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Effect of metabolic syndrome on the response to arterial injury

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ABSTRACT

Background: Metabolic syndrome is now an epidemic in the United States population. Intimal hyperplasia remains the principal lesion in the development of restenosis after vessel wall injury. The aim of this study is to characterize the changes induced in wall morphology in the developing intimal hyperplasia within a murine model in the presence of diabetes (type 1) and metabolic syndrome.

Methods: Control (wild type B6), Non Obese Diabetic, and metabolic syndrome (RCS-10) mice were used. The murine femoral wire injury model was used in which a micro wire is passed through a branch of the femoral and used to denude the common femoral and iliac arteries. Specimens were perfusion fixed and sections were stained with hematoxylin and eosin and Movat stains such that dimensional and compositional morphometry could be performed using an ImagePro system. Additional stains for proliferation and apoptosis were used.

Results: In control mice, the injured femoral arteries develop intimal hyperplasia, which is maximal at 28 d and remains stable to day 56. Sham-operated vessels do not produce such a response. In diabetic mice, the intimal response increased 5-fold with a 2-fold increase in proteoglycan deposition, whereas in the metabolic syndrome mice there was a 6-fold increase in the intimal response and a similar increase in proteoglycan deposition. Collagen deposition was different with a 22-fold increase over control in collagen deposition in diabetes and a 100-fold increase over control in collagen deposition in metabolic syndrome as compared with the control injury mice. Maximal vascular smooth muscle cell (VSMC) proliferation was decreased in both diabetes and metabolic syndrome compared with controls, whereas early cell apoptosis in both diabetes and metabolic syndrome was sustained over a longer period of time compared with wild-type mice.

Conclusions: These data demonstrate that development of intimal hyperplasia is markedly different in diabetes and metabolic syndrome compared with controls, with an increase in

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collagen deposition, a reduction in VSMC proliferation, and an increase in early VSMC apoptosis. These findings suggest that preventative strategies against restenosis must be tailored for the diabetic and metabolic syndrome patients.

1. Introduction

Diabetes and metabolic syndrome are considered the disease of the 21st century. Metabolic syndrome is a prediabetic state, which is a constellation of well-defined metabolic risk factors as follows: insulin resistance, atherogenic dyslipidemia, central abdominal obesity, hypertension, and development of vascular prothrombotic and pro-inflammatory states [1,2]. It is characterized by high plasma levels of free fatty acids in association with high blood glucose levels. These patients present frequently for revascularization due to atherosclerotic occlusive disease [2]. The presence of metabolic syndrome can be correlated with increased carotid intima-media thickness in both men [3] and women [4]. Metabolic syndrome has also been demonstrated to amplify vascular wall thickness and stiffness [5]. Diabetes and insulin resistance have been shown to be independent predictors of early restenosis after coronary stenting [6–9]. After controlling for age, sex, previous myocardial infarction, stent length, current smoking, and statin therapy in a population of patients from the GENetic DEterminants of Restenosis study, metabolic syndrome was also associated with a greater target vessel revascularization and the combined endpoint of death, myocardial infarction, and target vessel revascularization after percutaneous coronary intervention [10]. We recently reported our results with lower extremity intervention in both diabetes and metabolic syndrome and demonstrated poorer anatomic outcomes in the presence of either diabetes or metabolic syndrome [12–15]. Animals models of diabetes and metabolic syndrome show changes consistent with an exaggerated intimal hyperplasia response [11]. Given these clinical findings, this study is designed to test the hypothesis that both diabetes and metabolic syndrome enhanced intimal hyperplasia through different cellular means. To test this hypothesis we used the murine femoral wire injury model and examined the changes in vessel morphology and cell kinetics under normal, diabetic, and metabolic syndrome conditions.

2. Materials and methods

2.1. Experimental design

Control (wild type, B6), Non Obese Diabetic (NOD), and metabolic syndrome (RCS-10) mice were used. The murine femoral wire injury model was used in which a micro wire is passed through a branch of the femoral and used to denude the common femoral artery. Specimens were perfusion fixed and sections were stained with hematoxylin and eosin and Movat stains such that morphometry could be performed using an ImagePro system. Animal care and procedures were conducted at Houston Methodist Research Institute in accordance with state and federal laws and under protocols approved by the Houston Methodist Research Institute Animal Care and Use Committee. Animal care complied with the "Guide for the Care and Use of Laboratory Animals" issued by the Institute of Laboratory Animal Resources.

2.2. Mouse strains

2.2.1. Control wild-type mouse

Mice with a C57BL/6J background (Jackson Laboratory, Harbor, ME) served as the primary mouse in our control group.

2.2.2. NOD mouse

Diabetes in NOD-LtJ mice (Jackson Laboratory, Harbor, ME) is characterized by insulitis, a leukocytic infiltrate of the pancreatic islets. Marked decreases in pancreatic insulin content occur in females at about 12 wk of age and several weeks later in males. Onset of diabetes is marked by moderate glycosuria and by a non-fasting plasma glucose higher than 250 mg/dL. Diabetic mice are hypoinsulinemic and hyperglucagonemic, indicating a selective destruction of pancreatic islet beta cells. NOD-LtJ females are more widely used than males because the onset of Insulin Dependent Diabetes Mellitus symptoms occurs earlier and with a higher incidence (90%–100% by 30 wk of age). NOD-LtJ males develop Insulin Dependent Diabetes Mellitus at a frequency of between 40 and 60% by 30–40 wk of age.

2.2.3. RCS-10 mouse

In the NONcNZO10-LtJ mouse (Jackson Laboratory, Harbor, ME), the onset of hyperglycemia occurs between 12 and 16 wk on a 6% fat diet, with >85% being diabetic by 24 wk. Males exhibit increased serum triglycerides, moderate to severe liver steatosis, and pancreatic islet atrophy similar to NZO-HlLt males. Serum insulin and leptin values are significantly lower than in NZO-HlLt, and are only moderately elevated above those recorded in NON-Lt males.

2.3. Blood glucose and lipid level measurement

All mice were monitored weekly for the development of diabetes by blood glucose measurement with blood glucose monitoring system Fast Draw test strips (J&J LifeScan, Milpitas, CA). Two consecutive non-fasting glucose measurements

Table – Metabolic panel.

Strain	Blood sugar (mg/dL)	Cholesterol (mg/dL)	Triglycerides (mg/dL)
Wild type	145 ± 20	109 ± 18	75 ± 23
RCS10	382 ± 40**	226 ± 50*	448 ± 71**
NOD	343 ± 56**	130 ± 30	316 ± 34**

*P <0.05 **P <0.01 compared with wild type