Numerous studies have demonstrated that poor glycermic control is associated with elevated plasma cholesterol levels in diabetic patients. Experiments have shown that cholesterol synthesis is increased in the small intestine of various diabetic animals. This increase is a generalized phenomenon occurring in all segments of the small intestine. Insulin therapy that normalizes blood glucose levels markedly decreases intestinal cholesterol synthesis in diabetic animals to a level similar to that observed in control animals. Studies have suggested that the hyperphagia that accompanies poorly controlled diabetes is the chief stimulus for the increase in intestinal cholesterol synthesis. However, the direct contact of the intestinal mucosa with nutrients is not the sole trigger for increasing cholesterol synthesis in the small intestine, suggesting that circulating and/or neurological factors play a role. The transport of newly synthesized cholesterol, most of which is in the chylomicron lipoprotein fraction, from the intestines to the circulation is increased in diabetic rats. The sterols associated with these chylomicrons are rapidly cleared from the circulation and delivered to the liver. The increased transport of chylomicrons from the intestine to the circulation in diabetic patients could potentially result in several alterations in lipid metabolism that may increase the risk of atherosclerotic vascular disease. *Diabetes* 38:141-45, 1989

Much of the morbidity and mortality associated with diabetes is due to atherosclerosis (for review see ref. 1). In the Framingham study, the incidence of cardiovascular disease is significantly increased in diabetes, and even with adjustment for the other known risk factors for atherosclerosis, the risk of vascular disease is still increased (2). These results indicate that diabetic patients have an increased risk of vascular disease that is not simply the result of an increased prevalence of nondiabetic risk factors but rather an independent risk factor for atherosclerosis. The mechanisms by which diabetes increases atherosclerosis remain speculative despite intensive investigative efforts (Fig. 1).

However, it is essential to recognize that the interaction of diabetes with other risk factors, such as hypercholesterolemia, is of major importance. If a 40-yr-old diabetic man smokes cigarettes, has an increased blood pressure (systolic BP 195 mmHg), and has increased plasma cholesterol levels (336 mg/dl), the probability of his developing cardiovascular disease during the next 8 yr is 46% (2). However, if these other risk factors are not present, the probability of developing cardiovascular disease is very small. For example, if the 40-yr-old diabetic man does not smoke, has a BP of 135 mmHg, and has a plasma cholesterol of 185 mg/dl, the probability of developing cardiovascular disease over the next 8 yr is only ~3%, which is lower than the average risk in nondiabetic males (2). When risk factors are matched, the relative risk of cardiovascular disease is greater in diabetic than nondiabetic individuals. However, from the point of view of diminishing cardiovascular disease, these data demonstrate that the elimination of the other risk factors is of paramount importance. These observations are supported by studies in Third World and Eastern societies, where the prevalence of risk factors for atherosclerosis is decreased and vascular disease does not account for a large percentage of deaths in patients with diabetes (3).

**Increases in Plasma Cholesterol Concentrations**

Numerous reports have demonstrated that poor glycemic control is associated with elevated plasma cholesterol levels in both diabetic humans and diabetic animal models (1). For example, Sosenko et al. (4) compared plasma cholesterol levels in insulin-dependent diabetic (IDDM) patients with varying degrees of metabolic control with plasma cholesterol concentrations in nondiabetic siblings. In the patients with...
excellent glycemic control, plasma cholesterol levels were similar to those of nondiabetic subjects, whereas in poorly controlled diabetic patients, the plasma cholesterol levels were significantly increased. The elevation of plasma cholesterol levels in poorly controlled diabetic patients is due to increases in very-low-density lipoprotein (VLDL) and low-density lipoprotein (LDL) levels (4).

Even more impressive than cross-sectional studies are the interventional studies that have demonstrated that, in both non-insulin-dependent diabetic (NIDDM) and IDDM patients, improvements in metabolic control result in a decrease in plasma cholesterol concentrations (1). For example, Dunn et al. (5) have shown that lowering fasting plasma glucose levels by continuous insulin infusion is associated with a decrease in total plasma cholesterol levels from 232 to 182 mg/dl. The decrease in plasma cholesterol levels are due to a decrease in both VLDL and LDL levels. The reduction in plasma cholesterol levels is greatest in the individuals who initially had the poorest glycemic control (5). Similarly, in NIDDM patients, Pfeifer et al. (6) demonstrated that there is a direct correlation between the decrease in glycosylated hemoglobin and the decrease in plasma cholesterol levels. Thus, there is substantial evidence from both epidemiological and interventional studies demonstrating that poor glycemic control is associated with increased plasma cholesterol levels. The increase in plasma cholesterol levels associated with poor glycemic control is relatively modest (20–50 mg/dl), but based on epidemiological studies in diabetic patients (2,3) and therapeutic trials in nondiabetic subjects (7), these modest elevations could have harmful consequences. For example, data from the Framingham study show that in a 40-yr-old diabetic man who does not smoke and has a systolic blood pressure of 120 mmHg, increasing the plasma cholesterol level from 185 to 235 mg/dl increases the risk of cardiovascular disease by 70%.

SMALL INTESTINAL CHOLESTEROL SYNTHESIS

Despite extensive investigation, the mechanism by which diabetes produces hypercholesterolemia is unknown. We describe studies from our laboratory and others suggesting that the intestine may play an important role in the diabetes-induced increase in plasma cholesterol levels (Fig. 1).

In intact rats, de novo cholesterol synthesis is increased two- to threefold in the gut of rats with streptozocin-induced diabetes (8). This increase in cholesterologenesis occurs in both the small and large intestine, but quantitatively, the small intestine is responsible for most of the observed increase (8). The enhancement of small intestinal cholesterol synthesis occurs soon after the onset of diabetes and persists for at least 5 wk. Most significant, insulin therapy that normalizes blood glucose levels markedly decreases intestinal cholesterol synthesis in diabetic rats to a level similar to that of control rats (8). Diabetes did not lead to an increase in cholesterol synthesis in other organs, including the liver (8). Other laboratories have shown that hydroxymethylglutaryl (HMG)-CoA reductase activity, the rate-limiting enzyme in cholesterol synthesis, is increased in the small intestine of diabetic rats (9–11). In the liver, HMG-CoA reductase activity is normal in moderately diabetic rats, but with worsening of glycemic control, hepatic HMG-CoA reductase activity decreases (12).

Studies of spontaneously diabetic chinese hamsters (13), diabetic db/db mice (13), spontaneously diabetic BB/W rats (14), and alloxan-induced diabetic rabbits (15) have also demonstrated that cholesterol synthesis is increased in the small intestine of diabetic animals. These observations suggest that in diabetic animals, an enhancement of intestinal cholesterol synthesis may be a general phenomenon. In obese insulin-resistant animal models of diabetes, we have also observed an increase in hepatic cholesterol synthesis (13).

LOCALIZATION AND REGULATION OF CHOLESTEROL SYNTHESIS IN INTESTINE OF DIABETIC ANIMALS

Diabetes leads to an increase in the size of the intestines. In animals with diabetes of relatively short duration the small intestine is hypertrophied only slightly, and small intestinal cholesterol synthesis is increased both on a per-total-organ basis and on a per-gram basis (16). In animals with diabetes for an extended duration the small intestine is markedly hypertrophied, and cholesterol synthesis on a per-total-organ basis is increased; on a per-gram basis, it can be increased, the same, or decreased (16). These observations indicate that soon after the onset of diabetes, an increased rate of cholesterol synthesis per unit mass is responsible for the increase in total small intestinal cholesterol synthesis. In contrast, after a longer period of diabetes, the increase in total small intestinal cholesterol synthesis is primarily due to an increase in intestinal mass.

Studies have further demonstrated that the increase in cholesterol synthesis in the small intestine of diabetic animals is a generalized phenomenon occurring in all segments along the longitudinal axis of the intestine (duodenum to ileum) (16). Diabetes enhances cholesterol synthesis in the distal segments of the small intestine to a greater degree than in the proximal segments (16). In the distal small intestine, cholesterol synthesis is increased in all cell fractions along the villus crypt axis, but the increase is greatest in the upper villus cells (17). In contrast, in the proximal small intestine the increase in cholesterol synthesis is chiefly due to an increase in the crypt cell fraction (17). These results demonstrate that, although cholesterol synthesis is increased in all segments along the duodenoileum axis, the cells responsible for this increase differ in the proximal and distal small intestine.
Various factors regulate cholesterol synthesis in the small intestine of diabetic animals. Cholesterol feeding decreases, whereas procedures that reduce bile acid pool size stimulate cholesterol synthesis in the small intestine (18). Pharmacologically lowering plasma cholesterol levels with 4-amino-pyrazol(3,4-d)pyrimidine stimulates small intestinal cholesterol synthesis in diabetic animals (19). The response to these experimental manipulations in diabetic animals is similar to that observed in control animals and indicates that the regulation of small intestinal cholesterol synthesis is not altered by the diabetic state.

ROLE OF HYPERPHAGIA IN INCREASING INTESTINAL CHOLESTEROL SYNTHESIS
The mechanism by which diabetes stimulates small intestinal cholesterol synthesis may be related to the increased food intake that accompanies poorly controlled diabetes. Experiments have indicated that limiting food intake by pair feeding can prevent the increase in small intestinal cholesterol synthesis seen in diabetes (16,20). Moreover, studies have demonstrated that other conditions that result in an increase in food intake also enhance small intestinal cholesterol synthesis (21). Specifically, in 3rd-trimester pregnant rats, 21-day lactating rats, obese rats, and animals infused intragastrically with 16 vs. 8 g glucose/day, cholesterol synthesis is increased in the small intestine (21). Thus, in four hyperphagic models, increased food intake is associated with increases in small intestinal cholesterol synthesis. These findings suggest that the hyperphagia that accompanies poorly controlled diabetes is the chief stimulus for the increase in intestinal cholesterol synthesis. Moreover, in other physiological or pathological conditions associated with increased food intake, small intestinal cholesterol synthesis may also be increased, a phenomenon that could potentially lead to detrimental effects on total-body cholesterol homeostasis.

We next examined whether the stimulation of intestinal cholesterol synthesis by increased food intake is due to an increased bulk of food or is related to caloric content (21). In both control and diabetic animals, when 50% Alphacel (nonnutritive bulk; ICN Biomedicals) is added to the diet, the quantity of food intake approximately doubled, whereas the caloric intake remained constant (21). Despite the marked increase in ingested food bulk, intestinal cholesterol synthesis in both control and diabetic animals was unchanged by the addition of Alphacel (21). These experiments demonstrate that increasing the bulk of food ingested is not a stimulus to increasing cholesterol synthesis in the intestine. In contrast, feeding glucose or fructose alone is sufficient to stimulate intestinal cholesterol synthesis in diabetic animals (22). In diabetic animals fed a 15% glucose solution as the sole source of calories, cholesterol synthesis in the intestine is increased greater than fourfold (22). Thus, the increase in cholesterol synthesis in the intestines of hyperphagic diabetic rats occurs in response to a single caloric source, indicating that dietary fiber, complex carbohydrates, protein, and lipids are not required for this phenomenon.

The increase in small intestinal cholesterol synthesis induced by increased food intake does not require the direct contact of the intestinal mucosa with nutrients (21). In diabetic animals in which either the proximal or mid intestine is excluded from contact with nutrients, we observed an approximately twofold increase in cholesterol synthesis in the bypassed segment (21,23). In another model of hyperphagia, i.e., lactating animals, we have also observed that cholesterol synthesis is increased in segments of the small intestine that have been excluded from contact with ingested nutrients (23). These results indicate that the direct contact of the intestinal mucosa with nutrients is not the sole trigger for increasing cholesterol synthesis in the intestine of hyperphagic animals, suggesting that circulating hormones and/or neurological factors play a role.

Very recently we observed that the characteristic increase in small intestinal cholesterol synthesis in diabetic animals is prevented by total gastrectomy (see Feingold et al., this issue, p. 219). Diabetic animals with total gastrectomies still have increased food intake and a hypertrophied small intestine, yet intestinal cholesterol synthesis is similar in gastrectomized diabetic and normal animals. The mechanism by which total gastrectomy prevents the characteristic increase in intestinal cholesterol synthesis in diabetic animals is unknown. Vagotomy and selective removal of either the antrum or the fundus of the stomach did not prevent the increase, indicating that the inhibition requires the removal of the entire stomach. The stomach possibly produces a circulating and/or luminal substance that is required for the diabetes-induced increase in intestinal cholesterol synthesis.

FATE OF CHOLESTEROL SYNTHESIZED IN INTESTINE OF DIABETIC ANIMALS
Cholesterol synthesized in the small intestine has three main fates: it can be transported via the lymphatics to the bloodstream and contribute to the extraintestinal cholesterol pool, it can be utilized in the formation of cellular membranes in the intestines, or it can be excreted into the intestinal lumen. Studies from our laboratory with control and diabetic animals whose thoracic ducts have been cannulated demonstrated that the rate of transport of newly synthesized cholesterol from the intestine to the circulation is increased fourfold in diabetic rats (24). Additionally, the percentage of newly synthesized cholesterol transported via the lymphatics to the bloodstream is greater in the diabetic animals. Thus, the increased quantity of labeled cholesterol transported in the lymph of diabetic rats is accounted for by two factors, an enhancement of intestinal cholesterol synthesis and an increased percentage transport of newly synthesized cholesterol from the intestines to the circulation. In both control and diabetic rats, most (~80%) of the newly synthesized cholesterol was transported in the chylomicron lipoprotein fraction (24). Employing entirely different methods, Young et al. (25) also showed that cholesterol synthesized in the small intestine is transported to the circulation to a greater extent in diabetic animals than in control animals. In addition to the increased synthesis and transport of endogenous cholesterol, studies have demonstrated that the absorption of exogenous dietary cholesterol is also enhanced in diabetic animals (26–28). Thus, diabetes induces an increase in the transport of both endogenous and exogenous cholesterol from the small intestine to the circulation.
METABOLISM OF CHYLOMICRONS IN DIABETIC ANIMALS

Studies have demonstrated that the sterol component of chylomicrons obtained from diabetic animals is rapidly cleared from the circulation when administered to either control or diabetic animals (29). If the rate of disappearance of either [14C]cholesterol-labeled normal chylomicrons administered to control animals is compared with that of labeled diabetic chylomicrons administered to diabetic animals, the half-times are almost identical (vitamin A-treatment: control, 3.6 min; diabetic, 3.5 min; cholesterol-treatment: control, 5.7 min; diabetic, 4.4 min) (29). The rapid disappearance of labeled chylomicrons from the circulation in diabetic animals is accompanied by a parallel increase in label in the liver, with >85% of the [14C]cholesterol present in the liver 15 min after chylomicron administration. These results demonstrate that the sterols associated with chylomicrons are rapidly cleared from the circulation of diabetic animals and that most of the intestinally derived sterols are delivered to the liver.

POTENTIAL ADVERSE CONSEQUENCES OF INCREASED TRANSPORT OF CHYLOMICRONS FROM INTESTINE TO CIRCULATION

The increased transport of chylomicrons from the intestine to the circulation in diabetic patients could result in adverse consequences. First, it has been postulated by Zilversmit (30) that an increased flux of cholesterol in chylomicrons is therogenic. If this is so, then the increased transport of cholesterol in chylomicrons and chylomycin remnants in diabetes could be a potential mechanism by which diabetes increases the risk of atherosclerosis independent of serum lipid levels. Second, the cholesterol carried in the chylomicrons is rapidly transported to the liver, where it could lead to an increase in hepatic cholesterol content. Some but not all studies have reported that the concentration of cholesterol in the liver is increased in diabetic animals (26,31,32). Increases in hepatic cholesterol content result in a decrease in the number of hepatic LDL receptors (33). Thus, the increased transport of cholesterol from the intestine to the liver in diabetic animals could decrease the number of hepatic LDL receptors. This effect is superimposed on other factors affecting LDL-receptor number in diabetes. Insulin increases the number of LDL receptors on fibroblasts grown in tissue culture, and insulin infusions stimulate LDL catabolism in vivo in humans (34,35). These observations suggest that insulin deficiency might also lead to a decrease in hepatic LDL-receptor number. Hepatic LDL receptors play a crucial role in regulating serum LDL levels by affecting both the catabolism of LDL and the rate of LDL formation from VLDL (33). Decreases in the number of hepatic LDL receptors are accompanied by increased serum LDL cholesterol levels (33).

Finally, small intestinal triglyceride synthesis is increased in diabetic animals, which is accompanied by the increased transport in the lymph of triglycerides from the small intestine to circulation (36). This probably results in the increased delivery of triglyceride to the liver, which could stimulate hepatic VLDL secretion (37) and contribute to the increased serum VLDL levels that frequently accompany poorly controlled diabetes. Because VLDL is the precursor of LDL, increases in VLDL levels can indirectly contribute to an increase in serum LDL. In summary, the increased transport of lipids from the intestine to the circulation in poorly controlled diabetes could potentially result in alterations in lipid metabolism that may increase the risk of atherosclerosis.

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