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Enhanced adiponectin actions by overexpression of adiponectin receptor 1 in macrophages

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ABSTRACT

Objective: Adiponectin is one of several important, metabolically active cytokines secreted from adipose tissue. Epidemiologic studies have associated low circulating levels of this adipokine with multiple metabolic disorders including obesity, insulin resistance, type II diabetes, and cardiovascular disease. To investigate how enhanced adiponectin-mediated changes in metabolism *in vivo*, we generated transgenic mice which specifically overexpress the gene coding for adiponectin receptor 1 (AdipoR1) in mouse macrophages using the human scavenger receptor A-I gene (SR-AI) enhancer/promoter. We found that macrophage-specific AdipoR1 transgenic mice (AdR1-TG) presented reduced whole body weight, fat accumulation and liver steatosis when these transgenic mice were fed with a high fat diet. Moreover, these macrophage AdR1-TG mice exhibited enhanced whole-body glucose tolerance and insulin sensitivity with reduced proinflammatory cytokines, MCP-1 and TNF-α, both in the serum and in the insulin target metabolic tissues. Additional studies demonstrated that these macrophage AdR1-TG animals exhibited reduced macrophage foam cell formation in the arterial wall when these transgenic mice were crossed with a low-density lipoprotein receptor (Ldlr) deficient mouse model.
Conclusions: These results suggest that AdipoR1 overexpressed in macrophages can physiologically modulate metabolic activities *in vivo* by enhancing adiponectin actions in distal metabolically active tissues. The AdipoR1 modified macrophages provide unique interactions with the resident tissues/cells, suggesting a novel role of macrophage adiponectin receptor in improving metabolic disorders *in vivo*.

1. Introduction

Metabolic Syndrome is present in an estimated ~35% of adults in the United States [1], and is a powerful risk factor not only for the future development of type II diabetes but also cardiovascular disease [2–4]. Metabolic Syndrome, prediabetes, type II diabetes, and cardiovascular disease are linked by a common pathophysiological process that involves insulin resistance, dyslipidemia, and inflammation, and the progression of these diseases constitute the spectrum of cardiometabolic disease. Even so, the factor(s) that is largely responsible for the disparate trait cluster that comprises the Metabolic Syndrome, and the mechanistic link between insulin resistance and vascular disease, have not been fully elucidated. A better understanding of cardiometabolic disease pathophysiology is critical for developing improved modalities for treating and preventing type II diabetes and vascular disease.

Adiponectin (also known as apM1, AdipoQ, Gbp28 and Acrp30) is one of several important, metabolically active cytokines secreted from adipose tissue, which circulates in high and low molecular weight multimeric forms [5–7]. Epidemiological evidence has indicated that plasma adiponectin levels are reduced in patients with insulin resistance, diabetes, obesity, or cardiovascular disease [8–10], and these relationships are more strongly related to a decrement in the high molecular weight form than the low molecular weight form [6,7]. In the circulation, adiponectin exerts bioeffects on multiple cell types and has insulin-sensitizing, anti-

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inflammatory, and anti-atherosclerotic properties. For example, adiponectin has been shown to augment insulin sensitivity and lipid oxidation in skeletal muscle and adipocytes [11,12] and to reduce hepatic glucose production in liver [13,14]. Accordingly, administration of adiponectin to intact rodents improved glucose tolerance and decreased plasma triglycerides [11,12]. In addition, adiponectin can inhibit both the inflammatory process and atherogenesis by suppressing the migration of monocytes/macrophages and their transformation into foam cells in the vascular wall [15,16]. Transgenic and knockout mouse models have confirmed the importance of adiponectin in metabolic diseases. Overexpression of the adiponectin gene protected ob/ob mice from diabetes and prevented apolipoprotein E-deficient mice from developing atherosclerosis [17]. Furthermore, overexpression of adiponectin in fat tissues resulted in increased circulating adiponectin levels, which in turn led to improved insulin sensitivity [18,19]. There is also evidence that adiponectin gene polymorphisms may be associated with hypoadiponectinemia, together with insulin resistance and type II diabetes [20].

Two cell-surface trans-membrane receptors have been identified for adiponectin, AdipoR1 and AdipoR2 [21], and adiponectin action is known to signal through these receptors and the docking protein APPL1 [22]. In muscle and liver cells, signal transduction involves the phosphorylation and activation of AMP-activated kinase (AMPK) [21], which plays a pivotal role in nutrient sensing and substrate metabolism. Adiponectin interacts with T-cadherin in pro-B-cells, however, the biological significance of this non-transmembrane receptor is not clear in other cells since T-cadherin lacks the transmembrane and cytoplasmic domains [23,24]. AdipoR1 is abundantly expressed in skeletal muscle and macrophages, whereas AdipoR2 is predominantly expressed in the liver [21]. Simultaneous disruption of both AdipoR1 and AdipoR2 abolished adiponectin binding and actions, resulting in increased liver triglyceride content, inflammation and oxidative stress in adipose tissue, and thus leading to insulin resistance and marked glucose intolerance [25]. Recently, these two receptors have also been found to be expressed in human macrophages [26,27] with AdipoR1 predominating in these cells [27,28]. Even so, the functions of AdipoR1/2 in macrophages remain poorly defined.

Macrophages are a heterogenous and plastic population of phagocytic cells, which arise from circulating myeloid-derived blood monocytes, enter target tissues, and gain phenotypic and functional attributes partly determined by their tissue of residence [29]. Macrophages play a critical role in both metabolic and vascular components of cardiometabolic disease. In the vascular wall, macrophages accumulate lipid in the presence of excess oxidized and non-oxidized LDL, then transition to foam cells, and initiate the fatty streak which is the hallmark lesion of atherosclerosis [30]. Recent attention has also focused on the important role of macrophages in insulin resistance, the Metabolic Syndrome, and type II diabetes [31,32]. In obesity and insulin resistance, adipose tissue contains an increased number of resident macrophages [33,34], which secrete cytokines and other factors that cross-talk with adipocytes. This alters the systemic release of adipocytokines from adipose tissue, causing dysmetabolism in multiple organs. Thus, the proinflammatory state, established in adipose tissue as a consequence of the increase in resident activated macrophages (i.e., M1 macrophages), is believed to be instrumental in the development of systemic insulin resistance and other traits that characterize the Metabolic Syndrome [35]. There is also accumulating evidence that macrophages reside in skeletal muscle, and could contribute to insulin resistance by directly impairing insulin action in this key target tissue [36]. In support of the central role of the macrophages [37], mice fed a high-fat diet were protected against glucose intolerance and insulin resistance when inflammatory pathways in macrophages were genetically disrupted [38].

Recently, we engineered transgenic mice to synthesize and secrete adiponectin from macrophages in an attempt to augment adiponectin action in the microenvironment of the macrophage [39,40]. This resulted in a lean, insulin sensitive, diabetes-resistant, and atherosclerosis-resistant phenotype, and gave us the idea that the adiponectin-macrophage axis could regulate multiple key components of the Metabolic Syndrome. However, this model failed to rigorously test this hypothesis since the transgenic mice also exhibited increased circulating adiponectin concentrations, and, therefore, higher adiponectin could be influencing multiple tissues directly, not necessarily as a result of a specific interaction with macrophages. The current studies have developed a novel approach to manipulate adiponectin action at the level of the macrophage in order to examine systemic effects related to metabolic diseases by genetic manipulation of the major receptor for adiponectin in macrophages, AdipoR1. Our data suggest that alterations in adiponectin effects, solely directed at the macrophage, can explain the co-occurrence of multiple components in the Metabolic Syndrome trait cluster and provide a novel unifying explanation for the pathophysiology of metabolic diseases. Our data, for the first time, suggest that overexpression of AdipoR1 can alter macrophage biology and impact systemic metabolism *in vivo*. These data point to the central role of macrophages, and the singular ability of adiponectin to regulate macrophage function, in metabolic diseases. Therefore, our studies provide new insights for investigating the mechanisms of metabolism and inflammatory response *in vivo*.

2. Methods

2.1. Experimental animals

To generate the macrophage AdipoR1 transgenic mice, full length cDNA encoding the AdipoR1 gene with a V5 epitope tag fused at the 3′ end was sub-cloned into a plasmid vector containing the human scavenger receptor A-I gene (SR-AI) enhancer/promoter (5.0 kb) (kindly provided by Dr. Chris Glass, University of California at San Diego). DNA fragments (8.5 kb) starting from the enhancer/promoter to human growth hormone tail were purified before they were microinjected into mouse embryos.

Transgenic animals were created by injections of the appropriate DNA fragments into the pronucleus of inbred C57BL/6 single cell embryos at our Transgenic Animal/Embryonic Stem Cell (TA/ESC) Core at the University of Alabama at Birmingham. All of the confirmed transgenic mice were backcrossed more than 10 generations into the C57BL/6J genetic background before being used for our experiments.

Wild-type and transgenic mice at 20 weeks of age but fed with the high fat diet for 16 weeks were then euthanized and bled by cardiac puncture. Plasma was isolated by centrifugation at 12,000 *g* for 10 min and stored at −80 °C until analyzes were performed.

To investigate the atherosclerotic lesion formation, the AdipoR1 transgenic mice (AdR1-TG) were crossed with the low-density lipoprotein receptor (Ldlr) deficient mice (Jackson Laboratory, Stock Number 002207; Ldlr^{tm1Her}/J), a common model for cardiovascular research. The AdR1-TG/Ldlr and control (WT/Ldlr) mice were fed with a high fat diet (60% kcal% fat) from the Research Diets Company (New Brunswick, NJ) for six months before measuring and analyzing the atherosclerotic lesion regions.

All of these animals were housed in a specific pathogen-free facility with 12-h light/dark cycles and received a standard laboratory chow diet except for the high fat diet experiments. Only male mice were used for the experiments. All animal procedures were