INTRODUCTION

Heart disease is the leading cause of death for Americans with diabetes [1]. High blood glucose levels can cause thickening of blood vessels and decreases in heart function. Changes in the function and structure of the heart have been reported in animals and humans [2]. Much attention has been given to changes in the arteries and veins caused by diabetic heart disease, but little is known about the effects on the heart’s lymphatic system. The lymphatic system provides waste removal, immune responses, and fluid balance. An imbalance of fluid in the heart can promote fibrosis, tissue death, and decreases in heart function [3]. Therefore, the heart health of 24 million Americans with diabetes partially depends on a robust lymphatic system.

Functional lymphatic vessels counter inflammation in the heart. The chronic inflammation commonly related to diabetes may serve as a trigger for changes in protein levels of the lymphatic biomarker, lymphatic vessel endothelial receptor (LYVE-1). LYVE-1 is responsible for the uptake of hyaluronic acid (HA) from the spaces between cells. Hyaluronic acid provides the stickiness or “goopyness” of the liquid surrounding cells. Excessive hyaluronan can promote fibrosis and permanent losses in heart function [4]. Such remodeling results in decreased filling of the ventricles of the heart and the inability to move blood through the body, as seen in diabetes. The electrocardiogram (ECG) is a non-invasive diagnostic tool commonly used in medical offices to evaluate the function of the heart. The R wave indicates how hard the heart is working. Increases in the R wave amplitude are an early indicator of an increased risk for heart disease and thickening of the heart muscle [5]. Exercise improves heart function in healthy and diseased subjects [6]. However, knowledge is lacking about the impact of aerobic exercise training on lymphatic biomarkers.

The Zucker Diabetic Fatty (ZDF) rat is a model of type 2 diabetes. The ZDF rat develops high glucose and lipid levels by week 8 and diabetes by week 12 when the animals are fed a high fat diet. The diabetes in the ZDF rat mimics the diabetic process seen in humans. This project evaluated changes in protein levels of LYVE-1 under diabetic and aerobic exercise training conditions and the association with ECG changes related to diabetes.

MATERIALS AND METHODS

Animals: Male Zucker Diabetic Fatty (ZDF - type 2 diabetes animal model) and Zucker lean (control) rats were assigned to 4 groups (n=10 or 12 per group): sedentary control, exercised control, sedentary diabetic, and exercised diabetic (Charles River Laboratory, Saint Louis, MO). All animal procedures were performed according to the Institutional Animal Care and Use Committee guidelines at the University of Kansas Medical Center.

Methods: Treadmill exercise training began at diabetes onset, 12 weeks of age. ECG measures were taken at baseline and after 7 weeks of exercise training. (ADInstruments, Colorado Springs, CO). Protein levels of LYVE-1 (Santa Cruz Antibodies, Santa Cruz, CA) were evaluated in left ventricular tissue through immunoblotting. Immunoblotting is a technique which allows the detection of proteins from a sample of tissues or cells.

Statistical Analysis: Descriptive statistics were calculated on animals’ means for each group. One-way repeated measures ANOVA (group) analyzed within and between subject differences and interactions. Single-time point measurements or change scores were completed with one-way ANOVA (group) with Least Significant Difference (LSD) post-hoc analysis. Pearson correlations were utilized for relationship values. Statistics were conducted with PASW Version 17 software (SPSS Inc, Chicago, IL, USA). Significance was measured at p<0.05. Results are presented as means ± standard error (the average of the group, not the individual animal).

RESULTS

VanHoose et al group has previously reported that R wave amplitudes were similar for the groups at baseline except for the sedentary diabetic animals (p<0.001) [7]. Diabetes caused increased pressure and work for the heart as indicated by increases in R wave amplitudes at baseline. Diabetic
animals that completed exercised training had R wave amplitudes similar to control animals. LYVE-1 protein levels were unchanged with diabetes, but increased with exercise (p<0.05). A moderate relationship (r=0.48, p<0.05) existed between R wave amplitude change and LYVE-1 protein levels in diabetes.

**DISCUSSION**

Rudbeck visualized the lymphatic vessels of the heart in 1653 [8], and several researchers continued investigations into the structure and function of the lymphatic vessels with the use of dyes and inks [9]. However, the discovery of lymphatic markers in the last 15 years has allowed for extensive inquiries of structure and function. Lymphatic biomarkers are defined as molecules expressed on lymphatic vessels. They are involved in the growth and function of the lymphatic vessel. LYVE-1 is a lymphatic biomarker, and its regulation is crucial for fluid balance in the heart [10].

Blockage or decreased flow of the lymphatic vessel in the heart can result in edema and fibrosis in the heart [11]. Although the mechanisms are not completely known, the relationship between the lymphatics and the heart function is becoming increasingly clear. Edema decreases left ventricular function, which results in stiffness and delayed relaxation. Pressure-volume relationships under acute and chronic edema conditions show increased diastolic interstitial fluid pressure [11]. These alterations result in remodeling of the left ventricle and changes in heart rhythm, which can be identified with ECG [12]. The functional losses noted with myocardial edema are similar to those seen with diabetic heart disease [13]. Such similarities suggest a possible relationship between the symptoms and the disease. We hypothesized that diabetes causes modifications related to edema in the heart and that aerobic exercise training could combat such changes in the model of type 2 diabetes, ZDF rat.

LYVE-1 removes HA from the liquid surrounding the cells, and HA is elevated with diabetes [14]. HA fragments increases the inflammation in the heart and can result in declines in relaxation of lymphatic vessels [15] [16]. Relaxation of the lymphatic vessels is needed for strong contractions, which maintain fluid balance. Inefficient ventricular contractions, observed in the diabetic heart coupled with weak lymphatic vessels, can slow flow and cause edema and fibrosis development in the heart. This study showed that diabetes impacts heart function in the ZDF rat. At the same time, LYVE-1 protein levels did not increase to address fluid imbalances due to diabetes. However, LYVE-1 protein levels did increase with aerobic exercise training. These changes in LYVE-1 may indicate an increase in immature lymphatic vessels or a protective response to elevated HA levels with exercise under the diabetic condition. This study did not investigate the immune response, which may also play a role in the increase in LYVE-1 protein levels. Future studies on LYVE-1 expression and function under diseased and treatment conditions will shed light on the role of the lymphatic system in diabetic heart disease.

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