Diabetes-Induced Accelerated Atherosclerosis in Swine
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Patients with diabetes are at higher risk for atherosclerotic disease than nondiabetic individuals with other comparable risk factors. Studies examining mechanisms underlying diabetes-accelerated atherosclerosis have been limited by the lack of suitable humanoid animal models. In this study, diabetes was superimposed on a well-characterized swine model of atherosclerosis by injection of the β-cell cytotoxic streptozotocin (STZ), resulting in a >80% reduction in β-cells and an increase in plasma glucose to diabetic levels. Animals were maintained without exogenous insulin for up to 48 weeks. Plasma glucose and cholesterol levels and lesion extent and severity were quantified in swine with diabetes and hyperlipemia alone and in combination compared with controls. Diabetes had no effect on plasma cholesterol levels, but diabetic/hyperlipemic (D-HL) swine developed hypertriglyceridemia and showed a doubling in aortic sudanophilia over nondiabetic/hyperlipemic (N-HL) swine as early as 12 weeks (47.25 ± 4.5 vs. 24.0 ± 4.6%). At 20 weeks, coronary artery stenosis was significantly greater in D-HL than in N-HL animals (86 ± 10 vs. 46 ± 8%). Coronary lesions predominantly arose in the first 2–3 cm of the vessels and displayed humanoid morphology. Aortic lesions in D-HL swine had double the cholesterol content of those in N-HL swine, and incorporation of oleate into cholesteryl ester was significantly greater in D-HL than in N-HL animals (7–14,21). Therefore, by use of streptozotocin (STZ) as a diabetes-inducing agent that we superimposed on the atherosclerotic swine model, we set out to determine the following: 1) whether diabetes could be reproducibly produced and maintained over prolonged time periods in swine; 2) whether plasma glucose and cholesterol levels in the model were interdependent; 3) whether cholesterol content and lipid esterifying mechanisms in lesions and monocytes were altered by diabetes; and, most importantly, 4) whether induced diabetes accelerated atherosclerosis in this model.

RESEARCH DESIGN AND METHODS
Induction of hyperlipemia and diabetes. All procedures involving animals were approved by the Committee for Animal Use in Research and Education.
of the Medical College of Georgia and complied with those approved by the American Veterinary Medical Association Panel on Euthanasia.

Male Yorkshire swine (n = 152), initially 8–12 weeks old and weighing 15–20 kg, were sedated with Telazol (10 mg/kg) and injected via ear vein either with buffer or with filter-sterilized STZ (50 mg/kg in 0.1 mol/l Na-citrate, pH 4.5) each day for 3 days. STZ-injected swine were given 25 g glucose twice daily at feeding for 2 days to offset insulin release from β-cells. Five days after the last STZ or buffer injection, half of the swine from each group were placed on a diet containing 1.5% cholesterol and 15% lard, and the other half was fed a normal diet as previously described (7–14). The resultant four groups of swine, namely nondiabetic-normolipemic (N-NL), nondiabetic-hyperlipemic (N-HL), diabetic-normolipemic (D-NL), and diabetic-hyperlipemic (D-HL) were maintained for periods of 4, 8, 16, 20, 24, 32, and 48 weeks before sacrifice. Fasting (18 h) plasma glucose was monitored daily for 2 weeks, then weekly thereafter with an Ames Glucometer II. Blood (~500 μl) for this and weekly plasma cholesterol measurements were obtained without sedation by pricking an ear vein with a lancet and collecting drops in a hematocrit tube. Total plasma cholesterol and triglycerides were measured with a standard enzymatic assay kit (Sigma, St. Louis, MO). Lipoproteins were isolated and quantified using the methods of Mahley et al. (22).

Tissue and monocyte collection. At the end of the various time periods, animals were killed and necropsied as previously described (7–14). The thoracic and abdominal aortas were removed intact, opened longitudinally along the ventral surface, and fixed in neutral-buffered formalin after excision of samples from standard sites in the thoracic and abdominal aorta for histological examination and lipid composition and synthesis studies. After fixation, the abdominal aortas were stained with Sudan IV (20) and pinned out flat, and digital images were obtained with a TV camera connected to a computerized image analysis system (Image Pro Plus; Media Cybernetics, Silver Spring, MD) to determine the percentage of aortic surface involved with atherosclerotic lesions. After initial studies extending over the 48-week period were completed, detailed histological and biochemical studies were performed on 04 swine at 20 weeks, a stage at which D-HL swine consistently showed significantly enhanced lesion development compared with N-HL swine. The left anterior descending and right coronary arteries from half of the 20-week swine were dissected free of heart tissue at the time of necropsy, fixed in formalin, and cross-sectioned at 2-mm intervals. The 2-mm segments from each vessel were examined under a dissecting microscope, and those with lesions were embedded in paraffin, serially sectioned, and stained with hemotoxylin and eosin (H and E), Masson’s trichrome, and Verhoff-Van Giesen stains. Measurements were carried out on the section qualitatively showing maximal stenosis in each artery. Original lumen area was determined by tracing the internal elastic lamina. The residual lumen area was also traced and expressed as a percentage of original lumen area to determine percent stenosis. Differences between group means were analyzed using analysis of variance. Coronal vessels from the other 32 swine were snap-frozen in liquid N2 for biochemical studies to be reported elsewhere. Samples of iliac and carotid arteries were similarly processed.

Effect of STZ on β-cells. To examine the effect of injection of STZ on insulin-producing β-cells, samples of pancreas were fixed in formalin, paraffin-embedded, sectioned, and stained with an antibody to porcine insulin. Using a standard magnification, the percent area of pancreas in section occupied by insulin-producing cells was measured in all four groups of swine using computerized image analysis at 2, 8, and 20 weeks after STZ injection.

Isolation of blood monocytes and lipid analysis. Just before necropsy, 400 ml of blood was removed into heparinized syringes via a jugular catheter. Monocytes were isolated by counterflow centrifugation as previously described (14). Freshly isolated monocytes of >95% purity were incubated (1.5–2 × 106 cells/ml) at 37°C for 90 min in 2.0 ml of a mixture of Medium 199 (Life Technologies, Grand Island, NY) and normal swine plasma (1:1, vol/vol), which contained either [1-14C]-acetate or [1-14C]-oleate at levels of 2 or 3 μCi/ml (specific activity: 57.0 Ci/mol) (NEN Research Products). After incubation, the tissues were rinsed in PBS and extracted by homogenization in chloroform/methanol mixtures as previously described (15). One portion of the lipid extract was fractionated by thin-layer chromatography as described above, while a second portion was saponified and the total cholesterol content determined after precipitation of the sterols by digitonin (26).

RESULTS

Plasma glucose and lipid levels. The plasma glucose and cholesterol levels of swine over the 48-week period are shown in Figs. 1 and 2. Glucose levels in nondiabetic swine (N-NL and N-HL) ranged between 3.3–6.1 mmol/l and were not age- or diet-dependent (Fig. 1). Animals...
injected with STZ (D-NL and D-HL) typically maintained a three- to fourfold increase above controls in fasting plasma glucose, and there was no effect of age or HL diet on glucose level (Fig. 1). Similarly, plasma cholesterol levels in the two groups of swine fed normal diet (N-NL and D-NL) ranged from 1.8–3.1 mmol/l (48-week mean 2.1 ± 0.3 mmol/l), whereas both groups fed high-fat diet (N-HL and D-HL) established cholesterol levels ranging between 10.9–17.6 mmol/l (48-week mean 13.8 ± 1.5 mmol/l) within 4 weeks of initiating the diet (Fig. 2). There was no significant difference in cholesterol levels at any stage between diabetic and nondiabetic swine fed the same diet. The distribution of cholesterol among lipoprotein classes was examined at 20 weeks (Fig. 3), and although total cholesterol and both LDL/VLDL and HDL cholesterol were significantly elevated in both N-HL and D-HL, no differences were seen between the two HL groups. Diabetes tended to lower HDL cholesterol levels in both NL and HL swine, but differences were not significant. However, although HL alone did not alter triglyceride level, the combination of hyperlipemia and diabetes resulted in a significant (more than twofold) elevation in plasma triglycerides (Fig. 3).

**Effect of STZ on β-cells.** All swine injected with STZ showed peak fasting plasma glucose within 2 days after the third injection. Thereafter, the majority of swine showed a mild decline in glucose level but maintained diabetic levels of >11 mmol/l (Fig. 1). Examination of pancreas sections immunostained with porcine anti-insulin antibodies showed that control (N) swine (n = 10) contained 221 ± 86 insulin-producing β-cells per standardized field compared with 23 ± 4 β-cells at 2 weeks (n = 10 swine) after STZ injection and 43 ± 10 β-cells per field at 20 weeks (n = 10 swine) after injection (Fig. 4A–C). Insulin-producing cells in control animals were clearly predominantly clustered in islets (Fig. 4A), whereas at 2 weeks after STZ injection, single β-cells were scattered, with no evidence of clustering (Fig. 4B). By 8–20 weeks after STZ injection, some β-cell regeneration was evident, and, although the density of β-cells was greatly reduced compared with controls (Fig. 4A), some clustering of insulin-producing cells in islets was seen in addition to single cells (Fig. 4C).

**Lipid studies.** Cholesterol content and cholesteryl ester formation were measured in standardized segments of aorta, iliac, and carotid arteries from 20-week swine (n = 10/group). The patterns seen in iliac and carotid arteries and in lesion-susceptible and nonsusceptible areas of the aortic arch were similar to that seen in the thoracic aorta, and data from these vessel segments are not shown. In the aorta, diabetes alone had no effect on cholesterol content in normolipemic conditions (Fig. 5A and B). Hyperlipemia alone enhanced arterial cholesterol content two- to sixfold over control vessels even in grossly nonlesioned areas of thoracic and abdominal aorta in N-HL swine (Fig. 5A and B). Abdominal lesions had 10-fold higher cholesterol content than nonlesioned areas from the same N-HL swine even in the absence of diabetes (Fig. 5B and C). However, although superimposition of diabetes did not significantly increase cholesterol content in grossly nonlesioned areas of the abdominal aorta (Fig. 5B), the content in lesioned areas was double that seen in hyperlipemia alone (Fig. 5C).

**FIG. 3.** Effect of experimentally induced hyperlipemia and diabetes on plasma lipids in swine. Data are means ± SD from fasting plasma samples from swine after 20 weeks of treatment (n = 10 per group). Bars having similar symbols are not statistically different.

Cholesteryl ester synthesis from [14C]-oleate, which is regarded as an early biochemical index of the rate of atherogenesis, was increased 3- to 20-fold in all grossly
normal arterial beds by hyperlipemia alone (Table 1). However, superimposition of diabetes on hyperlipemia doubled oleate incorporation compared with hyperlipemia alone in both thoracic and nonlesioned abdominal segments (Table 1). Once lesions had been established, however, no differences were seen between nondiabetic and diabetic hyperlipemic animals (Table 1), suggesting that maximal incorporation rates had been reached.

**Synthesis by monocytes.** Because we have previously demonstrated that monocyte-derived macrophages are the major cell type present in the intima in early lesions (8,9,15) and that circulating monocytes have enhanced lipid biosynthesis, acyl-CoA-cholesterol acyltransferase (ACAT) activity and cholesterol esterification in hyperlipemic animals (15), we examined lipogenesis from [14C]-acetate and [14C]-oleate in monocytes from the four groups of swine at 20 weeks. Lipogenesis from [14C]-acetate was enhanced up to 40% above control by hyperlipemia alone in all lipid classes (Fig. 6A). In the absence of hyperlipemia, diabetes had little or no effect on lipogenesis. However, when superimposed on hyperlipemia, diabetes further augmented synthesis in all classes above that seen in hyperlipemia alone (Fig. 6A). Moreover, ACAT activity in monocytes, as measured by [14C]-oleate incorporation into cholesteryl esters (Fig. 6B), was responsive to both diabetes (40% increase over control) and hyperlipemia alone (threefold above control), and the combination of the two further augmented this response to fourfold over controls, findings which are consistent with those seen in nonlesioned areas in the vessel wall (Table 1).

**Accelerated atherosclerosis in diabetic swine.** This model of hyperlipemic diabetic swine is characterized by greatly accelerated atherosclerosis in the diabetic state. As expected, only hyperlipemic animals developed lesions. These differences are readily visualized qualitatively on Sudan IV–stained aortas viewed grossly, as in Fig. 7: panels A and B are thoracic and abdominal aorta from a representative 20-week D-HL animal; panels C and D show those from an N-HL animal at the same stage with comparable terminal cholesterol levels (15.2 vs. 14.7 mmol/l). Computerized image analysis of Sudan-stained aortas from all four groups (Fig. 8) demonstrates that the percent aortic surface area involved with lesion is, on average, twofold greater in D-HL compared with N-HL swine, even as early as 12 weeks. With prolonged (48 weeks) hyperlipemic conditions, the effect of diabetes is less pronounced as lesion coverage maximizes.

As in humans, coronary arteries in hyperlipemic swine typically develop major lesions in the first 2–3 cm from their origin (although severely diabetic swine frequently showed grossly visible focal lesions in the distal epicardial arteries). In hyperlipemic swine with comparable cholesterol levels between 10.9–17.6 mmol/l (Fig. 2), diabetic swine (D-HL) showed greatly accelerated coronary atherosclerosis compared with swine with hyperlipemia alone (N-HL), and frequently showed almost totally occluded vessels by 20 weeks (Fig. 9A, B, and D). Coronary artery lesions from 16 D-HL swine at 20 weeks showed an

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**FIG. 4.** Representative digital light photomicrographs of sections of swine pancreas immunostained with anti-insulin antibody to demon-
average of 86 ± 10% stenosis compared with 48 ± 8% in 16 N-HL swine at the same stage (P < 0.01). In a separate 20-week study in which plasma cholesterol levels were maintained in the 7.8–12.0 mmol/l range (n = 5 per group), D-HL swine showed 46 ± 8% coronary stenosis compared with 15 ± 4% in the nondiabetic (N-HL) group (P < 0.01). Lesions in D-HL swine were humanoid in appearance, demonstrating relatively acellular necrotic cores covered by fibrous caps with medial thinning at the base of the lesions (Fig. 9A–C), together with evidence of hemorrhage into the plaques (Fig. 9C) and severe calcification (Fig. 9A and B). Lesions frequently extended into the media (Fig. 9B). In comparison, coronary lesions from N-HL swine showed fatty streaking and lesions in early stages of progression without hemorrhage or calcification at 20 weeks (Fig. 9D). Similar findings were observed in lesions taken from standardized sites (arrows in Fig. 7B and D) in the lower abdominal aorta and in the first 2 cm of the iliac artery. As in coronary arteries, aortic lesions in D-HL animals showed greater progression, with extensive calcification and medial involvement at the base of the lesions and frequent hemorrhage into the lesion, which was not seen in N-HL lesions (Fig. 9E and F). Lesions in the iliac arteries of D-HL swine were frequently highly stenotic (Fig. 10A) and were intensely calcified compared with lesions from the same sites in N-HL swine (Fig. 10B). These features of complicated plaques were not observed in lesions from N-HL swine before 32 weeks, and they

![A] THORACIC AORTA

![B] ABD AORTA/NON-LESION

![C] ABD AORTA/LESION

**FIG. 5.** Total cholesterol content in intima-media segments stripped from standardized sites. Bars having different symbols are statistically different (P < 0.05). Diabetes had no effect on cholesterol content in thoracic and nonlesioned abdominal aorta, but abdominal lesions from D-HL swine had twofold greater cholesterol content than those with hyperlipemia alone.

**TABLE 1**

<table>
<thead>
<tr>
<th>Arterial site</th>
<th>N-NL (n = 4)</th>
<th>D-NL (n = 4)</th>
<th>N-HL (n = 4)</th>
<th>D-HL (n = 4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thoracic aorta</td>
<td>1.0 ± 0.4*</td>
<td>0.7 ± 0.5*</td>
<td>57 ± 12†</td>
<td>106 ± 18‡</td>
</tr>
<tr>
<td>Abdominal aorta (nonlesion)</td>
<td>—</td>
<td>—</td>
<td>99 ± 16*</td>
<td>92 ± 24*</td>
</tr>
<tr>
<td>Abdominal aorta (lesion)</td>
<td>—</td>
<td>—</td>
<td>57 ± 16*</td>
<td>42 ± 13‡</td>
</tr>
</tbody>
</table>

Data are means ± SD from 5 swine per group. Intima-media preparations from abdominal aortas from all four groups of swine at week 20 were incubated with [14C]-oleate, as previously described. Statistical analysis was performed on logarithmically transformed data (log10) by analysis of variance; pairwise comparisons between means were made with Student’s t test within horizontal rows, values with different symbols are statistically different (P < 0.05).

![A: 14C-Acetate](image1)  
![B: 14C-Oleate](image2)

**FIG. 6.** Lipogenesis from [14C]-acetate and [14C]-oleate in purified peripheral blood monocytes from 20-week swine (n = 5 per group). Results were calculated as disintegrations per minute divided by 2 × 10⁶ cells and expressed as a percent of N-NL control. A: Lipogenesis from acetate was increased up to 40% by hyperlipemia alone in all lipid classes. Diabetes alone had little effect in the absence of hyperlipemia, but further augmented synthesis above hyperlipemia alone in the presence of hyperlipemia. B: ACAT activity, as measured by oleate incorporation into cholesteryl esters, was increased by both diabetes (40%) and hyperlipemia (threefold). Combination of the two further increased incorporation to fourfold over controls. a, significantly different from controls (P < 0.05); b, significantly different from all other groups (P < 0.05).
were never as extensive as those in the D-HL group, even at 48 weeks. Occlusive lesions were also frequently observed in small epicardial branches of coronary arteries in D-HL swine (Fig. 10C). Also, of the 64 swine examined at 20 weeks, 2 D-HL animals had abdominal aortic aneurysms associated with almost occlusive plaques (Fig. 10D), and 1 D-HL swine had a dissecting aneurysm of the thoracic aorta resulting in a “double-barreled” aorta from the aortic valves to the level of the diaphragm.

DISCUSSION

Patients with diabetes are prone to the same recognized cardiovascular disease risk factors as nondiabetics, but are at a two- to sixfold greater risk of developing macrovascular disease (1,2). Diabetic women are fivefold more prone to coronary artery disease than nondiabetic individuals (2,3); myocardial infarction is twofold higher in younger diabetic subjects, and mortality after infarction is

FIG. 7. Representative digital photomicrographs of Sudan IV–stained aortas from 20-week swine. Compare area stained between thoracic (a) and abdominal (b) aorta from D-HL swine with thoracic (c) and abdominal (d) aorta from N-HL swine. Sudanophilic area is significantly greater in D-HL swine in both thoracic and abdominal aorta (Fig. 8). Excised areas of abdominal aortas immediately above the bifurcation (arrows in Fig. 7b and d) are sites taken for histological studies, as in Figs. 9e and f.

FIG. 8. Percent aortic surface area showing sudanophilia was measured using digital image analysis on aortic preparations as illustrated in Fig. 7. Only hyperlipemic swine formed lesions, and D-HL swine showed an approximate twofold greater sudanophilic area compared with N-HL at all stages (P < 0.001, n = 10 per group).
FIG. 9. Representative digital light photomicrographs of coronary arteries and abdominal aortas from swine showing degree of stenosis and lesion morphology at 20 weeks of treatment duration. All are stained with hemotoxylin and eosin. 

a: Left anterior descending (LAD) coronary artery from 20-week D-HL swine showing 95% stenosis. Plaque shows humanoid features of acentric lumen (L), fibrous cap (F), necrotic lipid core (N), areas of hemorrhage (H), and calcification (C) in the plaque, as well as medial thinning (arrowhead) at plaque base (×28).

b: As in panel a, but from the right coronary artery (RCA) of a second 20-week D-HL swine showing 97% occlusion of lumen. Lesion shows small foci of calcification and hemorrhage, cholesterol clefts (arrow), and necrotic lipid core, which extends into the media (arrowheads) (×28).

c: Higher magnification
also elevated (4). Diabetes is now considered an independent risk factor for atherosclerosis that is not necessarily related to the severity, duration, or type of diabetes (29,30). Diabetic patients also demonstrate increased LDL cholesterol and triglyceride levels and reduced HDL cholesterol levels (31,32); they are subject to abnormal platelet function and coagulation (33,34), and they tend to be hypertensive and overweight (29,35). However, these conventional risk factors do not completely explain the increased risk of vascular disease, epidemiologically, in diabetic patients, and there is no evidence that they are more important in diabetic than in nondiabetic subjects (36). Diabetes appears to act as a “multiplier” of risk factors, but even this concept appears dependent on geography and sex (2).

A major limiting factor in studying mechanisms responsible for accelerating atherosclerosis in diabetes has been the lack of suitable humanoid animal models. Although both the American Diabetes Association and the Juvenile Diabetes Foundation International made recommendations for the development of such models over a decade ago (37,38), the lack of appropriate animal models is still recognized (5,39). Diabetes appears spontaneously in numerous animal species, and several genetic models have been developed in rodents (40–42). These models have proven useful for studies on numerous aspects of diabetes, but they are poor models of diabetic atherosclerosis, as they develop nonhumanoid lesions, at best, and have lipid and lipoprotein metabolism and profiles very different from humans. A more successful approach has been the superimposition of β-cell ablation (by pancreatectomy or use of the diabetogenic agents alloxan and STZ) on animal models of atherosclerosis, including nonhuman primates (42–44) and rabbits (45). Surprisingly, few studies of the effects of chemically induced diabetes on atherosclerosis have been carried out in humanoid models, and these have largely been performed as part of other studies in nonhuman primates. Consequently, these studies frequently lacked suitable controls that consumed nonatherogenic diets or atherogenic diets alone (42), and they did not compare atherogenesis in diabetic and nondiabetic conditions. However, such studies have demonstrated severe atherosclerosis in diabetic animals (42–44), accompanied by atherogenic lipid profiles (42,47), which are in keeping with the present findings. Review of the literature has revealed no detailed studies on atherosclerosis in diabetic swine. Although spontaneous diabetes has been reported in Yucatan miniature swine (48–51), only one recent study (52) in a small number of alloxan-treated Sinclair miniature swine has demonstrated enhanced arterial sudanophilia at prelesion stages. To our knowledge, the current study is the first to describe and quantify diabetes-induced accelerated atherosclerosis in multiple arterial beds over an extended period of time.

In this study, intravenous injection of STZ resulted in a 10-fold reduction in insulin-producing β-cells and a concomitant increase in fasting plasma glucose to diabetic levels. These levels were maintained over the 48-week period despite a doubling in the number of β-cells at 20 weeks compared with 2 weeks after STZ injection. These findings indicate that β-cell regeneration occurs in this model, possibly because of the young age (8–12 weeks) of the swine at induction. Although the resultant 20% of the normal complement of β-cells achieved is insufficient to maintain normoglycemia, clearly the animals are producing (reduced levels of) insulin, as they survived for up to 48 weeks with no exogenous insulin. The animals also show impaired glucose tolerance in oral glucose tolerance tests (data not shown). Attempts were made to measure glycated hemoglobin, but, although generally elevated in diabetic compared with normal swine, the values were ~50% of human values and were highly variable. The swine erythrocyte is highly impermeable to glucose (53,54), such that glycated hemoglobin is not a useful indicator of diabetes in this model. Recent studies in alloxan-treated pigs (52), however, demonstrated increased levels of other glycated plasma proteins after only 1 week of diabetes.

The current results show that hyperlipemia had no effect on glucose levels but that it did induce a 47% increase in HDL cholesterol. Although statistically significant, this increase was minuscule compared with the 14-fold increase in LDL + VLDL cholesterol, which comprised >90% of the total cholesterol in hyperlipemic animals. We have no explanation for the increase in HDL cholesterol observed in fast-fed swine, but it has been previously observed by ourselves and others in both swine (19,20,52) and other diet-induced hyperlipemic animal models (55), and may simply be an effect of the huge increase in total cholesterol. As in humans (31,32), however, diabetic animals tended to have lower HDL cholesterol levels than nondiabetic animals, but differences in actual levels were not statistically significant, in accord with findings in alloxan-induced diabetes in swine (52). Perhaps of more relevance to the accelerated atherosclerosis in this model is that the total cholesterol–to–HDL cholesterol ratio, which is generally increased in human diabetic subjects, was increased significantly by diabetes in both normolipemic and hyperlipemic swine (38 and 28%, respectively). Although the diabetes-induced decrease in HDL cholesterol and increased total cholesterol–to–HDL cholesterol ratio may result in a higher atherogenic potential, there was no effect of diabetes on total or LDL + VLDL cholesterol. Moreover, the accelerated atherosclerosis seen in D-HL swine cannot be explained on the basis of an increase in atherogenic lipoproteins. These data differ somewhat from those of Dixon et al. (52) who showed elevated total cholesterol in fast-fed alloxan-treated compared with fast-fed nondiabetic swine at 8 weeks but, strangely, not at 12 weeks. Although the differences between D-HL and N-HL swine in lesion acceleration and progression cannot be explained on the basis of differing total cholesterol levels, it is of interest that, when swine were maintained at lower plasma cholesterol levels for 20 weeks, the degree of coronary of base of a RCA lesion showing erosion and thinning of the media (M) at arrowheads, as well as hemorrhage into the necrotic core of plaque (×90). d: Same as in panel a, but from a nondiabetic (N-HL) swine. A fatty streak lesion (arrows) is present along one side of the vessel, and a more advanced lesion with organizing fibrous cap and small lipid core is present. No hemorrhage or calcification is seen, and stenosis, including both lesions, is 48%. Compare relative stenosis and severity of lesions in panels a and b with that shown in panel d. e: Abdominal aortic lesion from just above bifurcation in 20-week D-HL swine. Lesion is equal in thickness to underlying media and shows extensive calcification and hemorrhage with erosion of media (arrow) (×28). f: The same as panel e, but from N-HL swine at 20 weeks. Lesion thickness is ~30% of media, with fibrous cap developing over necrotic lipid core. No calcification or hemorrhage is present (×18).
FIG. 10. Representative digital light photomicrographs of femoral artery lesion from 20 weeks D-HL swine showing 91% occlusion (a). Lesion displays well-developed fibrous cap (F) overlying necrotic lipid core (N) and extensive calcification at base of lesion (arrowheads). Stained with hemotoxylin and eosin (×18). b: Iliac lesion with 20% occlusion from a similar site in a 20-week N-HL swine. As in coronary arteries (9 days), a small fatty streak lesion (arrowhead) and a slightly more advanced lesion (arrow) with initiating fibrous cap and lipid core is seen. Stained with hemotoxylin and eosin (×18). c: Small epicardial branch of right coronary artery from 20-week D-HL swine with 98% occlusion. Lumen
stenosis was reduced by at least half in both D-HL and N-HL groups. However, even at the reduced (7.8–12.0 mmol/l) cholesterol levels, the 20-week D-HL group had coronary stenosis comparable with the N-HL groups (46 ± 8 vs. 48 ± 8%, respectively) maintained at the higher cholesterol levels (10.9–17.6 mmol/l) for the same time period. Thus, the acceleration and severity of diabetic atherosclerosis in this swine model is maintained at both low and high cholesterol levels, but is definitely linked to the level of hypercholesterolemia.

As we (7) and others (19,20) have previously demonstrated, high-fat diet feeding alone does not result in hypertriglyceridemia in swine despite the high lard content of the diet. However, as in alloxan-treated, fat-fed swine (52), superimposition of STZ-induced diabetes on hyperlipemia results in a significant increase, similar to that seen in human diabetic subjects, in plasma triglycerides. Although there was no effect of diabetes on the cholesterol content of grossly normal thoracic and abdominal aortic intima, abdominal aortic lesions from diabetic swine contained twofold more cholesterol than lesions from nondiabetic swine. Conversely, ACAT activity, as evidenced by incorporation of [14C]-oleate into cholesteryl esters, was not significantly different in the more advanced abdominal aortic lesions from diabetic and nondiabetic animals. Grossly normal areas of thoracic and abdominal aorta, which we previously (15) have shown to have thickened intimas containing macrophage foam cells in hyperlipemic animals, showed a twofold increase in oleate incorporation in D-HL compared with N-HL swine, although no differences in total cholesterol content were seen. These data suggest that accelerated formation of lesions in diabetes may be associated with increased lesion ACAT activity, which reaches maximal levels as lesions mature. Our previous studies (8,9,15) have demonstrated that blood monocytes are the major source of foam cells in developing plaques in N-HL swine and that they exhibit stimulated lipid synthesis and ACAT activity before entering the lesion and differentiating into macrophage foam cells (15). The present study demonstrates that lipid synthesis and ACAT activity in monocytes are further augmented by diabetes and probably account for the increased prelesion ACAT activity, thus implicating the monocyte in the enhanced atherogenesis seen in this diabetic model.

The present study clearly demonstrates that, under comparable conditions of hyperlipemia, diabetes vastly accelerates atherogenesis compared with that seen in nondiabetic swine. This acceleration is seen not only in the greater extent of aortic sudanophilia from 8 weeks onward, but also in the severity of aortic, coronary, and iliac lesions. As in humans, abdominal aortic lesions in swine are more advanced than those in the thoracic portion, even in hyperlipemia-alone swine. Superimposition of diabetes results in more rapid progression of abdominal plaques to complicated lesions, and the extensive necrosis, hemorrhage, and calcification, accompanied by medial thinning, makes them susceptible to aneurysm. In their recent study on alloxan-treated swine, Dixon et al. (52) showed 9% of carotid artery area was sudanophilic at 12 weeks’ duration of diabetes and hyperlipemia. In the present study, 47% of the aortic surface area was sudanophilic at 12 weeks. Because both plasma glucose and cholesterol levels were comparable in the two studies, it is unlikely that the large differences in sudanophilia can be explained on the basis of the diabetes-inducing agent or high-fat diet used. In our hands, Sinclair miniature swine and Yorkshire swine have comparable lesion development with hyperlipemia alone initiated at the same young age (8–12 weeks) as in the present study. The same may not hold true when they are sexually mature, as were the Sinclair swine used by Dixon et al. (52). The most likely reason for the difference is that, in our experience, the swine carotid artery is, compared with the aorta and iliacs, largely spared from atherogenesis, and lipid accumulation in this vessel is much slower. In contrast, striking differences in coronary atherosclerosis are seen between D-HL and N-HL animals with comparable cholesterol levels. Not only are coronary lesions twice as occlusive in D-HL compared with N-HL swine, but D-HL coronary arteries also develop severely complicated plaques containing hemorrhage and calcification, with extensive medial thinning as early as 20 weeks. In the present and previous (8,9) studies, these complications were not seen before 30 weeks in N-HL lesions, and N-HL lesions did not attain these levels of stenosis in the 48-week study period. Coronary lesions comparable with those seen in the D-HL swine have previously been produced in swine by high-fat diet alone (21,56,57). However, these were attained after 10–12 months of diet feeding, with maintenance of total cholesterol levels in the 21–26 mmol/l range, compared with the 5 months at 10.9–17.6 mmol/l cholesterol range in the present study. These findings further emphasize the rapid acceleration of atherosclerosis seen in this diabetic swine model, particularly as it affects the coronary vessels, many of which showed >95% occlusion at necropsy. Although swine are considered to have few native collateral coronary vessels (58,59), it is probable that collaterals develop under these conditions of high stenosis, given that <10% of the animals with severe stenosis died spontaneously of apparent coronary infarcts (R.G.G., unpublished data).

The swine has proven, over the past 3–4 decades, to be an excellent humanoid model of atherosclerosis (7–15,19,21), and the swine model has been instrumental in elucidating the key role of the blood monocyte in early atherogenesis (8,9,14) as well as the dynamics of smooth muscle and endothelial cell proliferation in lesion development (21). The location and severity of lesions are similar to those seen in humans, particularly in the coronaries, where lesions occur predominantly in the first 2–3 cm of the origin (21,60). Swine, like humans, demonstrate areas of predilection to atherosclerosis (7–12), and the morphology of swine plaques is similar to that seen in humans from early to late stages of progression (7–14,21).

(L) contains postmortem blood clot. Section is somewhat oblique through a branch point, but lesion can be seen to extend into media on external side of internal elastic lamina (arrows), with extensive medial thinning (arrowheads). Stained with hemotoxylin and eosin (×180). d: Longitudinal section through part of a large abdominal aortic lesion at site of aortic aneurysm in a 20 weeks D-HL swine. Ballooning of media (M) and internal elastic lamina (arrowheads) is evident. Lumen is visible at the top left. Arrow in lumen indicates direction of blood flow. A large calcific area (C) has been torn away during sectioning and is surrounded by intraplaque hemorrhage (H). Plaque shows extensive recanalization with small vessels (small arrows). Stained with Verhoff-Van Giesen (×18).
Swine develop spontaneous atherosclerosis in old age (17), and, in our experience, female swine are protected from atherosclerosis (R.G.G., unpublished results). The cholesterol levels (7), the lipoprotein patterns, and the lipoprotein metabolism (19,20) of normal swine are similar to those of humans, and, when fed a high-fat diet, swine develop a dyslipoproteinemia similar to that of the type II pattern seen in humans (7,19,20). Alloxan-treated swine develop a dyslipoproteinemia analogous to patterns seen at various stages of both types I and II diabetes (52). We now show for the first time that diabetes can be produced and maintained for prolonged periods (48 weeks) in swine without exogenous insulin by chemical ablation of β-cells with STZ. The results indicate that diabetic glucose levels and impaired glucose tolerance can be maintained if β-cell numbers are reduced to <20% of normal. The severity of coronary disease renders this model of particular relevance to the human condition, and the rapid calcification of lesions, generally not prominent in animal models, is a particularly humanoid characteristic. Moreover, the ability to control lesion acceleration and severity through control of cholesterol level provides an invaluable parameter for examination of the progression of diabetic atherosclerosis. This study also implicates monocyte/macrophage function, known to be altered by hyperlipemia (14,15), as being further altered in the diabetic state. Not only do monocytes from D-HL swine show elevation of lipid metabolism and ACAT activity, but also, as shown in other studies (61), enhanced 12-lipoxygenase and H2O2 production, resulting in increased oxidant stress in the lesion. Additionally, lesions in D-HL swine show greater smooth muscle cell proliferation and accumulation than that seen with hyperlipemia alone (62). Based on studies in tissue culture, this greater proliferation does not appear to be induced by hyperglycemia alone (62), but by one or more other factors present in the serum (63). Clearly, although the lack of insulin dependence, hypertriglycerideremia, and glucose intolerance appear to be reflective of type 2 diabetes, more investigation is needed to (1) determine whether the model best fits type 1 or type 2 diabetes, (2) determine whether insulin-resistance is developed, and (3) further clarify other metabolic and biochemical features of the model. However, the large number of animals studied (n = 152) at various stages demonstrates reproducibility of the quantitative findings and suggests that this model will be ideally suited for studies into the mechanisms of diabetes-induced accelerated atherosclerosis.

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