-fat diet
Lipoprotein mass (mg/L)		
VLDL	1273.0 ± 88.4	759.1 ± 61.0[2]
IDL	334.9 ± 16.6	328.6 ± 16.4
LDL		
LDL-I (S_f^0 7–12)	924.4 ± 39.1	1318.3 ± 45.6[2]
LDL-II (S_f^0 5–7)	1067.0 ± 34.8	1225.7 ± 38.1[2]
LDL-III (S_f^0 3–5)	812.6 ± 39.8	598.2 ± 37.6[2]
LDL-IV (S_f^0 0–3)	179.9 ± 15.2	109.5 ± 10.2[2]
HDL_2	246.4 ± 24.1	369.4 ± 33.9[2]
HDL_3	1819.8 ± 30.6	1907.3 ± 32.6[3]