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Hyperglycemia and loss of ovarian hormones mediate atheroma formation through endothelial layer disruption and increased permeability

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Benton J, Powers A, Eiselein L, Fitch R, Wilson D, Villablanca AC, Rutledge JC. Hyperglycemia and loss of ovarian hormones mediate atheroma formation through endothelial layer disruption and increased permeability. Am J Physiol Regul Integr Comp Physiol 292: R723–R730, 2007. First published June 29, 2006; doi:10.1152/ajpregu.00112.2006.—The overall goal of this project was to examine the interactions of hyperglycemia and loss of ovarian hormones on the artery wall in a type 1 diabetic mouse model. Intact or ovariectomized (OVX) female BALB/c mice were fed a high-cholesterol diet. Half the animals were treated with streptozotocin to induce insulin-deficient diabetes mellitus, generating four treatment groups: control, intact; control, ovariectomized; diabetic, intact; diabetic, ovariectomized (DOVX). We examined arterial structure and function and found that 1) diabetes and ovariectomy additively increased endothelial layer permeability, 2) arterial stiffening was increased in DOVX, 3) DOVX synergistically increased atheroma formation, and 4) ultrastructural evaluation revealed that the basal lamina was often multilayered and formed convoluted aggregates separating endothelium from the internal elastic lamina in diabetic, but not control arteries or arteries from OVX mice. Endothelium overlying these regions formed thin cytoplasmic extensions between these aggregates and was often separated from the basal lamina by electron lucent spaces. Our studies showed that diabetes and loss of ovarian function have additive and synergistic effects to worsen arterial pathophysiology by disrupting the arterial endothelial layer with increased permeability and increased atheroma formation.

atherosclerosis; cardiovascular; diabetes; female gender; sex hormones; hyperglycemia; diabetic, ovariectomized; female sex hormones; hyperglycemia-induced changes in these parameters would be potentiated by the loss of female sex hormones. We showed that at least one of the mechanisms for increased arterial pathophysiology in this setting was disruption of the arterial endothelial layer with increased permeability and increased atheroma formation.

Endogenous estrogens are widely regarded as atheroprotective and loss of female sex hormones has been strongly associated with the development of atherosclerotic cardiovascular disease (39). Recent randomized clinical trials have generated skepticism regarding hormone replacement therapy’s atheroprotective potential (19, 21, 29, 39); however, clear and powerful protective effects of estrogen on atherosclerosis development have been demonstrated in many animal models and in observational human studies (5, 16, 20). Previous work from this laboratory has demonstrated that 17β-estradiol reduces arterial endothelial layer permeability and arterial LDL accumulation during an oxidant stress (42, 43), attenuates arterial stiffening (28), and decreases glycoxidative damage to the arterial wall in euglycemic animals (28, 42). The effect of female sex hormones in the setting of diabetes mellitus is much less well defined. A previous study in male apolipoprotein E-mutant mice treated with streptozotocin (STZ) developed significantly more lesions in some (but not all) parts of the aorta and its main branches. Chronic treatment with 17β-estradiol lead to a significant decrease in blood glucose and triglyceride levels and reduced the lesion area in all vascular segments studied (38). Although much of the advantage of female gender in atherosclerotic cardiovascular disease is eliminated by diabetes, female sex hormones may still be playing a significant role in protection against vascular injury in diabetic women. The combination of DM1 and loss of female sex hormones has been postulated to be synergistic in its effect on artery pathophysiology, although the mechanism(s) for this synergistic effect are not known with certainty.

The overall goal of this project was to investigate the interactions of hyperglycemia and loss of ovarian function on arterial pathophysiology. Our studies were designed to examine artery permeability and LDL accumulation, arterial stiffening, endothelial layer ultrastructural changes, and atherosclerotic lesion development. We hypothesized that hyperglycemia-induced changes in these parameters would be potentiated by the loss of female sex hormones. We showed that at least one of the mechanisms for increased arterial pathophysiology in this setting was disruption of the arterial endothelial layer with increased permeability and increased atheroma formation.

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MATERIALS AND METHODS

Chemicals and Materials

Krebs-Heinsleit buffer consisting of (in mM): 116 NaCl, 5 KCl, 2.4 CaCl$_2$·H$_2$O, 1.2 MgCl$_2$, 1.2 NaH$_2$PO$_4$, and 111 glucose. BSA and dextran [65,000 mol wt fluorescently labeled with tetramethylrhodamine isothiocyanate (TRITC)] was obtained from Sigma (St. Louis, MO).

Animal Care and Treatment

Ten-week-old ovarioctomized and intact BALB/c mice were obtained from Charles River Laboratories (Boston, MA) and housed in an approved facility. All protocols used in this study were approved by the Animal Use and Care Committees of the University of California, Davis, CA, and conformed to Guidelines set by the American Physiological Society and Animal Welfare Act. Animals were kept on a 12:12-h light-dark cycle and had access to food and water ad libitum. All mice were placed on a high-fat diet (Purina 15% butter, 1.25% cholesterol, 0.5% cholate) upon arrival and continued on the diet throughout treatment. At 12 wk of age, mild hyperglycemia was induced in a subset of animals by intraperitoneal injection of STZ (40 mg/kg) for five consecutive days. Control mice were given saline as a vehicle control. To monitor blood glucose levels, ~100 µl of blood were taken from the saphenous vein of each animal every 4 wk throughout the study. Injections were repeated at 7 wk to maintain the hyperglycemic state, and mice were killed following 12 wk of treatment. Treatment groups for all experiments were as follows: control, intact (CI); control, ovarioctomized (COVX); diabetic, intact (DI); and diabetic and ovarioctomized (DOVX).

Preparation of Fluorescently Labeled LDL

Blood was obtained from fasting human males by venipuncture in tubes containing 15 mg EDTA. All procedures were conducted under a protocol approved by the Human Subjects Review Committee at the University of California, Davis, CA. Low-density lipoproteins (LDL) were isolated by sequential density gradient ultracentrifugation and labeled with the fluorescent hydrocarbon probe 1,1-diocadecyl-3,3',3'-tetramethylindocarbocyanine (DiI) (33). The spectral properties of DiI are excitation maximum 540 nm and emission maximum 556 nm. Freshly labeled DiI-LDL (~1 mg/ml) was prepared weekly. Prior to perfusion, 2 ml DiI-LDL was added to 30 ml Krebs-Heinsleit buffer containing 1% wt/vol BSA (final concentration, ~66 µg/ml) for use in all experiments.

Perfusion Experiments

Mice were anesthetized with nembutal (50 mg/kg) and carotid arteries were isolated and removed for perfusion experiments as described previously (44). Briefly, the carotid artery was isolated, and both ends of the vessel were cannulated, with the distal cannula placed just proximal to the bifurcation of the internal and external carotid. The artery then was removed, placed into a perfusion chamber, and bathed in oxygenated Krebs-Henseleit buffer (pH 7.4, 37 °C).

Measurement of Arterial LDL Accumulation and Measurement of Arterial Permeability

Quantitative fluorescence microscopy for measurement of lipoprotein influx in mouse carotid arteries has previously been described in detail (44). Arterial permeability was estimated by the rate of accumulation of 65,000 mol wt dextran labeled with TRITC-dextran. TRITC-dextran was diluted to a final concentration of ~95 µg/ml in Krebs-Henseleit buffer containing 1% wt/vol BSA. All perfusion experiment protocols are identical to those described for measurement of arterial LDL accumulation. Dextran accumulation was used as an index of arterial permeability because dextran is a nonlipid reference molecule that does not specifically bind to the arterial matrix.

Plasma Assays

Following removal of the carotid arteries, blood was collected from each animal through the right ventricle using a 23-gauge needle and heparinized syringe. Blood was transferred to vacutainers and centrifuged at 2,800 rpm for 10 min at 4°C. Plasma was separated and stored at −20°C until analysis. Plasma glucose was measured using an Analox Instruments GM7 analyzer. Plasma lipid levels were determined by IDEXX Veterinary Services (Sacramento, CA).

Tissue Assays

Immediately after exsanguination, the aorta and uterus were removed, rinsed in Krebs-Henseleit buffer, and stored at −80°C until used. The aorta was used for atheroma quantification and analysis of vascular stiffening. The uterus was dried, weighed, and used as a functional index of female sex hormone status. Following arterial compliance measurements, the remaining aorta was dried and weighed. This tissue was used for determination of total protein (modified Lowry assay) and total collagen (Woessner assay).

Assessment of Vascular Stiffening

Maximum passive stiffness. Maximum passive stiffness (MPS) of mouse thoracic aortic rings was determined through load–strain analysis ex vivo (6, 36). The vessels were hydrated overnight in Krebs buffer at 2°C. Two stainless steel rods were inserted through the lumen of a 1-mm thoracic aortic segment in a parallel fashion while the vessel was immersed in Krebs buffer. One rod was attached to a motorized controller, while the other to a force transducer (model FT10; Grass Instruments, Quincy, MA). As the motorized controller pulled the rods apart, the vessel tension was recorded. In preparation for each stretch, the aortic segments were conditioned three times to a standard strain (10% of maximal strain) that was determined in previous experiments. The vessel was stretched until breakage. Load vs. strain curves were generated for each vessel. MPS is defined as the maximal slope of the load vs. strain relationship.

Quantitative and Qualitative Analysis of Atheroma

Aortas were characterized for qualitative assessment of lesion morphology and quantification of atheroma (40). Briefly, after careful removal of periadventitial fat, aortas were fixed (4% followed by 10% paraformaldehyde overnight), and cut into three segments: proximal segment including all of the aortic arch; midsegment consisting of the thoracic and suprarenal aorta; and distal segment consisting of the infrarenal aorta proximal to the iliac bifurcation. Aortic segments were placed side-by-side in cryomolds with optimum cutting temperature compound. In this manner, cryosections (10-µm thick taken at ~30°C, discarding every other section) sample all three segments of the aorta, permitting direct comparisons of each. For each aorta, we analyzed four sections per slide for a total of nine slides per aorta. In this manner we achieved extensive sampling of each aorta for atheroma (36 sections per segment and 108 cryosections per aorta), for a total of ~360 µm from each segment and 1,000 µm from each aorta. Quantitative morphometric analyses of aortic lesions were performed by staining cryosections with oil-red-O (Sigma) to define and characterize neutral lipid in the vessel wall. Cryosections were counterstained with Gill’s hematoxylin (Sigma) permitting histological assessment of lesions in artery segments. Sections were analyzed for lesion location and area by direct imaging (Olympus BX-40 microscope with a DP11 camera with smart media chip). Atheroma were identified by a single observer blinded to experimental parameters, and mean lesion area (µm$^2$ ± SE) determined for each atheroma by morphometric analysis with a computer-assisted imaging system (Image Pro Plus, version 4.1). We’ve previously determined the quantification data to be highly reproducible having an intraobserver error of <6%. To assess for possible regional differences in atheroma development with the various experimental treatments, we determined
lesion area as a function of location in the aorta (proximal, mid, or distal segment). Atheroma distribution was expressed as percentage of lesions per segment. Qualitative morphologic assessment of lesion complexity was performed with light microscopy for the following parameters: absence or presence of foam cells, wall thickness, vessel wall architecture integrity, nuclear proliferation, and luminal extension. Lesion morphology, complexity, and severity were used to classify the stage of lesions.

**Electron Microscopy**

Isolated carotid arteries and aortas were perfused with oxygenated, supplemented cell culture medium to rinse away blood and were subsequently fixed with 10% formalin for 10 min. The vessels were then stored in 10% formalin until embedding at the Electron Microscopy Laboratory, Department of Pathology and Laboratory Medicine, School of Veterinary Medicine, University of California, Davis, CA. In brief, the tissues were secondarily fixed with 1% OsO4 in 0.1 M PO4 buffer for 60 min. After rinsing in DHB2O, the vessels were placed in 0.1% tannic acid for 30 min and subsequently treated after rinsing in DHB2O with 1% uranyl acetate in DHB2O for 90 min. The tissues then were dehydrated through graded acetone. Finally, the vessels were embedded in an epoxy resin mixture with a cross-section orientation. Ultra-thin sections were cut with a diamond knife and picked up on copper grids. They were stained with uranyl acetate and lead citrate before viewing on a Phillips CM120 Biotwin (Hillsboro, OR). Micrographs were taken with a Gatan MegaScan, model 794/20, digital camera (Pleasanton, CA). The aortas and carotids from three animals per treatment group were analyzed, and representative pictures were chosen for each group.

**Statistical Analyses**

All statistical analyses utilized SigmaStat 2.0 software by Jandel Scientific. Mean ± SE was determined for each treatment group. Treatment groups were compared using one-way ANOVA. Student-Newman-Keuls post hoc test was used to analyze for significant effects ($P = 0.05$). Data were analyzed by the Kruskal-Wallis ANOVA on ranks. Dunn’s post hoc test was used to analyze for significant effects (95% confidence limits).

**RESULTS**

**Plasma Glucose and Lipoprotein Levels**

STZ injected mice experienced three- to fourfold increase in plasma glucose levels over saline-injected animals that was maintained throughout the treatment period. Glucose values 4 wk after initiation of the study were: CI, 100 ± 5 mg/dl; COVX, 110 ± 5 mg/dl; DI, 415 ± 20 mg/dl; and DOVX, 380 ± 80 mg/dl. These values were not significantly changed at 8 and 12 wk after treatment. Ovariectomy had no effect on plasma glucose levels in diabetic animals. While glucose levels increase was not statistically significant. Both DI and DOVX animals had significantly higher plasma glucose levels than CI or COVX animals at 4, 8, and 12 wk. There were no significant differences in plasma glucose concentration between the DI and DOVX groups or the CI and COVX groups.

Total cholesterol, triglycerides, and HDL were determined for six animals per group. Results of these measurements in summarized in Table 1. The DI group had significantly greater total cholesterol than the CI and COVX groups. The DOVX group also was significantly greater than the CI group. Triglycerides were not significantly different. HDL levels in the DI group were significantly greater than in the CI group.

Estriol values for CI and DI animals were similar, 38.05 ± 0.25 pg/ml and 38.14 ± 0.48 pg/ml (means ± SE), respectively. Ovariectomy significantly reduced estriol levels in COVX animals (8.4 ± 0.44 pg/ml; 78.1% reduction) and in DOVX animals (11 ± 0.4 pg/ml; 71% reduction). $N = 5$ mice in each group. Estradiol levels were not significantly different between COVX and DOVX mice, but were significantly different ($P < 0.05$) from intact control animals.

**Body and Uterine Weights**

Body dry weights were CI, 21.3 ± 0.9 g; COVX, 24.0 ± 0.4 g; DI, 17.5 ± 0.9 g; and DOVX, 19.5 ± 1.0 g. DI body dry weights were significantly less than CI and COVX. DOVX weights were significantly less than COVX. Uterine dry weights were CI, 0.0595 ± 0.009 g; COVX, 0.0108 ± 0.001 g; DI, 0.0085 ± 0.002 g; and DOVX, 0.0041 ± 0.0009 g. COVX animals demonstrated a significant 81% decrease in uterine weight relative to CI animals. DI uterine weights were also significantly less than CI. Uterine weights for diabetic animals (DI and DOVX) were significantly lower than control groups;
however, DOVX animals still experienced a 56% reduction in uterine weight relative to DI, as expected due to the ovariec-
tomy.

**Effect of Hyperglycemia and Ovariectomy on Arterial Endothelial Layer Permeability**

Using quantitative fluorescence microscopy, we measured the rate of accumulation of 65,000 mol wt TRITC-dextran in individually perfused carotid arteries. Dextran accumulation rate was used as an index of arterial permeability. Accumulation rates for the four treatment groups were as follows: CI, 2.67 ± 0.178; COVX, 4.04 ± 0.367; DI, 4.29 ± 0.363; and DOVX, 5.16 ± 0.224 ng·mm²·min⁻¹. Endothelial layer permeability in COVX, DI, and DOVX arteries were significantly increased compared with CI arteries (P < 0.001). The DOVX arteries demonstrated a 32% increase over DI arteries (P < 0.05). Arterial permeability resulting from ovariectomy (COVX) vs. diabetes alone (DI) was not significantly different (P = 0.68). Values are expressed as means ± SE for n = 12–14 vessels per group. Dextran accumulation rates for the four treatment groups are summarized in Fig. 1.

**Effect of Ovariectomy and Diabetes on Arterial LDL Accumulation**

Following vascular permeability measurements, DiI-LDL accumulation rates were determined for the same arteries. Arterial LDL accumulation rates were as follows: CI, 1.281 ± 0.106; COVX, 1.572 ± 0.145; DI, 2.213 ± 0.166; and DOVX, 2.215 ± 0.121 ng cholesterol·mm²·min⁻¹. Values are expressed as means ± SE for n = 12–14 vessels per group. Arterial LDL accumulation results are summarized in Fig. 1. While the pattern of accumulation between experimental groups appeared to be similar to that observed for dextran accumulation, no significant differences in LDL accumulation were observed between the 4 groups.

**Effect of Ovariectomy on Arterial Stiffening in Control and Diabetic Mice**

Aortic stiffness measured by MPS ex vivo, was significantly increased in diabetic, ovariectomized mice (0.59 ± 0.06 N) compared with control mice (0.34 ± 0.03 N) (P < 0.05).

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![Fig. 2. A: orientation of aorta sample in vessel elastigraph. Note that the vessel is stretched radially between two foot processes, with one foot process movable. B: maximum passive stiffness (MPS) of thoracic aorta from 6-mo-old male mice determined by the maximum slope of the load vs. strain relationship. DOVX significantly increased MPS. Results are shown as means ± SE. One-way ANOVA (P < 0.05) followed by Newman-Keuls post hoc test (*P < 0.05, DOVX vs. CI).](http://ajpregu.physiology.org/)

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Ovariectomy (0.42 ± 0.04 N) and diabetes (0.47 ± 0.06 N) individually increased MPS slightly, but not significantly (Fig. 2).

Atheroma Development

We analyzed aortas from each of the four study groups (n = 10 aortas per group) by qualitative morphology and quantification of atheroma. The atherogenic diet in the presence or absence of hyperglycemia resulted in aortic lesions after 12 wk, a time frame consistent with previously published reports for atherosclerosis susceptible mouse strains (31). However, no atheromatous lesions developed in CI or COVX mice. Early atherosclerotic lesions were present in the intimal layer of aortas of only the two diabetic groups after 3 mo of cholesterol feeding: DOVX and DI. In these mice, atheromatous lesions were distinguished by lipid infiltration into the subendothelium of the vessel wall and the presence of fatty streaks, but not raised intimal lesions. In DOVX mice, lesions were more advanced with larger deposits of intracellular fat and foam cells, but were not complicated lesions. Compared with DI mice, atherosclerotic lesions in DOVX mice were more advanced and larger, but did not have cellular architecture disruption, cellular disarray, or proliferation of nuclei in the intima. Representative lesions of CI, DI, and DOVX mouse aortic segments are shown in Fig. 3.

We also characterized atheroma by size and location in the aorta (proximal, mid, and distal segments) by using quantitative morphometry. Mean lesion area and distribution of atheroma in aortic segments of DOVX, DI, COVX, and CI mice (n = 30 segments/aorta and 10 aortas/study group) after 12 wk of cholesterol feeding. The bar denotes statistically significant (P < 0.01) differences between groups.

Electron Microscopy of Arterial Intima

The overall ultrastructural organization of both carotid and aorta samples from all groups was similar. The endothelium overlaid a contiguous elastic lamina. The media consisted of three (carotid) or four (aorta) laminar aggregates of elastin separating elongate overlapping profiles of smooth muscle cells. Smooth muscle cells had focal intercellular contacts but otherwise were separated by irregularly oriented fibrillar-to-amorphous extracellular matrix. The media was discretely delimited from the adventitia by an external layer of elastin.
The adventitia was composed of fibrillar matrix occasionally demonstrating periodic banded collagen fibrils. This matrix invested stellate cells with numerous, thin cytoplasmic extensions that partially underlay the external elastic layer of the media.

Intercellular junctions between endothelial cells were formed in regions of cytoplasmic overlap and were characterized by closely opposed cell membranes containing fine densities (Fig. 5A) and segmental tight junctional complexes (Fig. 5B). These structures were intact in all groups. Control and ovariectomized mice had only slight undulations of the cell at its basal attachment to the underlying basal lamina. The basal lamina consisted of thin sheets of finely granular material arranged in one or two distinct layers that were consistently closely opposed to the endothelial cell membrane.

Ultrastructural changes in STZ-treated mice were limited to the endothelium and underlying basal lamina. Similar changes were seen in samples from both aorta and carotid arteries. In these groups, the basal lamina was often multilayered and formed convoluted aggregates separating endothelium from the internal elastic lamina (Fig. 5C). Endothelium overlying these regions formed thin cytoplasmic extensions between these aggregates and was often separated from the basal lamina by electron lucent spaces (Fig. 5C). In rare instances, endothelial cells were partially dissociated from the basal lamina leaving clear spaces (Fig. 5D). No difference between intact and ovariectomized mice was evident in the nature or extent of these changes.

**DISCUSSION**

Increased arterial permeability is one mechanism that appears to be important in early atherogenesis in this new mouse model. This is consistent with previous studies in animal models and in human patients that have shown increased microvascular permeability with DM1 (3, 11, 25, 45). Previous work from this laboratory and others have shown that estrogen withdrawal increased and estrogen treatment reduced endothelial layer permeability (9, 10, 14, 15, 18, 27, 28, 35, 47). Thus, these studies predicted that diabetes and loss of ovarian estrogens would act to increase endothelial layer permeability and provide a mechanism for the devastating vascular effects of diabetes and loss of ovarian hormones on the arterial wall. Indeed this was supported by the current experiments. However, other studies investigating the effects of diabetes or estrogen on vascular permeability have failed to find this relationship (17, 34). These differing findings may be due to length of treatment, use of different models, or differences in other experimental conditions.

LDL accumulation in the artery wall was not statistically different in any of the groups, although there appeared to be a trend toward increased LDL accumulation in the diabetic

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**Fig. 5.** A: endothelium, basal lamina, and internal elastic membrane from a control mouse carotid artery. Two adjacent cells intercalate at their junctions (arrowheads). The basal surface of the endothelium has modest undulations and is closely opposed to a linear slightly aggregated basal lamina. Bar = 200 nm. B: carotid endothelium from streptozotocin-treated mouse. While intercellular junctions (arrowheads) are similar to controls, the basal cytoplasm is convoluted and separated from the basal lamina by electron lucent spaces. C: carotid endothelium from ovariectomized mouse treated with streptozotocin. Tight junctional complexes are evident (arrowheads) in intercellular junctions. Gaps between basal projections of endothelial cytoplasm are filled with interlacing and irregularly arranged basal lamina-like material. D: aortic endothelium from ovariectomized mouse treated with streptozotocin. The endothelial cell is focally separated from the underlying basal lamina and elastic lamina by empty spaces.

**EC**-endothelial cell  
**BM**-basement membrane  
**EL**-electron lucent spaces  
Arrow-interlacing and irregularly arranged basal lamina-like material
groups. LDL accumulation in the artery wall is a complex process involving endothelial layer permeability, binding to glycosaminoglycans, cells, and other lipoproteins, and efflux of the LDL from the artery wall. The studies that we have conducted suggest that in diabetes the mechanisms for early atherogenesis are more strongly dependent changes in endothelial layer permeability than binding to artery wall components or reduction of LDL efflux.

Independently, both diabetes mellitus and estrogen withdrawal have been shown to increase artery stiffening. No studies of this type have ever been attempted in mouse models of diabetes or estrogen status. Our studies show that in combination these two factors actadditively to increase arterial stiffening. Further studies are needed to determine mechanisms for this pathophysiological effect.

Our data showed what can be most closely related to fatty streaks in this new animal model. These fatty streaks were primarily distributed in the proximal and thoracic aorta of diabetic mice and more commonly covered greater surface area in the DOVX mice. These studies highlight the synergistic effect of diabetes and loss of ovarian hormones on arterial pathobiology. Our studies also suggest that the mechanism for greater atheroma formation at this stage of atheroma development is related to increased arterial permeability rather than LDL binding.

The arterial ultrastructural studies showed significant changes only in diabetic animals. No ultrastructural changes were identified when control and ovarioctomized arteries were imaged. The ultrastructural observations raise questions regarding the mechanisms of endothelial barrier permeability alteration suggested by the lipoprotein accumulation experiments. While occludens and adherens junctions were normally formed and intact in all groups, there were significant alterations in both the structure and amount of basal lamina-like structures in the intima and in the interaction of the endothelium with the underlying matrix. This would suggest potential alterations in the function of the integrin-based adhesion complexes in diabetic animals as well as potential stimulus for synthesis of increased basal lamina by affected endothelial cells. The consequences of these alterations on the luminal side of the internal elastic lamina could include a greater propensity for lipid entrapment under endothelium in this less tightly bound potential space, as well as other uncharacterized alterations in lipid interactions with basal lamina material.

Our studies show that the metabolic milieu associated with hyperglycemia in a model of DM1, in combination with loss of ovarian hormones, provides a potent injury to the artery wall and specifically to the endothelial cell layer. The injury to the artery wall manifests itself most dramatically by increased endothelial layer permeability, arterial stiffening, and atheroma formation. Further studies are needed to determine whether tight control of hyperglycemia or hormone supplementation will reverse these specific examples of vascular injury.

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