Adipokines, Myokines and Cardiovascular Disease

Kenneth Walsh, PhD

It is recognized that obesity contributes to cardiovascular and metabolic disorders through alterations in the levels of adipocyte-derived cytokines (adipokines). Adiponectin is an adipokine that is downregulated in obese individuals. It has beneficial actions on the cardiovascular system by directly acting on the heart and blood vessels, and acute administration of adiponectin can minimize the tissue damage resulting from myocardial infarction. More recent research has been aimed at identifying novel adipokinin-like factors involved in metabolic and cardiovascular regulation. Activation of Akt, a protein kinase involved in cell signaling, has been implicated in the control of skeletal muscle hypertrophy. An experimental mouse model demonstrates that substantial increases in muscle fiber hypertrophy, weight and strength occur upon induction of Akt signaling in skeletal muscle. In a mouse model of obesity, the increase in muscle mass caused by myogenic Akt induction results in diminished fat deposition and improvements in whole body metabolism. Based on these findings a protocol to identify novel muscle-secreted proteins (myokines) that confer the phenotypic changes brought on by myogenic Akt induction has been devised. One of these newly discovered factors, referred to as follistatin-like 1, is able to promote revascularization in ischemic limbs and protect the heart from ischemic stress. (Circ J 2009; 73: 13–18)

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The “Adipokine” Adiponectin and Metabolic Regulation

It is recognized that adipose tissue functions as both energy storage and secretory tissue producing a variety of bioactive substances including leptin, tumor necrosis factor (TNF)-α, plasminogen activator inhibitor type 1, and adiponectin1–4 These bioactive molecules are generally referred to as adipokines, and several are involved in the pathophysiology of various obesity-linked disorders. Adiponectin is an adipokine whose mRNA is largely expressed in adipose tissue.5–7 It is abundantly present in human plasma at a range between 3 and 30 μg/ml.8 Adiponectin shares structural homology with the collectin family of proteins and it multimerizes to form stable higher-order complexes.

Clinical studies implicate lower plasma levels of adiponectin in the pathogenesis of obesity-related diseases.8–11 Conversely, plasma adiponectin concentrations increase following weight loss.12,13 In patients with diabetes mellitus, plasma adiponectin concentrations are lower than in age- and BMI-matched nondiabetic patients.12 A number of studies have established inverse correlations between the circulating levels of adiponectin and those of the inflammatory markers C-reactive protein and IL-6.14–17 Several studies using experimental models have examined the metabolic actions of adiponectin. A strain of the adiponectin-knockout mouse exhibits more severe insulin resistance than wild-type mice when fed a high-fat/sucrose diet.18,19 The adiponectin-knockout mouse displays delayed clearance of free fatty acid in plasma, low levels of fatty-acid transport protein-1 expression in muscle, higher levels of TNF-α mRNA in adipose tissue and higher plasma TNF-α concentrations than wild-type mice. Restoration of adiponectin expression in knockout mice by adenovirus-mediated gene transfer results in a reversal of the metabolic phenotype. Other studies have shown that adiponectin regulates glucose metabolism and insulin sensitivity, at least in part, through the phosphorylation and activation of the 5’-AMP-activated protein kinase (AMPK) in muscle and liver.20,21 Of note, another strain of adiponectin-deficient mouse does not display detectable changes in insulin resistance or glucose intolerance.22

Adiponectin and Vascular Function

Adiponectin appears to protect against the development of various vascular diseases. A number of experimental studies have shown that adiponectin has an anti-atherogenic function. Administration of an adenovirus expressing adiponectin (Ad-APN) reduces atherosclerotic lesion size in apolipoprotein E-deficient mice, and this coincides with reduced VCAM-1, SR-A and TNF-α expression in the aortic sinus.23 Conversely, adiponectin-deficiency in apolipoprotein E-deficient mice leads to an increase in vascular lesion area.24 Adiponectin knockout mice also develop increased neointimal thickness and display increased vascular smooth muscle cell proliferation following acute arterial injury, whereas overexpression of adiponectin inhibits neointimal lesion formation in wild-type mice.25 Importantly, adiponectin inhibits intimal hyperplasia in this model when mice are fed a normal diet. Thus, the protective actions of adiponectin on the vessel wall can be differentiated from its normalizing effects on glucose and lipid metabolism. Adiponectin-knockout mice exhibit a greater degree of hypertension than wild-type mice when they are placed on a high-salt diet.26 The same study also found that the levels

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Molecular Cardiology Unit, Whitaker Cardiovascular Institute, Boston University Medical Campus, Boston, MA, USA
Mailing address: Kenneth Walsh, PhD, Molecular Cardiology/Whitaker Cardiovascular Institute, Boston University Medical School, 700 Albany Street, Room 611, Boston, MA 02118, USA. E-mail: kwalsh@bu.edu
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of endothelial nitric oxide synthase (eNOS) and prostaglan-
din (PG) I2 synthesis (PGIS) were downregulated in the aorta of adiponectin-knockout mice fed a high-salt diet. 
Restoration of adiponectin expression in the knockout mice by adeno virus mediated gene transfer increased the expres-
sion of eNOS and PGIS and reversed the hypertensive phe-
notype. Furthermore, adiponectin stimulates the intracellular 
signaling kinases Akt and AMPK, which phosphorylate and activate eNOS. Treatment with the eNOS inhibitor, 
Nω-nitro-L-arginine methyl ester (L-NAME), blocks the 
amelioration of hypertension that results from Ad-APN 
administration in the knockout mouse model. Thus it 
seems that the anti-hypertensive effects of adiponectin are 
mediated by its ability to upregulate the production of NO, 
and possibly PGIS, by endothelial cells. 
Consistent with adiponectin’s ability to promote NO-
dependent endothelial cell function, adiponectin-knockout 
mice display an impaired angiogenic response to chronic hindlimb ischemia. Furthermore, overexpression of adi-
ponectin promotes revascularization in this model. The revas-
cularization–stimulatory action of adiponectin overexpres-
sion can be blocked by administration of adenosine 
expressing dominant negative AMPK, indicating that 
AMPK signaling has an important role in adiponectin-
mediated stimulation of blood vessel growth. In addition, 
adiponectin promotes endothelial cell survival through its 
ability to stimulate the AMPK signaling pathway. 
Although adiponectin is probably not an angiogenic factor 
per se, as it is not sufficient to stimulate blood vessel growth 
in normal tissue, its ability to promote endothelial cell sur-
vival and function are presumed to favor the revasculariza-
tion response under conditions of ischemic stress.

Cardiac-Protective Properties of Adiponectin

A number of experimental studies have found that adi-
ponectin exerts beneficial actions on the heart under patho-
logical conditions. Adiponectin-deficient mice develop 
severe cardiac hypertrophy and there is increased mortality 
in response to pressure overload because of transverse aortic con-
striction. Conversely, overexpression of adi-
ponectin will attenuate cardiac hypertrophy in response to 
pressure overload in wild-type and diabetic db/db mice. 
Adiponectin-deficient mice also exhibit increased cardiac 
hypertrophy in response to angiotensin II infusion, whereas 
adiponectin overexpression reduces the hypertrophy in this 
model. Adiponectin’s anti-hypertrophic actions can be 
attributed, at least in part, to the modulation of intracellular 
AMPK signaling cascade. Although AMPK is activated as 
the heart undergoes pressure overload hypertrophy, this 
activation is attenuated in adiponectin-deficient mice. Cell 
culture experiments in cardiac myocytes show that adipon-
ectin activates AMPK, and inhibits the hypertrophic 
response to α-adrenergic receptor stimulation. The inhibit-
ion of hypertrophic growth by adiponectin can be reversed 
by transduction with dominant-negative AMPK. These 
anti-hypertrophic actions of adiponectin on AMPK are 
thought to occur via the Adipo R1 and R2 receptors.

In response to myocardial ischemia–reperfusion injury, 
adiponectin-deficient mice display increased myocardial 
infarct size, myocyte apoptosis and myocardial TNF-α 
expression, compared with wild-type mice. Conversely, 
overexpression of adiponectin reduces infarct size, apop-
totic cell frequency and TNF-α levels in both wild-type and 
adiponectin-deficient mice subjected to ischemia–reperfu-
sion injury. In cultured cardiac myocytes, adiponectin inhib-
ited apoptosis, mediated by its ability to activate AMPK 
signaling, and also suppressed TNF-α production, through 
its ability to stimulate COX-2-dependent synthesis of PGE2. 
More recently, it has been shown that adiponectin protects 
against the development of systolic dysfunction following 
myocardial infarction in a mouse model of heart failure.

The findings from the experimental studies are consistent with 
some, but not all, epidemiological studies that have 
evaluated the association of serum adiponectin levels with 
cardiac disease. For example, it has been shown that high 
plasma adiponectin levels are associated with a lower risk 
of myocardial infarction in men, and that adiponectin levels 
rapidly decline following acute myocardial infarction. 
Recently, it was shown that myocardial salvage following 
successful reperfusion in acute myocardial infarction patients 
was positively associated with plasma adiponectin levels. 
Low levels of adiponectin are also associated with a further 
progression of left ventricular hypertrophy in patients pre-
senting with hypertension, left ventricular diastolic dysfunc-
tion and hypertrophy. Finally, high adiponectin levels may 
be predictive for mortality in patients with chronic heart fail-
ure. 
However, this interpretation of the epidemiological 
data is complex and may be related, in part, to the observa-
tion that wasting, which can elevate adiponectin levels, is 
strongly associated with the increased risk of death in the 
final stages of chronic heart failure.

The Myokine Concept

A few years ago, my laboratory embarked on a project to
identify novel secreted proteins that are functionally similar to adiponectin. Although it has long been recognized that adipose can function as an endocrine tissue, most of the factors produced are pro-inflammatory and harmful in the setting of obesity-induced metabolic and cardiovascular disease. In this regard, adiponectin is relatively unique as an adipokine because it is expressed at highest levels in lean, healthy individuals. Thus, we posed the question, “If adipose tissue in the obese state predominantly produces secreted factors that are harmful, what tissue would predominantly produce factors that are protective and provide a counterbalance to the pro-inflammatory factors that are produced by adipocytes?” We reasoned that skeletal muscle might serve such a role because individuals with well-developed muscles are generally at a low risk of developing metabolic and cardiovascular diseases. In other words, the well-established protective effects of exercise could be partly mediated by increased secretion of protective proteins from skeletal muscle cells. We speculate that these protective factors, referred to as “myokines” (derived from the Greek words “muscle” and “motion”), would oppose the harmful effects of the pro-inflammatory adipokines (Fig 1). Presumably these factors would correct metabolic abnormalities and protect against cardiovascular disease. As such, myokines could represent new targets for therapies that mimic the benefits of exercise training on obesity and diabetes. To test this hypothesis, we established a research program that involved the construction of a new mouse model of inducible skeletal muscle growth. We then tested whether muscle growth could reverse the adverse effects of obesity. We are now using this model to identify myokines with potential therapeutic and diagnostic utilities.

The “MyoMouse” Model and Myokine Isolation

Exercise training promotes improvements in whole-body metabolism and cardiovascular system function. Endurance exercise training elicits a variety of metabolic and morphological responses, including mitochondrial biogenesis and an increase in the proportion of type I muscle fibers. These fibers, also referred to as slow/oxidative fibers, are widely appreciated as beneficial in the context of metabolism through their inherent ability to efficiently burn fatty acids. In contrast to endurance exercise, resistance exercise training is associated with an increase in protein synthesis and hypertrophy of type II fibers that are characterized as fast/glycolytic. These type II fibers are preferentially lost upon aging leading to frailty and wasting. Increased type II muscle mass may promote favorable effects on glucose metabolism. However, little is known about the mechanisms by which resistance exercise training affects whole-body metabolism.

Akt1 is a serine-threonine protein kinase that is activated by various extracellular stimuli through the phosphatidylinositol 3-kinase pathway. Numerous studies have implicated Akt1 signaling in the control of organ size and cellular hypertrophy. Overexpression of constitutively active Akt1 induces muscle hypertrophy both in vitro and in vivo. Akt signaling in skeletal muscle is preferentially activated in response to resistance training suggesting that Akt1 signaling may function as a mediator of type II muscle hypertrophy.

To investigate the role of fast/glycolytic muscle fibers in the control of obesity and obesity-related metabolism, we generated a conditional transgenic mouse, known as the “MyoMouse”, that can reversibly grow functional type IIb muscle by switching Akt1 signaling on and off in a skeletal muscle-specific manner using the tetracycline regulatory system. Akt1 activation resulted in modest muscle growth exclusively because of type IIb muscle fiber hypertrophy. This muscle growth was accompanied by an increase in strength, but the mice did not show improvements in running performance.

To elucidate the metabolic-regulatory properties of fast/glycolytic muscle fibers, the mice were maintained on a high-fat/high-sucrose diet prior to gene activation. Akt1 transgene activation in obese mice led to muscle growth that was accompanied by marked reductions in accumulated body weight and white adipose tissue. Type IIb muscle growth in obese mice also led to improvements in metabolic parameters such as better glucose clearance and reduced levels of circulating leptin. These metabolic improvements were independent of physical activity or changes in the level of food intake. Other measurements showed that the muscular mice had an elevated metabolic rate and that they displayed an increased rate of fatty acid oxidation. Because the muscle growth in this model is fast/glycolytic, no increase in fat oxidation was observed in muscle. Transcript profiling of skeletal muscle showed that transgene activation led to the induction of genes involved in glycolysis, but decreased expression of genes associated with mitochondrial biogenesis and fatty acid oxidation. In contrast, an increase in fatty acid β-oxidation could be observed in livers excised from these mice. Our study also found that diet-induced hepatic steatosis was dramatically resolved following transgene-induced growth of type II muscle, indicating that the role of the liver had converted from a lipid storage to a lipid oxidation phenotype. Furthermore, transcript profile analysis of the liver showed that an increase in fast/glycolytic fiber growth had numerous effects on the expression of hepatic metabolism genes.

Collectively, these metabolic improvements occurred through the ability of added type IIb fibers to orchestrate changes in the phenotypic and transcriptional profiles of the liver and fat tissues of the animal. These data suggest that type IIb fibers have a previously underappreciated role in systemic metabolism and that the MyoMouse model is useful for investigating the role of type IIb fibers in obesity-linked metabolic disorders. The data also suggest that strength training, in addition to the widely-prescribed therapy of endurance training, may be of particular benefit to overweight individuals. The metabolic improvement in the MyoMouse model may result from the systemic metabolic consequences of increased glucose uptake by muscle. In addition, it is tempting to speculate that type II skeletal muscle allows the organism to cope with excess adipose tissue through the production of hormonal factors released by muscle (ie, myokines) that act on adipose, hepatic or central nervous system tissues.

To identify novel secreted proteins that are functionally significant in the context of metabolic and cardiovascular diseases, we developed a screening assay in which candidates are selected from the MyoMouse transcriptome. In this screen, a microarray analysis is performed using skeletal muscle of the MyoMouse model to identify differentially regulated transcripts. Among the transcripts regulated by Akt1 induction, functionally unknown genes with full-length open reading frame cDNAs are then selected for further analysis. Predicted amino acid sequences are exami...
**Fstl1 and Vascular Function**

Fstl1, also referred to as TSC36, is an extracellular glycoprotein that has been grouped into the follistatin family of proteins. However, Fstl1 exhibits little amino acid sequence homology with follistatin (7%), and Fstl1 is poorly understood with regard to its functional significance. Transduction of cancer cell lines with Fstl1 has resulted in suppression of growth and invasion. Furthermore, an increase in circulating Fstl1 protein could be detected in the sera of mice, and circulating levels of Fstl1 increased in the MyoMouse model following Akt1 transgene-induced skeletal muscle hypertrophy. Fstl1 expression was also upregulated at the levels of protein and mRNA in ischemic adductor muscle following femoral artery excision. Hindlimb surgery also led to increased Fstl1 levels in serum.

Because Fstl1 was upregulated by muscle ischemia, we tested whether Fstl1 might be involved in the revascularization process. Fstl1 overexpression was found to enhance endothelial cell differentiation and migration, and diminish endothelial cell apoptosis. Administration of Fstl1 improved revascularization in ischemic limbs of wild-type mice. Mechanistically, Fstl1 exerted these actions on the vascular endothelium through its ability to activate Akt-eNOS signaling in these cells. Consistent with this hypothesis, Fstl1 overexpression was incapable of stimulating the revascularization of ischemic tissue in mice that were deficient in eNOS expression.

Thus, several lines of evidence suggest that Fstl1 can be designated as a myokine that acts on vascular endothelial cells. Both Akt transgene-induced myofiber hypertrophy and ischemic hindlimb surgery lead to an increase in tissue-resident and serum levels of Fstl1. Furthermore, Fstl1 is secreted into the media by cultured skeletal muscle cells, and it can directly act on endothelial cell signaling pathways that promote function and survival. Finally, it should be noted that although Fstl1 overexpression accelerates revascularization in ischemic muscle, it did not stimulate vessel growth in normoxic muscle. Thus, it appears that Fstl1 promotes the revascularization process in response to chronic tissue ischemia because it has salutary effects on the endothelium under conditions of stress. Based on these considerations, we propose that the secretion of Fstl1 by skeletal muscle under conditions of hypertrophic growth or ischemic stress will contribute to revascularization through its ability to promote endothelial cell function.

**Fstl1 and Cardiac Protection**

In addition to our observations of Fstl1 expression by skeletal muscle, we also discovered that Fstl1 is secreted by cardiac muscle under conditions of Akt-mediated hypertrophy and injury. Cultured cardiac myocyte cultures display Fstl1 protein in the cell media and this signal is increased when cultures are transduced with the Fstl1 gene. Furthermore, an increase in circulating Fstl1 protein could be detected in mouse serum following myocardial infarction. It is widely recognized that the heart secretes factors that maintain its performance and produce systemic actions. Examples include atrial natriuretic peptide and brain natriuretic peptide that serve as therapeutic or diagnostic agents. Therefore, Fstl1 could be considered a clinical candidate for cardiac disease therapy.

Fstl1 overexpression protected the heart from ischemia–reperfusion injury in mice. Consistent with that observation, Fstl1 activated Akt signaling in cultured cardiac myocytes and inhibited apoptosis. Thus, Fstl1 can function as a survival factor for both cardiac myocytes and endothelial cells via the activation of Akt signaling. Because increasing vascular supply represents a strategy for treatment of heart diseases, Fstl1 may be candidate therapeutic agent for ischemic cardiac diseases based on its ability to directly stimulate blood vessel formation and inhibit the death of cardiovascular cells.
Conclusions

It has been estimated that as many as 10% of encoded genes express proteins that are secreted from cells. Many of these secreted factors are involved in the cell to cell communication that is required for homeostasis in a complex organism. Adipocytes from lean, healthy individuals secrete cytokines that coordinate systemic metabolic processes and protect cardiovascular tissues from stress. Obesity causes an imbalance in this cytokine secretion, contributing to metabolic dysfunction and cardiovascular disease. Recently, the role of skeletal muscle in reversing the pathologcal consequences of obesity has been analyzed. Specifically, our research has focused on the type IIB muscle fibers that are classified as "glycolytic". These are the fibers that undergo hypertrophic growth in response to resistance exercise training and are selectively lost during the aging process. Recent studies have highlighted that type IIB muscle fibers have a previously unappreciated role in controlling systemic metabolism and the effects are mediated by the ability of type IIB fibers to modulate the behavior of remote, metabolically active tissues. Using this model, novel secreted proteins, or myokines, that potentially coordinate these processes have been sought, and their identification and characterization may lead to therapeutic or diagnostic uses.

References


