Rate of gastric emptying influences dietary cholesterol absorption efficiency in selected inbred strains of mice

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Abstract **This study compared the physiological process of cholesterol absorption in different strains of inbred mice with the goal of identifying novel mechanism(s) by which cholesterol absorption can be controlled. The rate and amount of cholesterol absorption were evaluated based on [^{14}C]cholesterol appearance in plasma after feeding a meal containing [^{14}C]cholesterol and by the percentage of [^{14}C]-cholesterol absorbed over a 24 h period. Results showed that the rate of [^{14}C]cholesterol appearance in plasma was slower in 129P3/J mice than in SJL/J mice. However, more dietary cholesterol was absorbed over a 24 h period by 129P3/J mice than by SJL/J mice. In both strains of mice, cholesterol delivered with medium-chain triglyceride was absorbed less efficiently than cholesterol delivered with olive oil. The strain- and vehicle-dependent differences in cholesterol absorption efficiency correlated negatively with stomach-emptying rates. Furthermore, inhibition of gastric emptying with nitric oxide synthase inhibitor increased cholesterol absorption efficiency in SJL/J mice. These results document that stomach-emptying rate contributes directly to the rate of dietary cholesterol absorption, which is inversely correlated with the total amount of cholesterol absorbed from a single meal. Additionally, genetic factor(s) that influence gastric emptying may be an important determinant of cholesterol absorption efficiency.**—Kirby, R. J., P. N. Howles, and D. Y. Hui. **Rate of gastric emptying influences dietary cholesterol absorption efficiency in selected inbred strains of mice.** ***J. Lipid Res.*** **2004.** 45: **89–98.**

Supplementary key words medium-chain triglyceride • mouse genetics • intestine • stomach

Dietary cholesterol contributes ~50% of the circulating cholesterol in humans and ~30% of the circulating cholesterol in mice (1, 2). Despite the importance of this process in determining plasma cholesterol level, the mechanisms dictating dietary cholesterol absorption efficiency have not been completely elucidated. This incomplete information is attributable in part to the complexity of the cholesterol absorption process, which involves lipid emulsification in the stomach, lipolytic digestion and bile salt solubilization in the intestinal lumen, enterocyte uptake of the dietary cholesterol, and its assembly into lipoproteins before secretion into the circulation. Thus, differences in the rate and efficiency of each of these processes will contribute to variations in cholesterol absorption efficiency among different individuals.

Cholesterol absorption efficiency in humans varies among different individuals even though they may be consuming a similar diet (3–6). Individual differences in cholesterol absorption efficiency were also observed among various inbred strains of rabbits (7, 8), rats (9), and mice (10–15). These results suggested a genetic component in the regulation of cholesterol absorption. Early studies on the identification of cholesterol absorption genes have focused on enzymes important for cholesterol ester metabolism in the gastrointestinal tract. Studies with gene knockout mice and specific enzyme inhibitors revealed that both the cholesterol esterification enzyme acyl-CoA:cholesterol acyltransferase and the cholesterol ester hydrolytic enzyme cholesterol esterase modulate the type of intestinal lipoproteins produced (16, 17). However, both of these enzymes play only minor roles in determining cholesterol absorption efficiency under normal dietary conditions (16–20). In contrast, enzymes that modulate cholesterol solubility in the intestinal lumen, such as the bile acid synthetic enzymes cholesterol 7α-hydrolase and sterol 27-hydroxylase, impact cholesterol absorption indirectly by modulating the physical structure of the cholesterol carrier in the intestinal lumen (21, 22). More recently, the ATP binding cassette transporter proteins, including ABCA1, ABCG5, and ABCG8, have been implicated as important regulators of dietary cholesterol absorption. These transporters may limit the amount of cholesterol absorbed by enterocytes by catalyzing cholesterol efflux to the intestinal lumen (23–26).

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Abbreviations: LCT, long-chain triglyceride; L-NAME, *N*-nitro-L-arginine; MCT, medium-chain triglyceride.

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Several lines of evidence suggested that the current list of genes and gene factors that dictate cholesterol absorption efficiency is incomplete. Studies with selective cholesterol absorption inhibitors have suggested the possibility of specific transporters on enterocyte membranes that mediate cholesterol uptake (27, 28). Schwarz et al. (12) have performed genetic linkage analysis of inbred mice and their backcross offspring and identified seven quantitative trait loci that influence cholesterol absorption efficiency. Importantly, these seven quantitative trait loci map to different chromosomes and are located at sites distinct from the aforementioned genes (12). The current study compared the physiological process of cholesterol absorption in these inbred strains of mice with the goal of identifying possible novel mechanism(s) by which cholesterol absorption can be controlled. The results showed that the rate of gastric emptying after feeding is an important determinant of cholesterol absorption efficiency.

EXPERIMENTAL PROCEDURES

Animals

The 129P3/J and SJL/J mice were obtained from Jackson Laboratories (Bar Harbor, ME). 129/SvEv mice were from Taconic Farms, Inc. (Germantown, NY). The mice were housed at our facility in a temperature- and humidity-controlled room with a 12 h light/dark cycle and fed basal chow diet for at least 3 weeks before experiments. Male mice between the ages of 8 and 12 weeks were used for all experiments. Mice were fasted overnight before the initiation of absorption studies.

Lipid absorption studies

The rate of dietary cholesterol absorption was determined based on plasma appearance of radiolabeled cholesterol fed into the stomach after retro-orbital injection of 100 μl of Triton WR1339 mixture [Triton WR1339-saline (1:7; v/v)]. A typical test meal contained 2 μCi of [^{14}C]cholesterol, 1 μCi of [^{3}H]triolein, and 2 g/l cholesterol suspended in 50 μl of olive oil. Blood was collected hourly from the tail, and plasma was isolated for liquid scintillation counting.

To determine the plasma appearance of lipids infused directly into the duodenum, a silicone tube was passed through the fundus of the stomach and extended into the duodenum under ketamine and xylazine anesthesia. The fundal incision was closed using a purse-string suture. Postoperatively, the animals were infused with a 5% dextrose saline solution (145 mM NaCl, 4 mM KCl, and 0.28 mM dextrose) at a constant rate of 0.3 ml/h. The animals were maintained overnight at 30°C before lipid infusion. On the day of experiments, 100 μl of Triton WR1339 mixture was injected retro-orbitally, and a 50 μl bolus emulsion of olive oil containing 2 mg/ml cholesterol, 2 μCi of [^{14}C]cholesterol, and 1 μCi of [^{3}H]triolein was infused into the duodenum. Infusion of dextrose saline solution was continued. Blood was collected after 2.5 h in heparinized microfuge tubes and centrifuged to separate plasma, and radioactivity in plasma was determined by scintillation counting.

Cholesterol absorption efficiency was measured in vivo by the dual-isotope 24 h fecal output method (11, 12). Mice were fed a 50 μl test meal by stomach gavage containing 2 μCi of [^{14}C]cholesterol (Perkin-Elmer Life Sciences, Boston, MA), 0.5 μCi of [^{3}H]sitostanol (American Radiolabeled Chemicals, St. Louis, MO), and 2 g/l cholesterol (Sigma Chemicals, St. Louis, MO) suspended in either olive oil [long-chain triglyceride (LCT)] or medium-chain triglyceride (MCT) (Powerhouse Supplements, Cincinnati, OH). Mice were housed individually after gavage in cages with raised wire platforms to collect feces and minimize coprophagy. Food was returned, and feces were collected for 24 h. Samples were homogenized in water and extracted with an equal volume of chloroform-methanol (2:1, v/v), then re-extracted with an equal volume of chloroform. An aliquot of the organic phase from each sample was dried under N_2, and the amount of radioactive sterols was determined by scintillation counting. The percentage of cholesterol absorbed was calculated by the formula $100 \times [1 - (^{14}C/^{3}H \text{ excreted})/(^{14}C/^{3}H \text{ administered})]$.

To determine the absorption efficiency of cholesterol delivered directly into the duodenum, mice were fasted overnight and a 1 cm abdominal incision was made under ketamine and xylazine anesthesia. A 50 μl test meal containing 2 μCi of [^{14}C]cholesterol, 0.5 μCi of [^{3}H]sitostanol, and 2 g/l cholesterol suspended in olive oil was injected directly into the duodenum at <1 cm distal to the pylorus. The incision was closed by suture, food was returned, and feces were collected over a 24 h period. The percentage of the dietary cholesterol absorbed was determined based on the amount of nonabsorbed cholesterol present in the feces as described above.

Gastric emptying and tissue distribution of the dietary lipids

Gastric emptying and tissue distribution of the gavaged lipids were determined by removal of the stomach and specified tissues at various times after delivery of the radiolabeled test meal by stomach gavage. At the end of each experiment, the stomach was removed from each animal and flushed with PBS, and the contents were homogenized with a tissue homogenizer. Blood was centrifuged to separate plasma. Small intestine was flushed with PBS to collect luminal contents and then homogenized. Liver was weighed and homogenized in PBS. The amount of radioactivity in each sample was determined by scintillation counting and expressed as the percentage of total radioactivity administered. In experiments to determine the effect of nitric oxide synthase inhibition on gastric emptying and cholesterol absorption, SJL/J mice were injected retro-orbitally with 0.1 ml of a PBS solution with or without 5 mg/ml *N*-nitro-L-arginine (L-NAME) before administration of the test meal.

RESULTS

Previous studies comparing cholesterol absorption efficiency among inbred strains of mice reported that 129P3/J mice absorbed cholesterol from a bolus meal with the highest efficiency and SJL/J mice had the lowest absorption efficiency (12). The physiological mechanism that may account for the difference in cholesterol absorption between the 129P3/J and SJL/J mice was explored by determining the rate of [^{14}C]cholesterol and [^{3}H]triolein appearance in plasma after their delivery to the stomach of these animals. Inhibition of postprandial lipoprotein lipolysis and clearance was accomplished by intravenous injection of Triton WR1339 (29). Surprisingly, a rapid appearance of the dietary [^{14}C]cholesterol and [^{3}H]triolein in plasma was observed in the low-absorption SJL/J mice (**Fig. 1**). In contrast, the high-absorption 129P3/J mice actually displayed a very slow rate of lipid transport, with

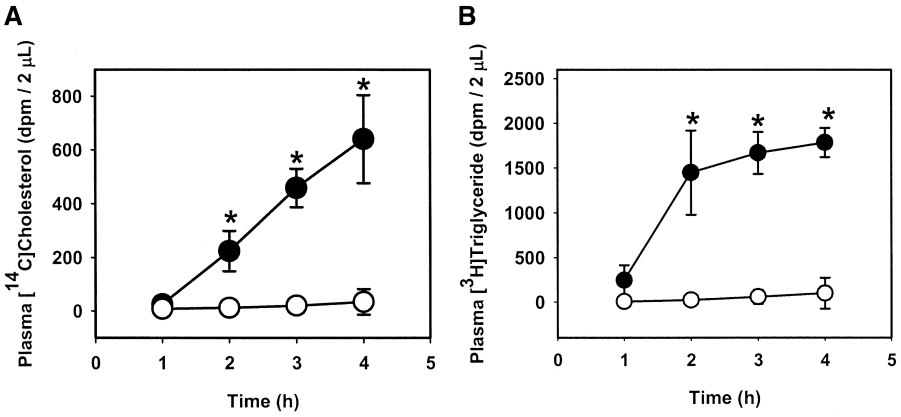

Fig. 1. Lipid absorption rates in SJL/J and 129P3/J mice. Male SJL/J mice (closed circles) and 129P3/J mice (open circles) were injected retro-orbitally with 0.1 ml of Triton WR1339 in PBS (1:7, v/v) followed by stomach gavage with a 50 µl test meal containing 2 µCi of [^{14}C]cholesterol, 1 µCi of [^{3}H]triolein, and 2 g/l cholesterol suspended in olive oil. Blood samples were collected hourly for 4 h for liquid scintillation counting. Cholesterol absorption (A) and triglyceride absorption (B) rates were determined based on the appearance of [^{14}C]cholesterol and ^{3}H radiolabel in plasma. The data are presented as means ± SEM from four mice in each group. Asterisks indicate differences from 129P3/J mice at $P < 0.01$.

minimal levels of [^{14}C]cholesterol and [^{3}H]oleate appearing in plasma over a 4 h period (Fig. 1).

The potential mechanism for the difference in the rate of lipid transport between 129P3/J and SJL/J mice was explored by determining the tissue distribution of radiolabeled lipids at various times after stomach gavage of these animals. More than 40% of the gavaged lipids were found to be retained in the stomach of 129P3/J mice after 6 h, whereas <15% remained in the stomach of SJL/J mice after this same period (**Fig. 2**). Importantly, the [^{14}C]cholesterol that rapidly exited the stomach of SJL/J mice did not result in its increased absorption, as no significant difference was observed in the amount of [^{14}C]cholesterol present in stomach, intestinal lumen, small intestine, plasma, or liver between SJL/J and 129P3/J mice at 6 h after radiolabeled lipid gavage (**Fig. 3**). Therefore, it is likely that the [^{14}C]cholesterol rapidly entering the intestinal lumen of SJL/J mice was not absorbed by the intestinal mucosa and excreted in feces over time. This interpretation is consistent with previous reports of lower cholesterol absorption efficiency in SJL/J mice compared with 129P3/J mice (12). We further compared stomach-emptying rates in several additional inbred mouse strains. The 129/SvEv mice were similar to 129P3/J mice, displaying a greater retention of lipid nutrients in the stomach compared with other strains, whereas C57BL/6, AKR/J, and DBA/2 mice emptied stomach contents at a rate similar to that of SJL/J mice (data not shown). Thus, delayed gastric emptying appears to be a characteristic trait of the 129 mouse lines, which may account of the high cholesterol absorption efficiency in these animals (12).

Consideration was given to the fact that in experiments measuring cholesterol absorption efficiency by the dual-isotope fecal output method, mice are permitted access to food after gavage of the lipid test meal, whereas the stomach-emptying experiments were performed initially in the gavaged animals with no subsequent access to food. To determine whether ad libitum feeding after stomach gavage of lipid test meals affected the rate of gastric emptying, mice were fasted overnight before administration of a lipid test meal by stomach gavage, then fasting was continued or mice were permitted access to food. **Table 1** shows that feeding had no effect on stomach emptying in the short term. The 129P3/J mice retained >80% of the lipid test meal at 1 h after gavage regardless of whether fasting was continued or mice were permitted to feed. After 24 h, ~50% of gavaged radiolabeled lipid remained in the stomach of 129P3/J mice when fasting was maintained continuously throughout the entire 24 h period (Table 1). However, if the animals were allowed to eat ad libitum after stomach gavage of the radiolabeled lipid, very little [^{14}C]cholesterol was found in the stomach of 129P3/J mice after 24 h (Table 1). These results indicated that additional feeding was required for complete emptying of the primary test meal in 129P3/J mice, probably via replacement of the initial test meal in the stomach with contents from the subsequent meal. In contrast to the results with the 129P3/J mice, <10% of the [^{14}C]cholesterol was found in the stomach of SJL/J mice after 24 h regardless of their fasting/feeding status after the initial test meal (Table 1). Taken together, these studies suggest that gastric emptying, as well as the total amount of cholesterol absorbed from a single meal, is a critical determinant of cholesterol absorption rate. Whereas an efficient stomach-emptying mechanism results in an increased rate of cholesterol absorption, a higher percentage of cholesterol is absorbed from each meal in animals with a less-efficient stomach-emptying mechanism.

The previously reported differences in cholesterol absorption efficiency (2) between ad libitum-fed 129P3/J and SJL/J mice were verified by comparing the tissue distribution of the [^{14}C]cholesterol test meal at 24 h after feeding. Results showed that approximately twice as much [^{14}C]cholesterol was found in the plasma and liver of the

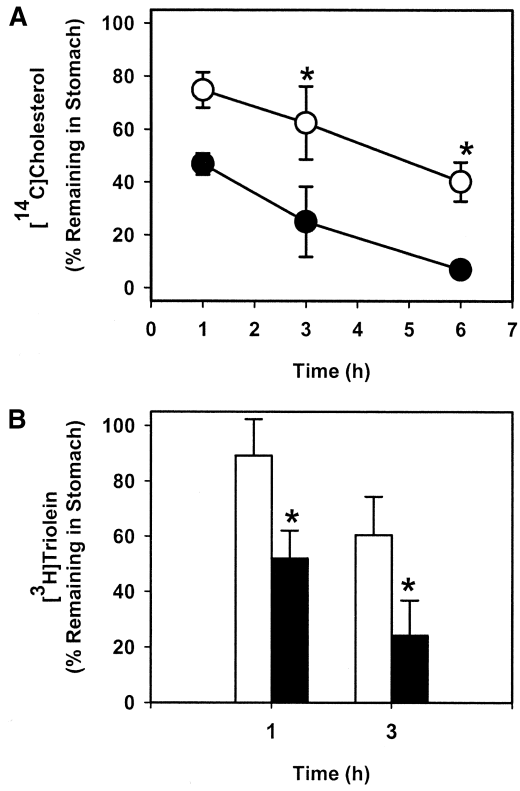

Fig. 2. Distribution of [^{14}C]cholesterol (A) and [^{3}H]triolein (B) in stomach of SJL/J (closed symbols) and 129P3/J (open symbols) mice after stomach gavage of 50 μl of olive oil with 2 g/l cholesterol and 2 μCi of [^{14}C]cholesterol or 1 μCi of [^{3}H]triolein. The mice had no additional food access after the test meal. The data are presented as means ± SEM from four animals. Asterisks indicate differences from 129P3/J mice at $P < 0.01$.

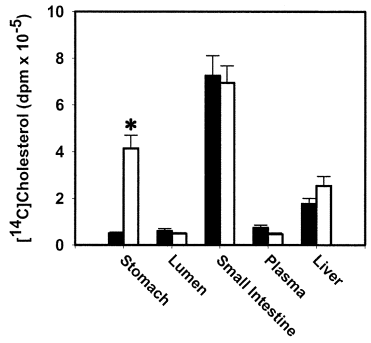

Fig. 3. Tissue distribution of [^{14}C]cholesterol in SJL/J (closed bars) and 129P3/J (open bars) mice at 6 h after stomach gavage of a 50 μl test meal containing 2 g/l cholesterol and 2 μCi of [^{14}C]cholesterol in olive oil. Blood was collected and centrifuged to separate plasma. Stomach and small intestine were flushed with PBS to collect contents. Small intestine and liver tissue were homogenized in PBS. Tissue distribution of [^{14}C]cholesterol was determined by scintillation counting and is presented for stomach content, lumenal content, small intestine (dpm/whole tissue), plasma (dpm/ml), and liver (dpm/g). The data are presented as means ± SEM (n = 4). The asterisk indicates a difference from the SJL/J group at $P < 0.01$.

129P3/J mice as in SJL/J mice (**Fig. 4**). Interestingly, the amount of [^{14}C]cholesterol present in the small intestine after 24 h was similar between the two strains of mice (Fig. 4). The latter observation further suggested that there is no difference in intestinal cholesterol transport between 129P3/J and SJL/J mice.

The hypothesis that differences in cholesterol absorption efficiency between 129P3/J and SJL/J mice are attributable primarily to differences in gastric emptying and not to differences in intestinal transport was further examined by infusing the lipid test meal directly into the duodenum of the animals and then monitoring the appearance of the radiolabeled lipids in their plasma after blocking lipolysis with intravenous injection of Triton WR1339. The results, illustrated in **Fig. 5**, showed no difference in cholesterol and triglyceride absorption between the two strains when the stomach was not involved in the processing of the test meal. These results are consistent with the interpretation that the difference in cholesterol absorption efficiency between 129P3/J and SJL/J mice is attributable to their different stomach-emptying rates. This interpretation was confirmed by measurement of cholesterol absorption efficiency in 129P3/J and SJL/J mice by the dual-isotope fecal output method when the test meal was injected directly into the duodenum compared with delivery by stomach gavage (**Fig. 6**). When the meal was delivered by stomach gavage, 129P3/J mice absorbed cholesterol with much higher efficiency (84.4 ± 1.1%) than did SJL/J mice (66.2 ± 2.2%), confirming previous reports (2). However, when the stomach was bypassed and the test meal was injected directly into the duodenum, absorption efficiency was decreased significantly in 129P3/J mice to a level similar to that observed in SJL/J mice (Fig. 6). Cholesterol absorption efficiency in SJL/J mice was not affected when the stomach was bypassed, perhaps because the test meal was rapidly emptied when delivered to the stomach. Delayed gastric emptying in 129P3/J mice, however, increases absorption efficiency by limiting the amount of cholesterol presented to the intestine at any given time.

Previous studies in humans have reported that lipid test meals containing MCT oil transit through the gastrointestinal tract more rapidly than do meals with LCT (30). Comparison of stomach emptying of lipid test meals delivered with MCT versus LCT (olive oil) demonstrated that test meals delivered with MCT were emptied faster than

TABLE 1. Effect of feeding and fasting after stomach gavage on gastric emptying of cholesterol

	Percentage of [^{14}C]cholesterol remaining in the stomach			
Strain	Fasted	Fed	Fasted	Fed
	1 h		*24 h*	
129P3/J	82.50 ± 0.88	89.46 ± 4.38	49.25 ± 12.13	0.43 ± 0.06[a]
SJL/J	45.14 ± 4.74[b]	30.78 ± 9.72[b]	4.86 ± 1.63[b]	0.25 ± 0.06[a]

129P3/J and SJL/J mice were fasted overnight before stomach gavage of 50 μl of olive oil containing 2 mg/ml cholesterol and 0.2 μCi of [^{14}C]cholesterol. Mice were then permitted to feed ad libitum or continued fasting.

[a] Fasted versus fed ($P < 0.05$).

[b] 129P3/J versus SJL/J ($P < 0.05$).

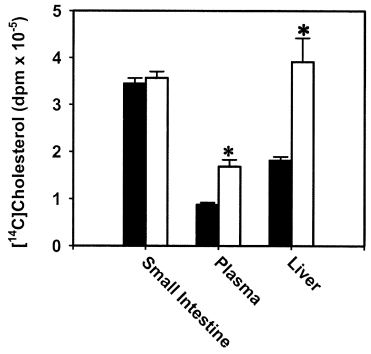

Fig. 4. Tissue distribution of dietary [^{14}C]cholesterol in SJL/J (closed bars) and 129P3/J (open bars) mice at 24 h after feeding. Male mice received a 50 μl test meal by stomach gavage containing 2 g/l cholesterol, 2 μCi of [^{14}C]cholesterol, and 1 μCi of [^{3}H]sitostanol in olive oil. Food was returned and mice were euthanized after 24 h. Blood was collected and centrifuged to separate plasma. Small intestine and liver tissue were homogenized in PBS. Tissue distribution of [^{14}C]cholesterol was determined by scintillation counting and is presented for small intestine (dpm/whole tissue), plasma (dpm/ml), and liver (dpm/g). The data are presented as means ± SEM (n = 4). Asterisks indicate differences from the SJL/J group at $P < 0.01$.

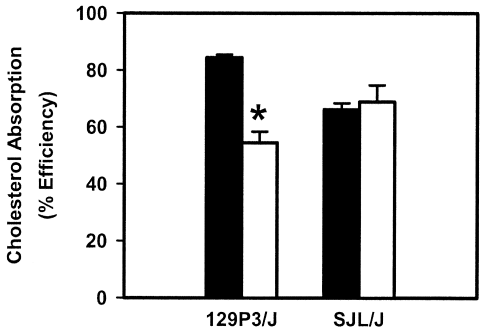

Fig. 6. Cholesterol absorption efficiency in 129P3/J and SJL/J mice after delivery of a bolus lipid test meal by either stomach gavage (closed bars) or directly into the duodenum (open bars). Male mice received a 50 μl test meal containing 2 μCi of [^{14}C]cholesterol, 0.5 μCi of [^{3}H]sitostanol, and 2 g/l cholesterol suspended in olive oil by either stomach gavage or injection into the duodenum. Cholesterol absorption efficiency was determined based on the amount of nonabsorbed sterols present in the feces after 24 h. The data are presented as means ± SEM (n = 13–15 for stomach gavage; n = 4–5 for duodenal injection). The asterisk indicates a difference between the stomach gavage and duodenum-infused groups at $P < 0.001$.

meals delivered with LCT in both 129P3/J and SJL/J mice (**Fig. 7A**). Interestingly, the amount of test meal remaining in the stomach of MCT-fed 129P3/J mice was similar to that observed in SJL/J mice fed with the LCT test meal (Fig. 7A). We then compared cholesterol absorption efficiency in 129P3/J and SJL/J inbred mice using either LCT or MCT as the cholesterol-delivery vehicle. The lipid substrates were delivered to the stomach of these animals by gavage tubes, and cholesterol absorption was determined based on the amount of nonabsorbed cholesterol excreted in the feces over a 24 h period. Results showed that the 129P3/J mice were 1.7-fold more efficient than SJL/J mice in cholesterol absorption when the substrate was delivered with MCT oil as the carrier (Fig. 7B). This observation is consistent with results reported previously by other investigators (12). Our experiments also showed that cholesterol absorption was increased by 20–30% in both strains of mice when LCT instead of MCT was used as the cholesterol carrier. A difference in cholesterol absorption from LCT-containing substrate was also observed between 129P3/J and SJL/J mice, albeit less dramatic than the difference observed with MCT-containing substrate. Nevertheless, this difference (18%) remained highly significant ($P < 0.001$). Interestingly, there was no significant difference in cholesterol absorption efficiency observed between MCT-fed 129P3/J mice and LCT-fed SJL/J mice (Fig. 7B). These results are consistent with the hypothesis that the difference in cholesterol absorption efficiency between 129P3/J and SJL/J mice, as well as that between LCT and MCT delivery vehicles, may be attributed to the different stomach-emptying rates between the two strains of mice. This hypothesis can be illustrated by the correlation of cholesterol absorption efficiency with stomach retention of lipid test meals in these animals (**Fig. 8**).

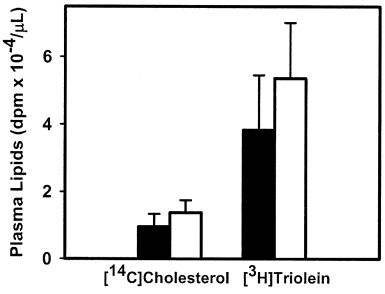

Fig. 5. Appearance of dietary [^{14}C]cholesterol and [^{3}H]oleic acid derived from infused [^{3}H]triolein in the plasma of male SJL/J (closed bars) and 129P3/J (open bars) mice after infusion of a bolus lipid test meal directly into the duodenum. Duodenal infusion of a 50 μl test meal containing 2 μCi of [^{14}C]cholesterol, 1 μCi of [^{3}H]triolein, and 2 g/l cholesterol suspended in olive oil was achieved through a silicone tube passed through the fundus of the stomach and extended into the duodenum. Lipoprotein lipolysis was prevented by retro-orbital injection of 0.1 ml of Triton WR1339 in PBS (1:7, v/v) before lipid infusion. Blood was collected after 2.5 h and spun, and radiolabel in plasma was determined by scintillation counting. The data are presented as means ± SEM (n = 3–4).

The relationship between stomach-emptying rate and cholesterol absorption efficiency was further explored by taking advantage of previous observations that inhibition of nitric oxide synthase delays gastric emptying in humans and mice (31–33). Thus, 20 mg/kg of the nitric oxide synthase inhibitor L-NAME was injected into SJL/J and 129P3/J mice before administration of the [^{14}C]cholesterol by stomach gavage. The extent of stomach emptying was measured after 1 h in one group of animals, whereas cholesterol absorption over a 24 h period was determined in another group. In SJL/J mice, L-NAME injection significantly increased the amount of [^{14}C]cholesterol remain-

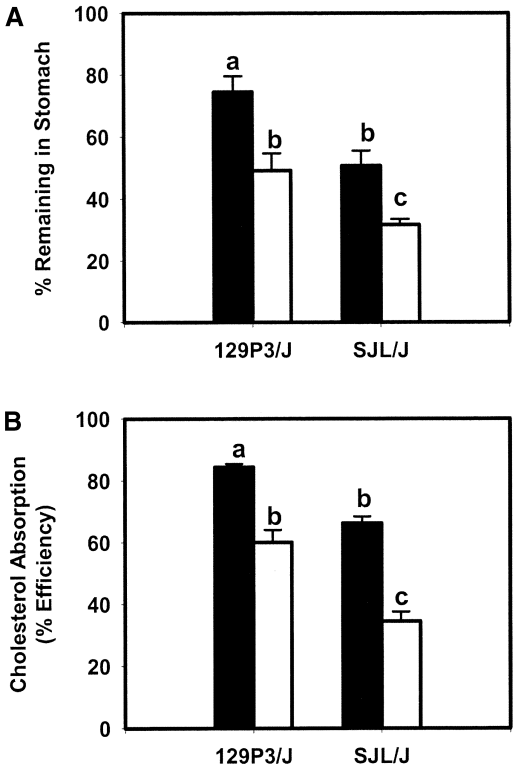

Fig. 7. Effect of long-chain triglyceride (LCT) or medium-chain triglyceride (MCT) carriers on gastric emptying (A) and cholesterol absorption efficiency (B) in 129P3/J and SJL/J mice. Male mice received a 50 μl test meal by stomach gavage containing 2 g/l cholesterol, 2 μCi of [^{14}C]cholesterol, and 0.5 μCi of [^{3}H]sitostanol in either LCTs (closed bars) or MCTs (open bars). Cholesterol absorption efficiency was determined based on the amount of nonabsorbed sterols present in the feces after 24 h. The animals had free access to food during this period. Gastric emptying was determined as the percentage of the radiolabeled lipids remaining in the stomach at 1 h after gavage with no additional food access. The data are presented as means ± SEM (n = 6–11 for stomach emptying; n = 11–15 for absorption efficiency). Bars with different letters indicate significant differences at $P < 0.01$.

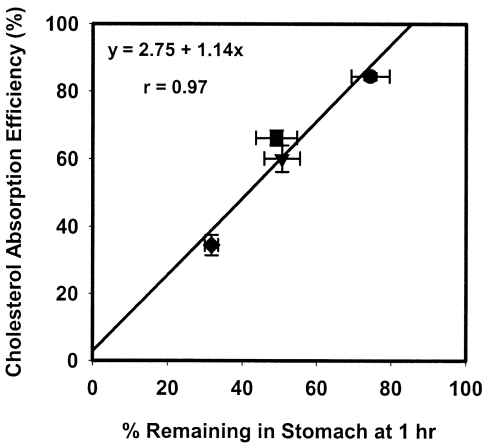

Fig. 8. Correlation of cholesterol absorption efficiency and stomach retention of radiolabeled lipids in 129P3/J and SJL/J mice. The data were derived from the composites of Fig. 7A, B.

ing in the stomach after 1 h (**Fig. 9A**) and concomitantly increased cholesterol absorption efficiency over a 24 h period compared with vehicle-treated mice (Fig. 9B). Delayed gastric emptying and increased cholesterol absorption efficiency after treatment with L-NAME was observed with either MCT or LCT oil as the vehicle. The correlation of cholesterol absorption efficiency with stomach retention of lipid test meals in SJL/J mice treated with L-NAME is illustrated in **Fig. 10**. Importantly, these data suggest that the fast rate of gastric emptying in SJL/J mice may be directly responsible for the lower cholesterol absorption efficiency in these animals. In contrast to results observed with SJL/J mice, L-NAME treatment had no effect on either stomach emptying (Fig. 9C) or cholesterol absorption efficiency (Fig. 9D) in the 129P3/J mice, suggesting that stomach-emptying rate may already be operating at a basal level in the 129P3/J mice.

Additional experiments were also performed to determine whether the difference in stomach-emptying rate between 129P3/J and SJL/J mice was specific for lipid nutrients or, alternatively, a physiological difference in the mechanism controlling general stomach emptying between the two strains of mice. In these experiments, [^{3}H]mannitol was delivered to the stomach of 129P3/J and SJL/J mice and stomach emptying of the radiolabel was monitored. The amount of [^{3}H]mannitol retained in the stomach of 129P3/J mice was found to be 3-fold higher than that observed in the SJL/J mice over a 4-h period (data not shown). Thus, the difference in dietary lipid retention in the stomach of 129P3/J and SJL/J mice was attributable to differences in a general mechanism of gastric emptying between the two strains.

DISCUSSION

Wang and Carey (15) recently reported an extensive study comparing methodologies for measurement of cholesterol absorption efficiency. These studies, in addition to previous reports (34), indicated that the method used to measure absorption contributes a large degree of variability to the measured efficiency. Although absolute values of cholesterol absorption varied depending on the methodology used, their studies found that relative differences in cholesterol absorption efficiency among the various inbred strains of mice were consistent regardless of the methods used for the analysis (15). It is interesting, however, that most previous studies characterizing the regulatory mechanisms that govern cholesterol absorption efficiency have focused on events that occur at the intestinal lumen and in the mucosa. These earlier studies have clearly documented that luminal lipid digestion and solubilization, mucosal uptake and efflux, and lipoprotein assembly and secretion are key determinants of cholesterol absorption efficiency (35–37). The current study used the 24 h fecal collection method and rate of radiolabeled lipid appearance in the plasma to show that events that oc-

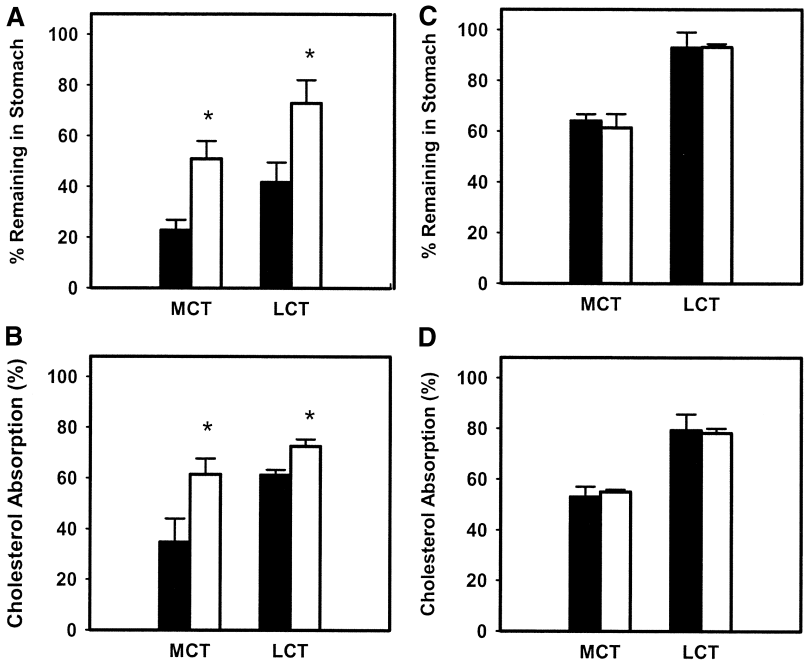

Fig. 9. Effects of the nitric oxide synthase inhibitor *N*-nitro-L-arginine (L-NAME) on gastric emptying and cholesterol absorption in SJL/J (A, B) and 129P3/J (C, D) mice. Male mice were injected retro-orbitally with 20 mg/kg L-NAME (open bars) or vehicle control (closed bars) followed by stomach gavage of a 50 μl test meal containing 2 μCi of [^{14}C]cholesterol and 0.5 μCi of [^{3}H]sitostanol in MCT or LCT (olive oil). Gastric emptying was determined as the percentage of radiolabeled lipids remaining in the stomach after 1 h (A, C). Cholesterol absorption efficiency was determined based on the amount of nonabsorbed sterols present in the feces after 24 h when the animals had free access to food after the test meal (B, D). The data are presented as means ± SEM (n = 3–4 in A, C; n = 3–7 in B, D). Asterisks indicate differences from the control group at $P < 0.05$.

cur before lipid nutrient entry into the intestinal lumen are also important regulatory steps in dictating cholesterol absorption efficiency. This conclusion is based on the observation that the 129P3/J mice, which have a slow gastric-emptying rate, absorbed more dietary cholesterol from a single meal than did the SJL/J mice, which have a faster gastric emptying rate. Cholesterol delivered with MCT was emptied faster and absorbed less efficiently than was cholesterol delivered with LCT in both strains of mice. Thus, overall cholesterol absorption efficiency is inversely correlated with gastric emptying rate in 129P3/J and SJL/J mice. Furthermore, inhibition of gastric emptying with nitric oxide synthase inhibitor was shown to increase cholesterol absorption efficiency in SJL/J mice.

The mechanism by which the rate of gastric emptying influences cholesterol absorption efficiency is not completely understood at present. It has been demonstrated previously that dietary cholesterol absorption is a saturable process (38, 39). This observation suggested the possibility of transporter protein-mediated cholesterol uptake by intestinal cells (40–42). Although the concept and exact identity of putative cholesterol transporter protein(s) in intestine remain highly debated, the ability of cholesterol absorption inhibitors to interact with specific proteins on intestinal brush border membranes strongly suggests that intestinal cholesterol absorption is protein mediated (27, 42). Rapid gastric emptying in SJL/J mice may result in higher concentrations of cholesterol delivered to the intestinal lumen passing quickly through the duodenum and proximal jejunum, where the majority of cholesterol absorption occurs. As a consequence, the transporter-mediated cholesterol uptake process may reach a saturation point, limiting the total amount of cholesterol that can be transported from the lumen to the intestinal mucosa. The excess cholesterol not taken up by the

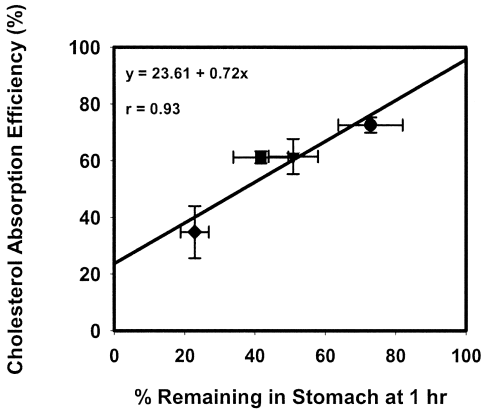

Fig. 10. Correlation of cholesterol absorption efficiency and stomach retention of radiolabeled lipids in SJL/J mice treated with 20 mg/kg L-NAME or vehicle control. The data were derived from the composites of Fig. 9A, B.

intestinal mucosa is then excreted in the feces, resulting in a lower level of cholesterol absorption in these animals. In contrast, the slower rate of gastric emptying in 129P3/J mice may result in lower concentrations of cholesterol delivered to the intestinal lumen, allowing the majority of the cholesterol to be taken up by the enterocytes through the active transporter-mediated high-affinity process. However, it should be noted that the existence of intestinal cholesterol transporters remains controversial. Thus, whether the effect of gastric emptying rate on intestinal cholesterol absorption is mediated via substrate delivery to these transporters or via other mechanisms remains to be determined.

Previous studies demonstrated that cholesterol absorption efficiency in mice (15) and hamsters (34) was also dependent on the fatty acyl chain length of the oil used as the cholesterol-carrying vehicle. Both studies revealed that cholesterol delivered with MCT was absorbed less efficiently than was cholesterol delivered with the LCT olive oil. However, no mechanistic insights were offered in these earlier studies to explain the difference between MCT and LCT in cholesterol delivery. In view of previous studies showing that long-chain fatty acids, but not medium-chain fatty acids, stimulate the production of intestinal hormones that are known to inhibit gastric emptying (43), such as cholecystokinin, peptide YY, and secretin (44–46), results from the current study suggest that differences in gastric mobility may explain the difference between MCT and LCT in mediating cholesterol absorption efficiency.

Factors that influence gastric emptying rate may also influence intestinal transit time. Previous studies have shown that the acceleration of intestinal transit time by pharmacological intervention reduces cholesterol absorption efficiency in humans (47). On the other hand, Wang, Paigen, and Carey (14) showed that variations in cholesterol absorption efficiency among selected inbred strains of mice were not attributable to differences in intestinal transit time. Thus, genes that control gastric emptying rate may be an independent determinant of cholesterol absorption efficiency. Further studies are required to determine whether gastric emptying rate and intestinal transit time are regulated by coordinate or independent mechanism(s).

The identity of the gene(s) responsible for dictating gastric emptying rate and cholesterol absorption efficiency has not been determined. In a recent study using a genetic mapping strategy to identify chromosomal regions harboring genes that influence cholesterol absorption efficiency in mice, backcrosses between SJL/J and 129P3/J mice revealed two different quantitative trait loci that influence cholesterol absorption (12). One locus maps to chromosome 1 in the region that contains the sterol 27-hydroxylase gene and a gene encoding an HDL binding protein. The second locus maps to chromosome 5 in the same region as the gene for the class B type 1 scavenger receptor SR-BI. Although products from these genes participate in bile acid and lipid metabolism (48–50), no overt difference in bile acid and HDL metabolism was associated with differences in cholesterol absorption efficiency between 129P3/J and SJL/J or among other inbred strains of mice (12). The SR-BI gene, which has been implicated previously as a cholesterol absorption gene (41, 51), is also unlikely to account for the difference in cholesterol absorption observed between SJL/J and 129P3/J mice, because only a marginal difference in cholesterol absorption was observed between wild-type and SR-BI knockout mice (52).

Other gene(s) within these two regions may be responsible for the difference in gastric emptying rate between SJL/J and 129P3/J mice and may consequently influence cholesterol absorption efficiency. The neuronal nitric oxide synthase gene (nNOS) is located within 8 centimorgans of the putative cholesterol absorption locus on chromosome 5. Gastric emptying has been reported to be significantly delayed in nNOS knockout mice, and chronic depletion of nitric oxide by nitric oxide synthase (NOS) inactivation results in delayed gastric emptying (33, 53, 54). In the current study, inhibition of NOS also resulted in delayed gastric emptying with a concomitant increase of cholesterol absorption in SJL/J mice. Treatment with the NOS inhibitor L-NAME had no effect on either gastric emptying or cholesterol absorption in 129P3/J mice, further suggesting a genetic difference between SJL/J and 129P3/J mice at this locus. Taken together, these results suggest that nNOS polymorphism may be a genetic determinant in the regulation of cholesterol absorption efficiency. These results also suggest that increasing nNOS activity may be one mechanism by which cholesterol absorption can be reduced.

Other mechanisms regulating the rate of gastric emptying also may be determinants of cholesterol absorption efficiency. Ghrelin and motilin are peptides known to stimulate gastric emptying (55, 56), and variations in their production or in the responsiveness of receptors to these peptides may contribute to variations in gastric emptying rate and consequently influence cholesterol absorption efficiency. Variation in the response to dietary components, as observed with olive oil and MCT oil, may further contribute to differences in gastric emptying rate and cholesterol absorption efficiency. Like MCT used in the current study, n-3 polyunsaturated fatty acids have also been reported to increase gastric emptying rate in humans (57). Thus, manipulation of gastric emptying by dietary or pharmacological means may also permit the modulation of cholesterol absorption efficiency.

These data emphasize that multiple genetic factors are involved in regulating cholesterol absorption. In addition to factors previously reported to influence cholesterol absorption efficiency, these results demonstrate that the rate of gastric emptying also contributes to variability among various strains of inbred mice and perhaps to interindividual variability. Identifying the physiological process that results in their different rates of gastric motility will facilitate the identification of additional genetic factors that influence cholesterol absorption.

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