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SUMMARY

Problem.

Digested fat is absorbed in the small intestine, where cells produce lipoprotein particles (chylomicra) that contain 300,000 to 500,000 triglyceride molecules. The composition of fatty acids consumed is reflected in the composition of fatty acids in the chylomicra.

In some mammals (e.g., rats, monkeys, and cattle), dietary saturated fats lead to the production of plasma chylomicra enriched in saturated triglycerides (TG). When these chylomicra are cooled to < 20°C *in vitro* they form solid-phase TG cores that melt well above physiological temperatures (> 45°C). Temperatures < 20°C have been documented in human extremities (e.g., fingers and toes) during occupational and recreational activities (e.g., scuba diving and open boat operation). Production of such chylomicra could have detrimental physiological consequences for humans during prolonged cold exposure. Such solidified core chylomicra (0.5 - 2.0 μ in diameter) would be expected to persist, and could aggregate and impede blood flow through arterioles (20-50 μm in diameter) and capillaries (2-8 μm in diameter) to the fingers, toes, and essential organs (e.g lungs, heart, and brain). Thus, personnel conducting operations in cold environments after consuming a meal high in saturated fats could be at increased risk of impaired blood flow and non-freezing cold injury.

Objective.

This study was conducted to determine if humans produce chylomicra capable of forming solid-phase TG cores of saturated fat when their plasma is cooled to < 5°C *in vitro* after consuming a meal high in saturated fat.

Approach.

Twelve volunteers were recruited from Naval Special Warfare commands at Naval Amphibious Base, Coronado. Subjects consumed a breakfast substantially enriched in saturated fats (68 g of total fat, 27 g saturated fat) and provided a blood sample 4.5 h later. Plasma was separated and analyzed for chylomicra of densities between 1.006 to 1.020 g/ml after chilling (< 5°C). Plasma was also analyzed for total cholesterol, high density lipoprotein (HDL) cholesterol, and TG concentrations.

Results.

Only 6 of 12 individuals had sufficient plasma triglyceride concentrations (>200 mg/dl) to permit fractionation of chylomicra. After cooling the plasma to < 5° C and separating the lipids by ultracentrifugation, no chylomicra were found with solid-phase cores (i.e., sedimented with intermediate density lipoproteins (IDL) between 1.006 and 1.020 g/ml). The remaining six individuals (with low plasma triglycerides) did not exhibit the anticipated rise in plasma triglycerides 4.5 h after eating the high-fat meal. In fact, plasma triglyceride concentration was found to be inversely related to high density lipoproteins (HDL) concentration (r = 0.785) for the group of 12 subjects.

Conclusion.

Several possibilities may explain the absence of the fat-enriched chylomicra in these individuals at the time of sampling: (1) the meal was not sufficiently rich in saturated fats to produce these chylomicra; (2) sampling blood at 4.5 h postprandial missed the peak levels of these chylomicra; and/or (3) their physical fitness levels enhanced their capacity to clear chylomicra from the plasma. In summary, we did not find fat-enriched chylomicra that form solid-phase triglyceride cores in the blood of Naval special warfare personnel after they ate a meal that was absolutely and relatively high in saturated fats.

INTRODUCTION

Digested fat is absorbed in the small intestine, where cells produce lipoprotein particles called chylomicra; each particle contains 300,000 to 500,000 triglyceride molecules. In the plasma, chylomicra are degraded by lipase, which releases fatty acids, glycerol, cholesterol, and phospholipids which are absorbed in the capillary beds of the major organs (Bachorik, Levy, & Rifkind, 1991; Puppione et al., 1982). The composition of fatty acids consumed is reflected in the composition of fatty acids in chylomicra. Chylomicra are produced and begin to appear in the blood 2 h after a meal, reaching a peak after 4 to 5 h, and continue to be released into the circulation for up to 6 to 8 h (Harris, Connor, Alam, & Illingworth, 1988).

Studies using mammals (i.e., rats, monkeys, and cattle) have shown that the lipid core of chylomicra can undergo a phase transition from liquid to a metastable solid upon exposure to temperatures below 22°C. These chylomicra with semi-solid centers of triglycerides became denser than other chylomicra and separated with the intermediate density lipoproteins (IDL). This was first discovered in studies of plasma and enterocyte lymph collected from cattle (Puppione et al., 1982). In another study, rats fed tripalmitin (a triglyceride composed solely of palmitic acid, a 16 carbon saturated fatty acid) produced chylomicra with cores that crystallized between 17 and 22°C in vitro (Clark et al., 1982). When monkeys were fed a diet containing 40% of the calories as butter fat (55% saturated fat), it was found that 20 to 30% of the chylomicra core crystallized when the blood was cooled to 16°C in vitro (Parks et al., 1981). The solidified core of these chylomicra did not melt until the temperature was increased to > 45°C. The solidifying core was not seen in chylomicra produced in monkeys when they were fed an isocaloric diet substituting safflower oil (8.6% saturated fat) for the butter fat (Parks et al., 1981).

To date, no research has been conducted to determine the metabolic fate of these solidified chylomicra in mammals. We speculate that chylomicra that undergo a physiologically irreversible phase transition to solid-phase triglyceride cores would result in potentially dangerous consequences. These transformed chylomicra would become resistant to lipase activity and, consequently, have a longer postprandial residence in the plasma. These persistant chylomicra may aggregate over time and impede blood flow to essential tissues and organs (e.g., heart, lung, and brain) (National Cholesterol Education Program, 1993). U.S. Navy Sea-Air-Land (SEAL) operators and Naval Special Boat personnel are often exposed to cold water and air for prolonged periods of time. Skin temperatures of the feet, forearms, and hands are often below 20°C for several hours during field operations (W. K. Prusaczyk, 1997). The temperature of blood circulating in these areas could drop to 25°C or less (2 cm into the muscular tissue) within 3 h during exposure to 20°C air or water (Ducharme & Tikuisis, 1991). Chylomicra in the blood circulating through the extremities during cold exposure would be exposed to these low temperatures. The objective of this research was to determine if this kind of chylomicron is produced in humans. Our approach was to provide a meal containing 132% of U.S. Dietary Guidelines for Americans (American Heart Association, 1986) for saturated fat and to analyze the blood at < 10°C for the presence of these chylomicra.

METHODS

Subjects.

Potential subjects from the Naval Special Warfare community were briefed on the background and purpose of the study. Twelve volunteers met the selection criteria and gave written, informed consent. Exclusion criteria were personal or family history of coronary heart disease, atherosclerosis, hyperlipidemia, and diabetes mellitus or tendency towards diabetes. Participants were required to avoid cold exposure and to refrain from exercise on the morning of the study until after their blood was drawn.

Protocol.

Subjects arrived at the test site and consumed a prescribed breakfast between 0700 and 0730. The meal consisted of two McDonald's® Sausage McMuffins® with egg, two military-issue Mars® Desert chocolate bars, and an unlimited amount of orange juice. A detailed description of the meal and percentage of USDGA are provided in Table 1 (McDonald's Corp., 1994).

Table 1. Composition of the prescribed high fat breakfast (solid food only)

□	Total Kcal	Fat (Kcal)	Fat (g)	Saturated Fat (g)	Cholesterol (mg)
Two Sausage McMuffin® with Egg	860	460	50	16	540
Two Mars® Desert Chocolate Bar	300	170	18	10.8	60
Total Solid Composition of Meal	1160	630	68	27	600
Percent USDGA (%)	n/a	103	103	123	200

Approximately 4.5 h after completing the meal, a 10 ml sample of venous blood was drawn by a Navy corpsman. Anthropometric measurements were also taken which included height, weight, and circumference of neck and abdomen (measured in triplicate) to estimate percentage of body fat (Hodgdon and Beckett, 1984). Subjects' anthropometric data are presented in Table 2.

Table 2. Subjects' anthropometric data and postprandial plasma lipid/lipoprotein concentrations.

Subject #	Age (yr)	Body Weight (kg)	Height (cm)	Body Fat (%)	Triglycerides (mg/dl)	HDL Cholesterol (mg/dl)	Total Cholesterol (mg/dl)
1	31	80.4	178.7	16	223	35	183
2	42	81.2	172.1	15	107	72	254
3	30	102.1	189.2	22	158	40	173
4	33	88.3	172.5	23	192	50	265
5	23	82.3	174.0	15	194	55	226
6	26	88.4	178.8	25	167	60	272
7	29	82.3	174.2	20	260	37	183
8	21	77.7	176.2	20	260	37	183
9	39	96.6	181.5	22	422	27	142
10	23	93.2	181.0	26	216	33	227
11	34	86.2	185.7	20	58	68	152
12	45	89.7	173.1	26	226	37	235
$\bar{x} \pm SD$	31±7.8	87.4 ± 7.2	178 ± 5.5	21 ± 4	203 ± 89	47 ±15	209 ± 44

Analysis of Blood Plasma.

Blood was drawn in vacutainer tubes treated with liquid EDTA (K_3), placed on ice, and transported to the laboratory (Lipid Laboratory, U.C.S.D. Medical Center, San Diego, CA 92161) for analysis. Plasma was separated by centrifugation and an aliquot removed to determine total cholesterol, HDL cholesterol, and triglyceride concentrations. HDL cholesterol was precipitated with heparin-Mn^{+2} (Bachorik et al., 1991; Warnick & Albers, 1979). Plasma and supernatant cholesterol were determined enzymatically with a coupled enzymatic system (Boehringer Mannheim Diagnostics No. 236691, 1987; Siedel et al., 1983; Wiebe and Bernert, 1984). Plasma triglycerides were also determined enzymatically with a coupled enzymatic system (A-GENT Triglycerides Test, 1975).

Six of the twelve participants were identified as having plasma triglyceride levels in excess of 200 mg/dl. Their plasma samples were fractionated further for IDL (densities of 1.006 to 1.020 g/ml) at < 5°C using ultracentrifugation (Puppione et al., 1982). IDL fractions were analyzed for cholesterol and triglyceride as described above. A high ratio of triglyceride to cholesterol (> 5) would indicate the presence of chylomicra. If their chylomicra had triglyceride cores that become metastable solids after exposure to temperatures less than 20°C, these chylomicra would be sedimented with the IDL fraction.

If chylomicra were indicated in the IDL fraction, microscopic examination of the samples would confirm their presence.

Statistics.

Correlation coefficients were calculated for physical characteristics and lipid/lipoprotein parameters, using SYSTAT software (Wilkinson, 1989). A 5% alpha error was selected as the criterion to declare significance.

RESULTS

Anthropometric Measurements.

The participants ranged in age from 23 to 45 years, and from 15% to 26% body fat (Table 2). No significant correlation was found between age, body fat, or any of the plasma lipid/lipoprotein parameters measured in these 12 participants.

Lipid Profiles.

Table 2 contains the results of plasma lipids analysis of the blood sampled approximately 4.5 h after consumption of the fat-enriched breakfast. The breakfast included 68 g of fat and 600 mg of cholesterol, which is considered sufficient to increase the concentration of plasma lipids 4.5 h postprandially (Harris, et al., 1988). Yet, the postprandial total cholesterol ranged from low levels of 142 to 272 mg/dl, and triglycerides from 58 to 422 mg/dl. The lowest triglyceride concentration is considered low for a postabsorptive level, and the highest triglyceride concentration is high even for a postprandial level (National Cholesterol Education Program, 1993). The total cholesterol and triglyceride concentrations were not significantly correlated with each other, or with estimated body fat ($r < 0.4$).

Postprandial triglyceride concentrations correlated inversely with HDL cholesterol (Figure 1). The highest concentration of plasma triglyceride, 422 mg/dl, corresponded with the lowest concentration of HDL cholesterol at 27 mg/dl, and the lowest plasma triglyceride concentration of 58 mg/dl corresponded with the second highest HDL cholesterol concentration of 68 mg/dl.

Figure 1. The concentration of plasma triglycerides plotted as a function of HDL cholesterol (n=12). The simple (i.e., product-moment) correlation coefficient (r) between plasma triglyceride and HDL was 0.785, where $r_{(0.05, 10)} = 0.576$.

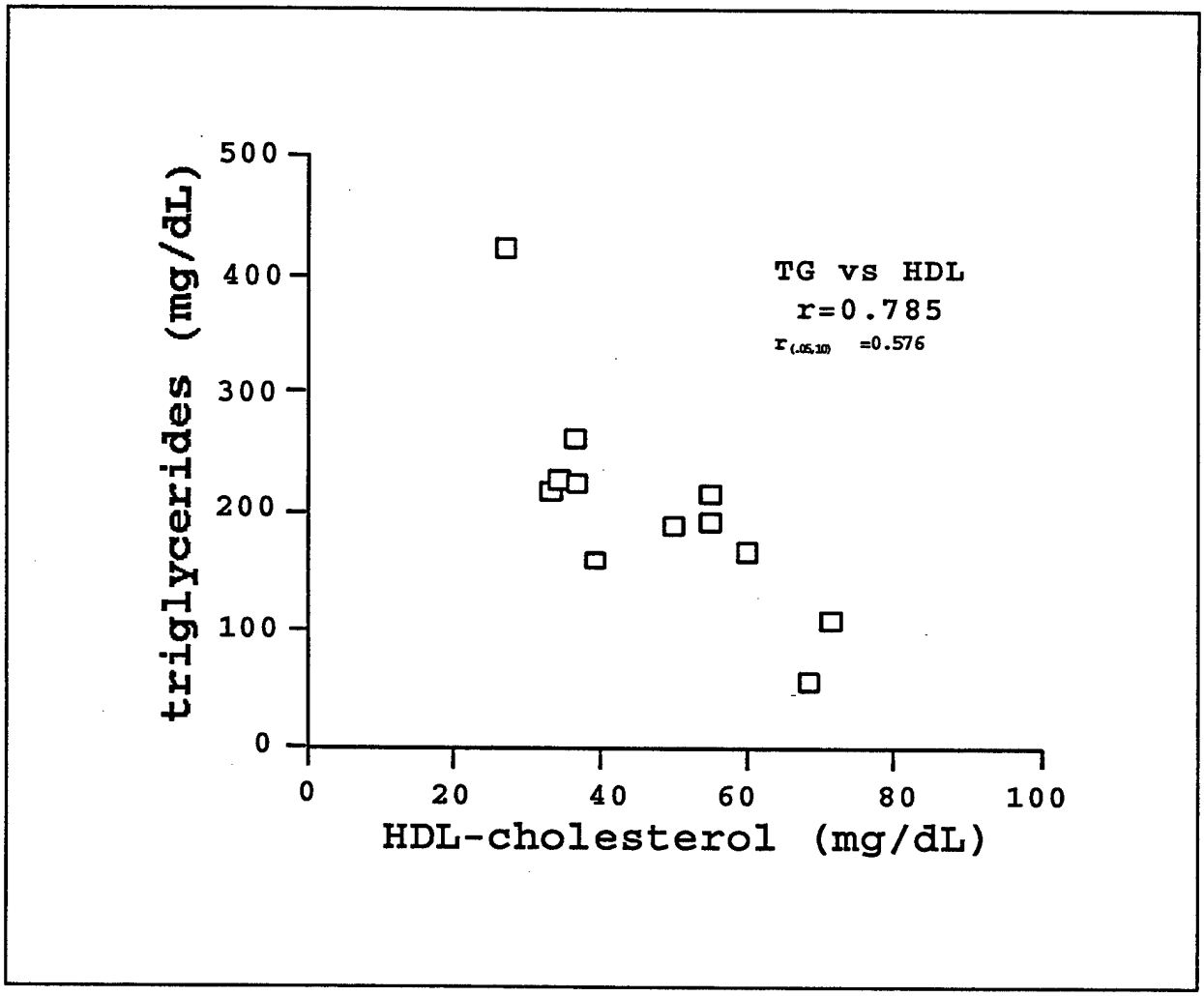

Table 3 shows the concentrations and ratio of triglyceride and cholesterol in the IDL fractions from the six participants with plasma trigylceride concentrations >200mg/dl. The ratios of triglyceride to cholesterol in the IDL were ≤ 2.5, which is well below the expected ratio of > 5.0 for chylomicra (Bachorik et al., 1991).

Table 3. Cholesterol and triglyceride concentrations in the IDL fraction from participants with triglyceride concentrations > 200 mg/dl (n=6).*

Subject #	Cholesterol (mg/dl)	Triglycerides (mg/dl)	Ratio (TG/C)
1	8.7	3.7	0.4
7	4.7	4.7	1.0
8	4.0	2.4	0.6
9	1.2	3.0	2.5
10	7.3	4.0	0.5
12	10.0	3.0	0.3

* The subjects numbers are the same as those used in Table 2.

DISCUSSION

Thermally metastable solid triglyceride core chylomicra were not found in any of the twelve subjects in this study. There are several possible explanations for this finding. First, chylomicra sufficiently enriched in the appropriate saturated fat to form a solid-phase core when chilled were not formed. The IDL isolated from the six participants with plasma triglyceride concentrations over 200 mg/dl had a low ratio (< 2.5) of triglyceride to cholesterol. Ratios > 5.0 are typical for chylomicra in normal individuals (Bachorik et al., 1991). We conclude that chylomicra were not present in the IDL fraction. If the suspect chylomicra had been present, chilling would have altered the density of the chylomicra, enabling them to sediment with the IDL fraction. It may be that dietary fatty acids absorbed by small intestine enterocytes became mixed with an existing pool of unsaturated fatty acids, or with fatty acids of markedly different chain lengths to form chylomicra triglycerides (Small, 1991). This would effectively decrease the concentration of triglycerides composed exclusively of saturated fatty acids and decrease the likelihood of forming a metastable solid core in the chylomicra when chilled.

A second possibility is that the meal did not contain the required composition and/or amount of saturated fat to form solid-phase triglyceride cores in the chylomicra. The composition of the breakfast consumed by subjects was 40% saturated fat (20% palmitin [16:0] and 20% stearate [18:0]) of the remaining 60%, 30% oleic acid (18:1), and 30% a mix of unsaturated fats. In contrast, butter fat fed to the monkeys (Parks et al., 1981) was 62% saturated fat, mostly palmitin. In the chylomicra isolated from the monkeys, only 20 to 30% of the core solidified when chilled. The finding that less than half of the triglyceride core solidified when two thirds of the fatty acids were saturated fatty acids suggests that the composition of triglycerides in chylomicra were altered by enterocytes of the small intestine. This is supported by the fact that the fatty acid composition of the diet is reflected in proportional terms in

plasma lipids; however, the composition of the triglycerides may be altered during reformation of triglycerides in the enterocytes (Small, 1991). Desaturation of palmitin and stearate is also well documented in humans, although the rates are low, *circa* 3% and 9%, respectively (Emken, 1994). Our test meal containing about 30% oleic acid and 20% stearate may have mixed unsaturated fatty acids with saturated fatty acids during the reformation of triglycerides in sufficient quantities to make solidification unlikely.

The amount of saturated fat consumed may not have been sufficient to form solid-phase chylomicra. The breakfast consumed by subjects contained 68 g of fat, over 100% of the RDA. However, for one meal, this is a considerable but not an unusual amount of fat compared to other possibilities. For example, as much or greater amounts of fat are found in a serving of cheese omelette with bacon (86 g), a serving of cheese and bacon quiche (92 g), 11 (medium) slices of bacon (67 g), or less than 1/4 lb of butter (68 g) (Pennington, 1994).

Our fat-enriched breakfast was 40% saturated fat, equivalent to the percentage of saturated fat contained in cheese omelettes and quiches. Butter and most natural cheeses are 62% saturated fat (Pennington, 1994), but few other sources of fat are as highly concentrated with saturated fat. Few meals could exceed the proportion of saturated fat fed to our subjects. However, if the chylomicra in question are produced by humans, the concentration of saturated fat in the prescribed meal may have to be greater than that used in this study.

Chylomicra produced and released into the plasma have previously been reported to appear as early as 1 h, postprandial, and persist for 6 to 8 h (Havel, 1994). Since our blood samples were collected at 4.5 h postprandial, they should have captured the chylomicra of interest.

The observed inverse relationship between HDL cholesterol and plasma triglycerides implies that those with higher HDL concentration assimilated the fat more rapidly (Figure 1). Current understanding of the production of HDL and assimilation of fat from plasma lipoproteins supports this conclusion. Higher levels of plasma HDL and more rapid assimilation of chylomicra fatty acids are the consequence of endurance training (Kiens & Lithell, 1989). Endurance-trained individuals generally have lower fasting triglyceride concentrations and have lower postprandial plasma triglyceride peak concentrations than untrained individuals (Thomson et al., 1986). Additionally, runners have higher levels of HDL (primarily increased HDL_2) and enhanced post-heparin lipoprotein lipase (LPL) activity than sedentary individuals (Williams et al., 1986).

Even a mild exercise program of jogging 15 miles per week for seven weeks was found to enhance the rate of chylomicron clearance in formerly sedentary subjects (Weintraub, Rosen, Otto, Eisenberg, & Breslow, 1989). Eight weeks of one-legged training on a cycle ergometer was found to enhance muscle LPL activity and significantly increase the arterial to venous difference of HDL_2 cholesterol in the quadriceps femoris compared to the untrained leg (Kiens & Lithell, 1989). Further evidence to suggest that skeletal muscles contribute to elevated plasma HDL is the observation that competitive road-racing cyclists have enhanced plasma HDL_2 concentrations after 4-6 h of competition (Mena, Maynar, & Campillo, 1991). Therefore, it seems that trained individuals have enhanced chylomicra clearance and elevated concentration of plasma HDL.

In conclusion, we were unable to detect the high melting point chylomicra in the plasma collected from Naval Special Warfare Personnel, 4.5 h after they consumed a meal highly concentrated in saturated fats. These personnel have been documented to follow a moderately intense training program (Jacobs, Prusaczyk, & Goforth, 1992; Prusaczyk, Goforth, & Nelson, 1990). Whether our negative finding results from interspecific differences (genetics), endurance training, or the fat composition of the meal is unknown. Clearly, more research is needed to determine if high melting point chylomicra particles are produced in humans in vivo or if dietary fatty acids are just metabolized differently than has been reported in monkeys, cattle, and rats.

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13. ABSTRACT *(Maximum 200 words)*

In rats, cattle, and monkeys, dietary saturated fats can lead to the production of plasma chylomicra that are enriched in saturated triglycerides (TG). Chylomicra are formed by solid-phase TG cores when cooled to < 20°C *in vitro*, and do not melt at physiological temperatures (> 50°C). Temperatures in human extremities have been documented at < 20°C during occupational and recreational activities. Chylomicra circulating in the extremities during cold exposure can form solid TG cores, which would be less likely to degrade. These persistent chylomicra would contribute to atherosclerosis. This study determined if this class of chylomicra could be produced in humans, *in vivo*. Twelve Navy SEAL personnel were fed a 1160-kcal breakfast containing 68 g of fat (40% saturated fat) and 600 mg cholesterol (CHO). A postprandial venous blood sample was taken 4.5 h after the meal. The TG:CHO ratios in the intermediate density lipoprotein fraction, where solid core chylomicra sediment, were < 2.5; chylomicra or their remnants were not found. In conclusion, high melting-point chylomicra were not found in the plasma of moderately endurance-trained humans 4.5 h after they consumed a meal concentrated in saturated fats. Whether this finding results from interspecies differences, endurance training, or the fat composition of the meal is unknown.

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