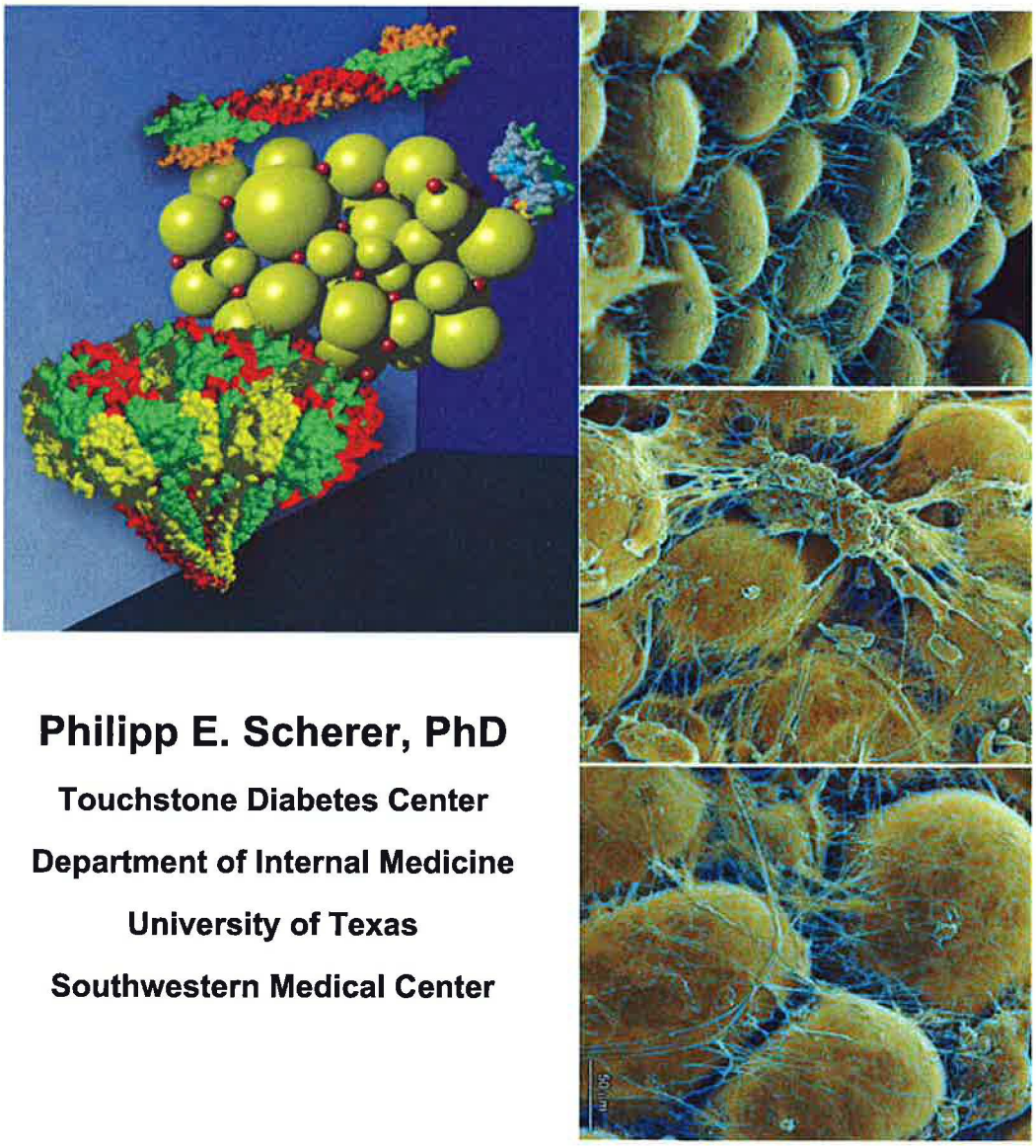
Internal Medicine Grand Rounds

August 29, 2008

Adipocyte-derived Factors
Physiological Role and Diagnostic Use



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This is to acknowledge that Philipp E. Scherer, PhD has not disclosed any finacial interests or other relationships with commercial concerns related directly or indirectly to this program. Dr. Scherer will not be discussing off-label uses in this presentation.

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Research Interests:

Adipose Tissue Physiology/Adipokines

β Cell Dysfunction in Diabetes

Obesity/Cancer Connection (Breast Cancer)

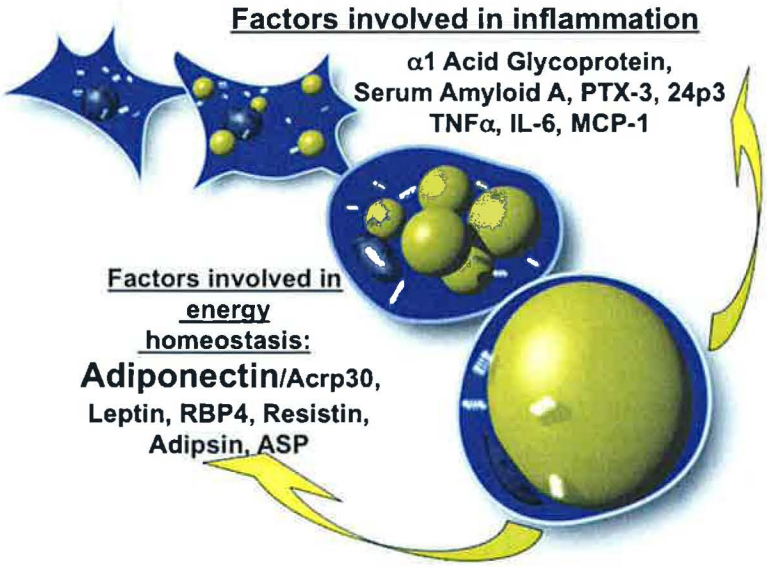
Mitochondrial Dysfunction in Diabetes

Introduction

The endocrine functions of the adipose organ are widely studied at this stage. The adipose organ, and in particular adipocytes, communicate with almost all other organs. While some adipose tissue pads assume the functions as distinct "miniorgans," adipocytes can also be present in smaller numbers interspersed with other cell types (Kirkland, 1996). While fat pads have the potential to have a significant systemic impact, adipocytes may also affect neighboring tissues through paracrine interactions. These local or systemic effects are mediated through lipid and protein factors. The protein factors are commonly referred as adipokines. Their expression and post-translational modifications can undergo dramatic changes under different metabolic conditions.

Adipose Tissue as an Endocrine Organ

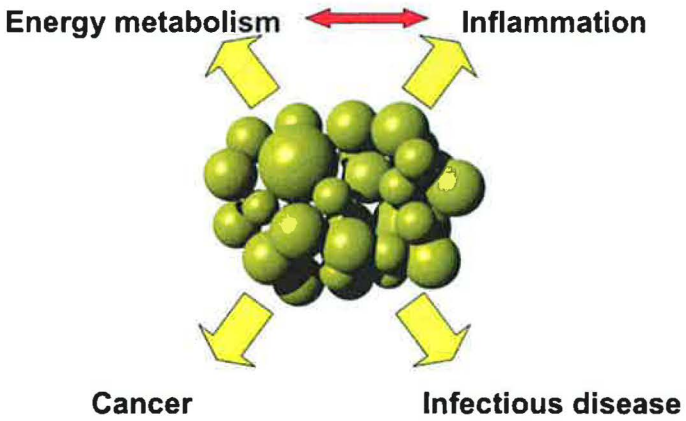
The adipocyte is unique among cells in that one "organelle," the lipid droplet, encompasses greater than 95% of the entire cell body. This lipid droplet serves as a storage vessel for triglycerides that can be released through lipolysis and added to by the process of triglyceride synthesis. Because the lipid droplet can assume such a large portion of the entire adipocyte, increases in lipid storage results in increased fat cell size. In fact, the capacity for triglyceride storage by the adipocyte is quite impressive as fat cell sizes range from 25-200μm in diameter. Hence, the adipocyte is traditionally viewed as a cell that is **primarily involved in energy storage**. However, it is now clear that the adipocyte has additional roles with the remaining 5% of its cellular mass. It has an exceptionally active secretory pathway whose function is not only to release products of conventional housekeeping genes, but also to **release many endocrine and paracrine factors commonly referred to as adipokines.** The endocrine functions allow the adipocyte to regulate processes in peripheral tissues such as the liver and the central nervous system such as the hypothalamus. The paracrine effects of adipokines have an impact on neighboring adipocytes as well as other local cell types within adipose tissue.



Complexity of the adipose organ

Cellular composition

Adipose tissue is comprised of adipocytes as well as many additional cell types. Even though these additional cells account for a significant proportion of the total cell number in adipose tissue, they are not highly visible in histological sections. Conventionally, these "stromal vascular cells" are being isolated using protocols that have been established many years ago in pioneering studies by Rodbell and colleagues. It involves collagenase digestion followed by flotation of the adipocyte fraction using a low g force (Rodbell, 1964). The resultant pellet of this cell suspension contains non-adipocytes. This fraction includes cell types such as preadipocytes, endothelial cells, pericytes, monocytes, macrophages, and others. These cells exert a number of important functions for adipose tissue homeostasis. For example, pericytes and endothelial cells make up the tissues vasculature and enable processes such as adipose tissue growth and development. Secretion of pro-angiogenic factors by adipocytes such as VEGF (Claffey, 1992), contributes to ongoing angiogenesis within adipose tissue at all times. In fact, blocking VEGF or angiogenesis has a significant impact on adipose tissue homeostasis (Rupnick, 2002), suggesting this process is crucial to sustain normal adipose tissue function and for the support of its metabolic and endocrine functions. Additional cell types including the monocytes and macrophages are present in adipose tissues and are thought to aid in the clearance of necrotic adipocytes, a role that appears to be of importance in adipose tissue in obesity (Cinti, 2005). Lastly, adipose tissue is a reservoir of pluripotent stem cells (Zuk, 2002). These cells, though poorly defined, may contribute to the pool of differentiated stromal vascular cells types as well as to the adipocyte pool. Whether a particular grouping of cells within the stromal vascular fraction is designated for adipocyte differentiation, such as a "preadipocytes," *in vivo* is not known and is currently under active research.

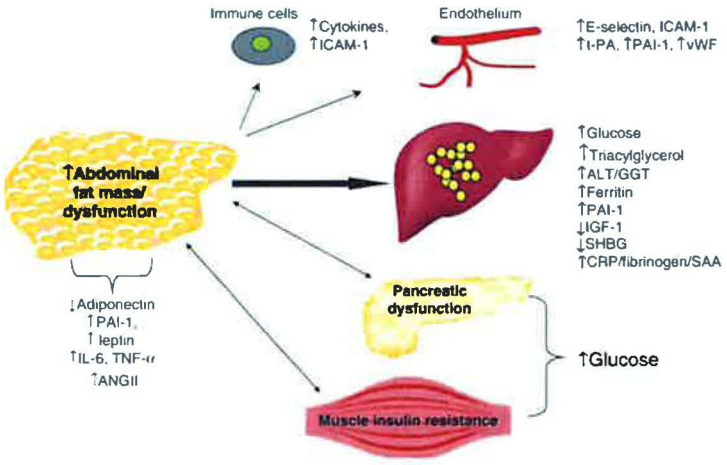


Endocrine function of the adipose organ

Studies in the late 1980's have demonstrated that adipocytes can secrete a number of factors and that the secretion of some of these factors is affected by metabolic dysregulation (Cook, 1987). However, the concept describing the adipocyte as an endocrine cell did not gain general acceptance until several additional factors were identified who expression was highly enriched in adipocytes, such as leptin and Acrp30/adiponectin (Zhang, 1994; Scherer, 1995). For the past 10 years, endocrine aspects of adipose tissue function have become an extremely active area of research and several additional hormones have been discovered (Scherer, 2006). Generally, these adipose tissue-derived factors are referred to as adipokines. These adipokines influence a number of important systemic phenomena and interact in the process with a large number of different organ systems. While the expression of a number of these secreted products is enriched in adipocytes, expression of only a few of these factors is consistently restricted to adipocytes in rodents and humans. Of these, adiponectin shows the most restricted expression pattern, with very few credible reports for expression in cells other than adipocytes except under conditions of severe hepatic steatosis during which the entire adipogenic program is induced in hepatocytes (Yoda-Murakami, 2001). Other adipocyte-derived hormones such as leptin and adipsin exhibit expression patterns that are not adipocyte specific (Cook, 1987). In fact, several adipose tissue -derived hormones such as resistin (also known as Adipocyte Secreted Factor (ADSF), and Found in Inflammatory Zone 3 (FIZZ3)), omentin, leptin, and visfatin (also known as pre-B-cell colony-enhancing factor (PBEF)) are produced by several other tissues as well (Rajala, 2003; Yang, 2006; Fukuhara, 2005).

The Adipokine Panel for Clinical Studies:
IL-6
MCP-1
PAI-1
Adiponectin
Leptin
RBP4
(Resistin)
(TNFα)

Combine with:
CRP
IL1β



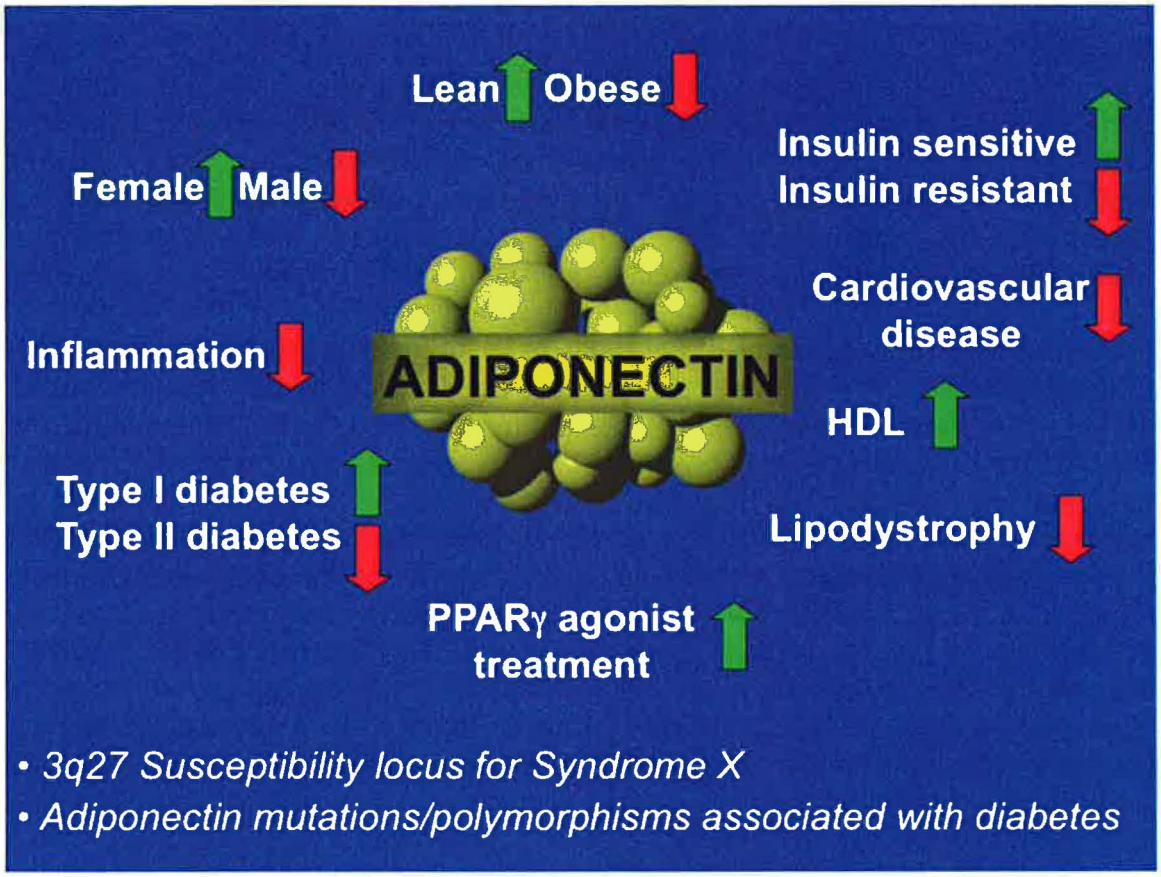
Diabetologia. 2008 Jun;51(6):926-40

Extracellular Matrix	*Metabolism*	*Immune System*	*Others*
Alpha 2 Macroglobulin[1]	Adipsin	Alpha 1 acid glycoprotein	Angiopoietin 1
Cathepsin B	Adiponectin	Colony Stimulating factor-1	Angiopoietin 2
Cathepsin D	Apelin	Complement component	Angiotensinogen
Cathepsin L	Apo E	inhibitor C1	Calcitonin
Cathepsin S	Cortisol	Complement C1	Chemerin
Collagen Alpha 1 (I)	Insulin like growth factor-1	Complement C2	Cyclophilin A
Collagen Alpha 1 (III)	Insulin like growth factor	Complement C3	Cyclophilin C
Collagen Alpha 1 (IV)	binding protein 7	Complement C4	Extra cellular SOD
Collagen Alpha 1 (VI)	Lipoprotein lipase	Complement C7	Galectin 1
Collagen Alpha 1 (XV)	Leptin	Complement factor B	Firbroblast growth factor
Collagen Alpha 1 (XIV)	Fasting induced adipose	Complement factor C	Hepatic growth factor
Collagen Alpha 1 (XVII)	factor	Complement factor D	Mineralocorticoid releasing
Collagen Alpha 2 (I)	Plasminogen activated	C reactive protein	factor
Collagen Alpha 2 (IV)	inhibitor-1	Haptoglobin	Nerve growth factor
Collagen Alpha 2 (VI)	Resistin	Interleukin 1beta	Pigment epithelium derived
Collagen Alpha 3 (VI)	Retinol binding protein 4	Interleukin 4	factor
Dystroglycan	Vaspin	Interleukin 6	Prostaglandin E2
Entactin	Visfatin	Interleukin 7	Prostaglandin I2
Fibulin-2		Interleukin 8	Prostaglandin 2alpha
Fibulin-3		Interleukin 10	Serum transferrin
Fibronectin		Interleukin 12	Stromal derived factor 1
Galectin-3-binding protein		Interleukin 18	TGF beta
Gelsolin		Lipocalin 24p3	Tissue Factor
Laminin alpha 4		Macrophage migration	Vascular endothelial growth
Laminin beta 1		inhibitory factor 1	factor
Laminin gamma		Serum Amyloid A3	
Lysyl oxidase		Tumor necrosis factor alpha[9]	
Matrilin-2			
Matrix Metalloproteinase 1			
Matrix Metalloproteinase 2			
Matrix Metalloproteinase 3			
Matrix Metalloproteinase 7			
Matrix Metalloproteinase 9			
Matrix Metalloproteinase 10			
Matrix Metalloproteinase 11			
Matrix Metalloproteinase 12			
Matrix Metalloproteinase 13			
Matrix Metalloproteinase 14			
Matrix Metalloproteinase 15			
Matrix Metalloproteinase 16			
Matrix Metalloproteinase 17			
Matrix Metalloproteinase 19			
Matrix Metalloproteinase 23			
Matrix Metalloproteinase 24			
Tissue inhibitors of metalloprotease 1			
Tissue inhibitors of metalloprotease 2			
Tissue inhibitors of metalloprotease 3			
Tissue inhibitors of metalloprotease 4			
Osteonectin (SPARC)			
Perlecan			
Procollagen C-proteinase enhancer protein			
Protein-Lysine 6-oxidase			
Spondin-1			
Tenacin			
Thrombospondin-1			
Thrombospondin-2			

Secretory Proteins Identified from Adipocytes

Adiponectin

Adiponectin is a 30kD protein that we first described in 1995 (Scherer, 1995). Other groups reported similar findings in later years, each imparting its own name for the molecule, such as Adiponectin, apM1, AdipoQ and GBP28 (Hu, 1996; Nakano, 1996; Maeda, 1996). At present, the molecule is most commonly referred to as adiponectin. Since this discovery, over two thousand papers have been published chronicling adiponectin's unique structure, function, and its physiological role under normal conditions and its dysregulation in and impact on pathological states. Though much has been learned, there are still many aspects of adiponectin physiology that remain to be elucidated.



Novel biochemical predictors of type 2 diabetes—narrative review of strengths of association with incident diabetes, adjustments made, correlated factors and caveats

Novel predictor	Evidence for association of baseline levels with incident diabetes	Strength of independent association from a representative study or summary meta-analysis*	Factors adjusted in analysis	Examples of observed correlations with other relevant parameters	Other points of relevance/ caveats
Adipose-derived					
Adiponectin	Consistent inverse associations independent of obesity, across ethnic groups and sex. Association may be stronger in obese individuals	Top tertile vs bottom tertile RR 0.40 (95% CI 0.23–0.70) [3]	Age, BMI, social class, physical activity, smoking status, alcohol intake, pre-existing CHD/stroke, statins, systolic BP, treatment for BP	+ve: HDL-C, age, renal dysfunction –ve: obesity, liver fat, ALT, PAI-1, ferritin, triacylglycerol	High adiponectin less strongly associated with lower CHD risk High adiponectin predicts higher all-cause mortality
Leptin	Inconsistent	High baseline leptin levels linked to elevated risk, no risk and low risk of incident diabetes [30–32]	Variations in statistical adjustments may partly explain differing results; more data needed	+ve: obesity, CRP, triacylglycerol, PAI-1, ALT –ve: HDL-C	Not a routine test, and thus not standardised, sexual dimorphism, with much higher levels in women
IL-6	Consistent associations independent of obesity and insulin resistance	Top tertile vs bottom tertile 2.02 (95% CI 1.14–3.58) [3]	Age, BMI, social class, physical activity, smoking status, alcohol intake, pre-existing CHD/stroke, statins, systolic BP, treatment for BP	+ve: obesity, CRP, triacylglycerol, PAI-1 –ve: HDL-C, adiponectin	IL-6 associated with higher all-cause mortality and other disease outcomes, i.e. non-specific
PAI-1 (also derived from liver, endothelium)	Consistent association with incident diabetes independent of obesity and insulin resistance	1.61 (95% CI 1.20–2.16) for 1 SD increase [33]	Age, sex, clinical centre, smoking, ethnicity, S_I, BMI, family history of diabetes, physical activity	+ve: liver fat, liver enzymes, triacylglycerol –ve: adiponectin, SHBG	Not a routine test and thus not standardised. PAI-1 rise over time is also associated with diabetes, but associations adjusting for LFTs or other markers of liver fat not tested

Diabetologia. 2008 Jun;51(6):926-40

Function

Initial studies designed to examine the function of adiponectin employed various forms of recombinant adiponectin. Some of these studies produced recombinant adiponectin through bacterial expression systems. These systems produce full length or a globular (a proteolytic cleavage product) form of adiponectin. Administration of these bacterially produced forms of adiponectin resulted in decreases in glucose, FFAs, and triglycerides in vivo and increased glucose uptake and fatty acid oxidation in muscles *in vitro* (Fruebis, 2001). However, the bacterially produced forms of adiponectin lack the ability to form higher order structures and are devoid of any relevant post-translational modifications (Shapiro, 1998). Therefore, to date it is not clear whether the effects attributed to the globular form are merely an interesting pharmacological observation or whether they have a relevant physiological counterpart. Adiponectin produced by mammalian expression systems is more likely reflecting the structure and function of endogenous adiponectin circulating in plasma. In the liver, administration of adiponectin results in glucose lowering due to decreased hepatic glucose output and suppression of gluconeogenic genes (Berg, 2001; Combs, 2001). Similar observations can be made in mice endogenously overexpressing adiponectin from adipocytes (Combs, 2004). In support of these findings, mice lacking adiponectin have reduced insulin sensitivity primarily at the level of the liver (Nawrocki, 2006). In the heart, adiponectin exerts potent cardio-protective effects and decreases myocardial infarct size in a cardiac ischemic reperfusion model (Shibata, 2004; Shibata, 2005). Adenoviral-mediated overexpression of adiponectin accelerates repair in a model of ischemic hind limb by increasing angiogenesis (Shibata, 2004).

Table 3. Estimated RRs of Myocardial Infarction During 6 Years of Follow-up According to Quintile of Baseline Adiponectin Levels (n = 796)

	Quintile*					*P* Value for Trend†
	1	2	3	4	5	
Plasma adiponectin level, mg/L, median (range)*	7.9 (2.4-10.5)	12.5 (10.6-14.5)	16.5 (14.6-18.5)	21.1 (18.6-24.8)	29.2 (24.9-56.1)	
Cases, No.	78	56	51	49	32	
Controls, No.	106	106	107	106	107	
Model, RR (95% CI)						
Adjusted for matched variables‡	1.00	0.70 (0.45-1.08)	0.63 (0.40-0.99)	0.61 (0.39-0.96)	0.39 (0.23-0.64)	<.001
Multivariable§	1.00	0.72 (0.46-1.14)	0.69 (0.43-1.09)	0.70 (0.44-1.13)	0.41 (0.24-0.70)	<.001
Multivariable, additionally adjusted for lipid levels§‖	1.00	0.75 (0.47-1.20)	0.73 (0.46-1.18)	0.82 (0.50-1.33)	0.56 (0.32-0.99)	.02

Abbreviations: CI, confidence interval; RR, relative risk.
*Quintiles, medians, and ranges of adiponectin levels are based on controls only.
†*P* value for trend based on log-transformed adiponectin levels.
‡Adjusted for matched variables (age, smoking status, and month of blood draw).
§Adjusted for matched variables, body mass index, family history of myocardial infarction before age 60 years, history of diabetes, history of hypertension, alcohol intake, and physical activity.
‖Lipids include low- and high-density lipoprotein cholesterol.

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Serum Adiponectin Levels Are an Independent Predictor of the Extent of Coronary Artery Disease in Men

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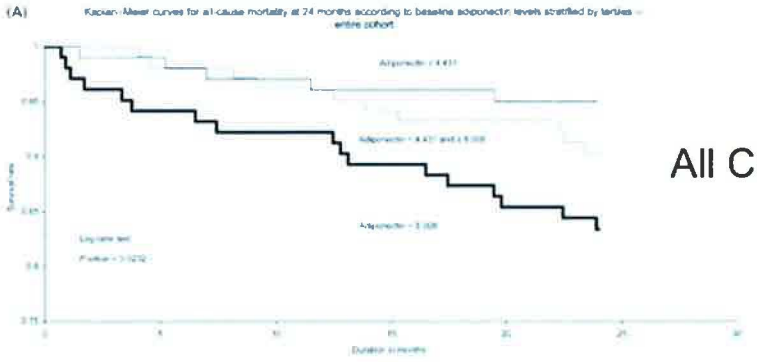
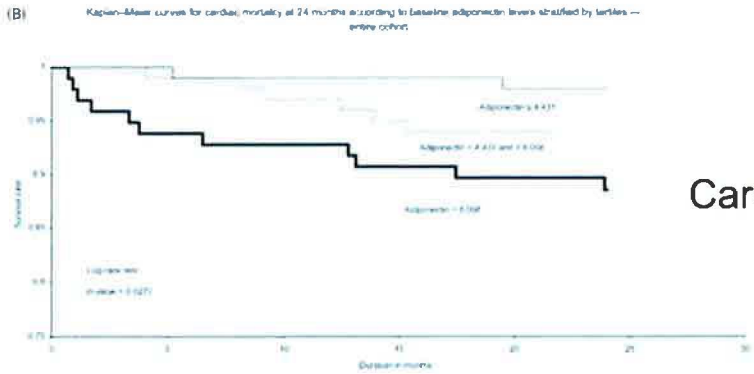
JACC Vol. 47, No. 10, 2006
May 16, 2006:2118–29

Our results indicate that the measurement of serum adiponectin might represent a novel diagnostic tool to stratify patients at risk for CAD and to identify those patients who would benefit most from preventive strategies.

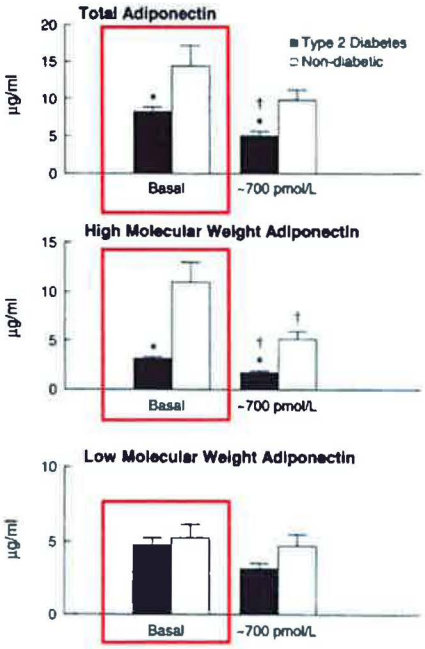
Adiponectin is present in microgram quantities in the serum and has a rapid turn over. Therefore, the use of adiponectin as a protein therapeutic is likely to be quite limited, except for defined acute applications such as in the context of myocardial infarctions. Nevertheless, it is clear that increasing adiponectin levels has many beneficial effects on insulin sensitivity, inflammation and lipid profiles (Trujillo, 2005). Early findings demonstrating serum adiponectin levels are inversely correlated with obesity spurned intense investigation of the relationship between adiponectin and all symptoms of the metabolic syndrome (Hu, 1996;Arita, 1999). Of these studies, strong evidence suggests hypoadiponectinemia is highly correlated with cardiovascular disease (Kumada, 2003). Though the precise mechanisms have just begun to be elucidated, adiponectin is inversely correlated with cardiovascular risk factors such as dyslipidemia, and high serum adiponectin levels decrease the risk of myocardial infarction (Pischon, 2004). Future studies examining the mechanisms mediating the chronic effects of adiponectin on the heart and vasculature in disease models will greatly facilitate our understanding of the role of adiponectin in cardiovascular disease.

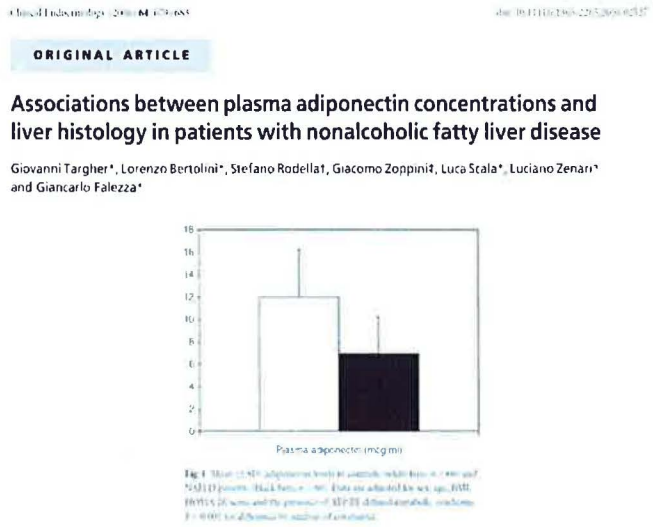


All Cause Mortality



Cardiac Mortality

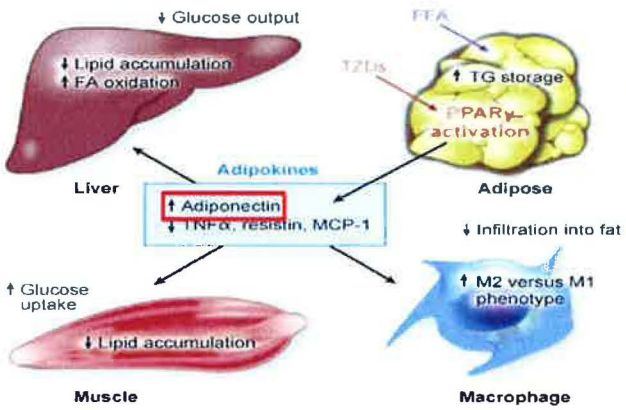




Basu.Pajvani.Rizza, and Scherer 2007 Diabetes, 56(8), 2174

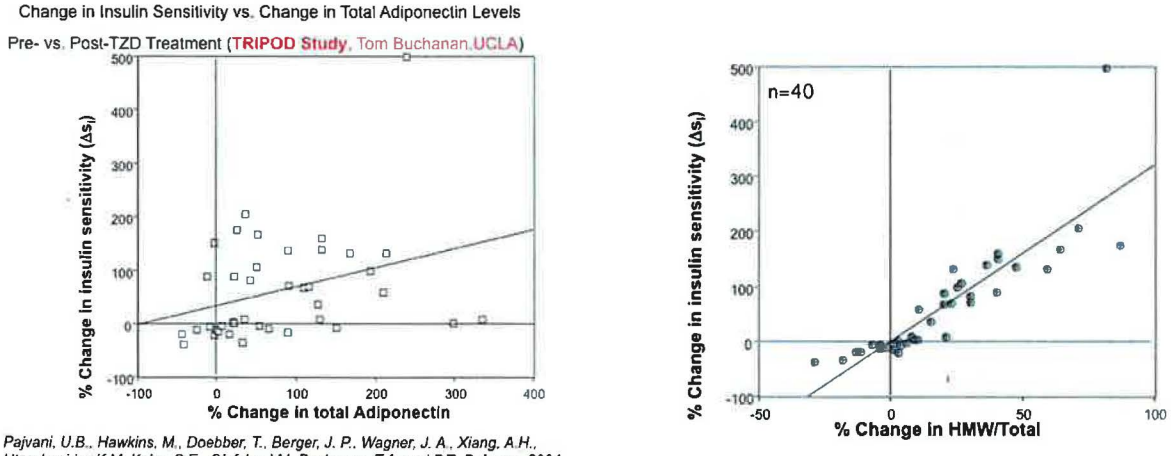
Hypoadiponectinemia is also associated with insulin resistance and diabetes (Weyer, 2001). Studies in rodents suggest that the anti-diabetic effects of adiponectin are likely due to decreased hepatic glucose output thereby contributing whole body glucose homeostasis. In support of this hypothesis, studies in humans show adiponectin levels correlate with basal and insulin-suppressed endogenous glucose production and not with β oxidation (Stefan, 2002). The relationship between adiponectin and insulin resistance are present in several different diabetic populations, several of which exhibit this relationship independent of obesity (Ranheim, 2004). In further support of these findings, studies in monkeys and humans show adiponectin levels drop prior to the decrease in whole body insulin sensitivity. Therefore, therapeutic treatments that increase adiponectin levels have great potential to enhance insulin sensitivity as well as exert cardio protective effects.

One of the most potent insulin sensitizing class of therapeutics include the thiazolidinediones ("TZD"). TZD treatment results in improvements in insulin sensitivity and improvements in the serum lipid profile. Several lines of evidence suggest that adiponectin mediates several of these beneficial effects. First, TZD treatment increases serum adiponectin (Combs, 2002). More specifically, the improvements in insulin sensitivity with TZD treatment correlates best with the levels of the HMW form of adiponectin (Pajvani, 2004), suggesting different forms of



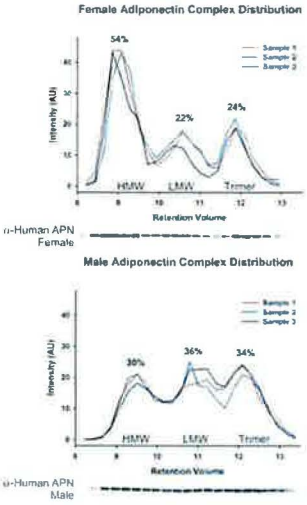
Tontonoz P, Spiegelman BM. 2008. Annu. Rev. Biochem. 77:289–312.

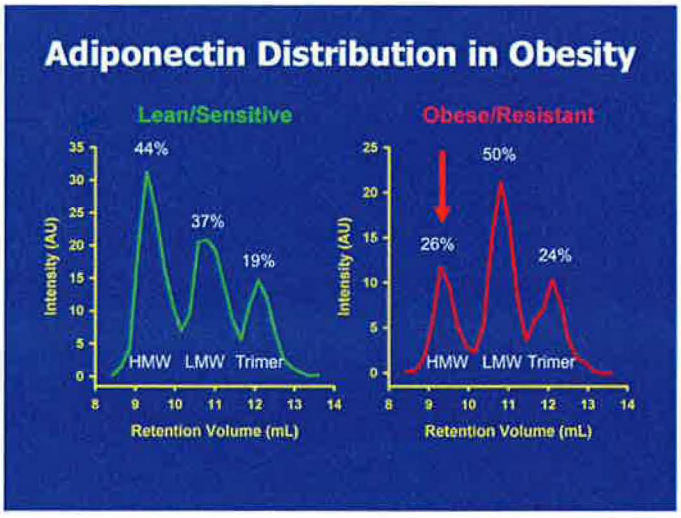
adiponectin possess different bioactivities and/or target organs. Beyond these merely correlative observations, we have recently reported that TZD treatment is not as effective in mouse models lacking adiponectin (Nawrocki, 2006). These observations have been confirmed and added value to the very elegant studies by Kadowaki and colleagues (Kubota, 2006). Finally, overexpression of adiponectin by adipocytes exerts TZD-like effects on lipid metabolism and insulin sensitivity. Increases in circulating adiponectin in these mice are coupled with improved lipid clearance, and insulin sensitivity. When crossed to *ob/ob* mice, adiponectin overexpression confers dramatic metabolic improvements. These improvements include decreased serum triglycerides, decreased fat cell size, and increased fat cell number, and improved glucose tolerance. Taken together, these data suggest adiponectin may mediate several of the beneficial effects of TZDs on metabolism.



Pajvani, U.B., Hawkins, M., Doebber, T., Berger, J. P., Wagner, J. A., Xiang, A.H., Utzschneider K.M, Kahn, S.E., Olefsky J.M, Buchanan, T.A. and ***P.E. Scherer****. 2004. J. Biol. Chem., 279(13):12152-12162.*

How does adiponectin exert its beneficial effects on lipid metabolism? While a number of different mechanisms may be contributing, we observe in many of our *in vivo* systems of adiponectin overexpression that there is a significant increase in adipose tissue lipoprotein lipase (LPL) activity. Similarly, TZD treatment also

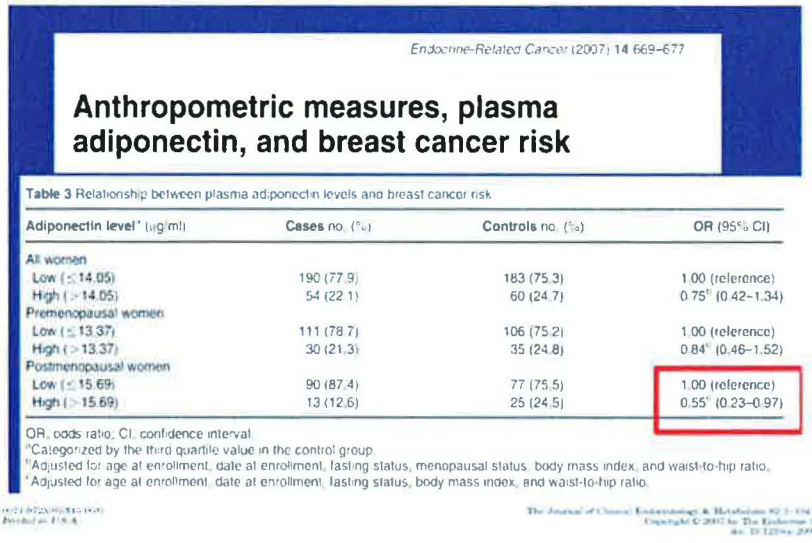




increases adipose tissue LPL activity *in vivo* and *in vitro*. While it is not clear whether this is an indirect effect exerted through increases in PPARγ activity or a reflection of a direct protein/protein interaction between adiponectin and LPL is not clear. However, the strong correlation between adiponectin levels and LPL activity can also be seen clinically (von Eynatten, 2004).

Our observations that TZD exposure primarily leads to an increase in the HMW form has highlighted the potential importance of the HMW which displays in many instance much more dramatic changes than other adiponectin complexes. While measurements of the HMW forms are clearly more informative under some circumstances, other circumstances suggest the measurements of HMW form and total adiponectin are comparable correlations. We will have to await the use of recently developed high throughput assays for the measurement of HMW forms in large epidemiological studies to better gauge the appropriate settings under which the HMW measurements will provide added value the measurements of total levels.

A significant body of literature also implicates adiponectin as protective agent against cancer.



Endocrine-Related Cancer (2007) 14 669–677

Anthropometric measures, plasma adiponectin, and breast cancer risk

Table 3 Relationship between plasma adiponectin levels and breast cancer risk

Adiponectin level* (μg/ml)	Cases no. (%)	Controls no. (%)	OR (95% CI)
All women			
Low (≤14.05)	190 (77.9)	183 (75.3)	1.00 (reference)
High (>14.05)	54 (22.1)	60 (24.7)	0.75† (0.42–1.34)
Premenopausal women			
Low (≤13.37)	111 (78.7)	106 (75.2)	1.00 (reference)
High (>13.37)	30 (21.3)	35 (24.8)	0.84‡ (0.46–1.52)
Postmenopausal women			
Low (≤15.69)	90 (87.4)	77 (75.5)	1.00 (reference)
High (>15.69)	13 (12.6)	25 (24.5)	0.55‡ (0.23–0.97)

OR, odds ratio; CI, confidence interval.
*Categorized by the third quartile value in the control group.
†Adjusted for age at enrollment, date at enrollment, fasting status, menopausal status, body mass index, and waist-to-hip ratio.
‡Adjusted for age at enrollment, date at enrollment, fasting status, body mass index, and waist-to-hip ratio.

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Total and High-Molecular-Weight Adiponectin in Breast Cancer: *In Vitro* and *in Vivo* Studies

Antje Körner, Kalliopi Pazaitou-Panayiotou, Theodoros Kelesidis, Iosif Kelesidis, Catherine J. Williams, Athina Kaprara, John Bullen, Anke Neuwirth, Sofia Tseleni, Nicholas Mitsiades, Wieland Kiess, and Christos S. Mantzoros

TABLE 2. Odds ratio for risk of breast cancer by quartile of adiponectin concentration for all subjects (n = 150)

	Q1 and Q2	Q3	Q4	P value
Total adiponectin, range (μg/ml)	0.13–9.95	10.13–15.57	15.46–20.04	
Model 1	1.0	0.46 (0.19–1.13)	0.35 (0.14–0.88)	0.04
Model 2	1.0	0.46 (0.19–1.13)	0.35 (0.14–0.87)	0.04
Model 3	1.0	0.40 (0.16–1.03)	0.30 (0.11–0.80)	0.03
Model 4	1.0	0.37 (0.14–1.00)	0.28 (0.10–0.75)	0.02
Model 5	1.0	0.38 (0.14–1.04)	0.23 (0.08–0.66)	0.02
HMW adiponectin, range (μg/ml)	0.00–3.74	3.76–5.66	5.80–15.24	
Model 1	1.0	1.02 (0.46–2.27)	0.30 (0.11–0.82)	0.05
Model 2	1.0	1.02 (0.46–2.29)	0.31 (0.11–0.84)	0.05
Model 3	1.0	0.95 (0.42–2.15)	0.29 (0.10–0.83)	0.06
Model 4	1.0	0.77 (0.33–1.83)	0.25 (0.09–0.75)	0.04
Model 5	1.0	0.78 (0.32–1.88)	0.27 (0.09–0.83)	0.05

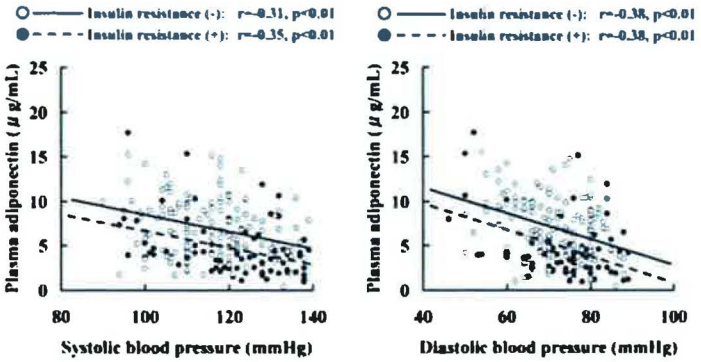
Model 1, adjusted for age; model 2, adjusted additionally for BMI; model 3, adjusted additionally for age at menarche, nulliparous status, menopausal status, and family history of breast cancer; model 4, adjusted additionally for insulin; model 5, adjusted additionally for leptin. Q, Quartile.

In summary, adiponectin is an important adipokine that has the potential to be an important mediator of many physiologically relevant processes. Modulation of adiponectin levels has a profound impact particularly in pathological settings, such as in diabetes, cardiovascular disease as well as in the context of cancer.

Hypertension

Adiponectin may also be involved in the progression of hypertension. On a high salt diet, Ohashi et al. showed that adiponectin deficient animals display significantly higher systolic blood pressure compared to wild type controls independent of insulin resistance (Ohashi, 2006). Reconstitution of adiponectin expression by adenoviral infection restored normal blood pressure. Overexpression of adiponectin can also decrease the systolic blood pressure in genetically obese KKAy mice. The association between adiponectin and hypertension is also evident in clinical studies by showing that hypoadiponectinemia is a risk factor for hypertension independent of insulin resistance and diabetes (Iwashima, 2004; Chow, 2007).

Correlation between plasma adiponectin concentration and blood pressure in normotensives without diabetes



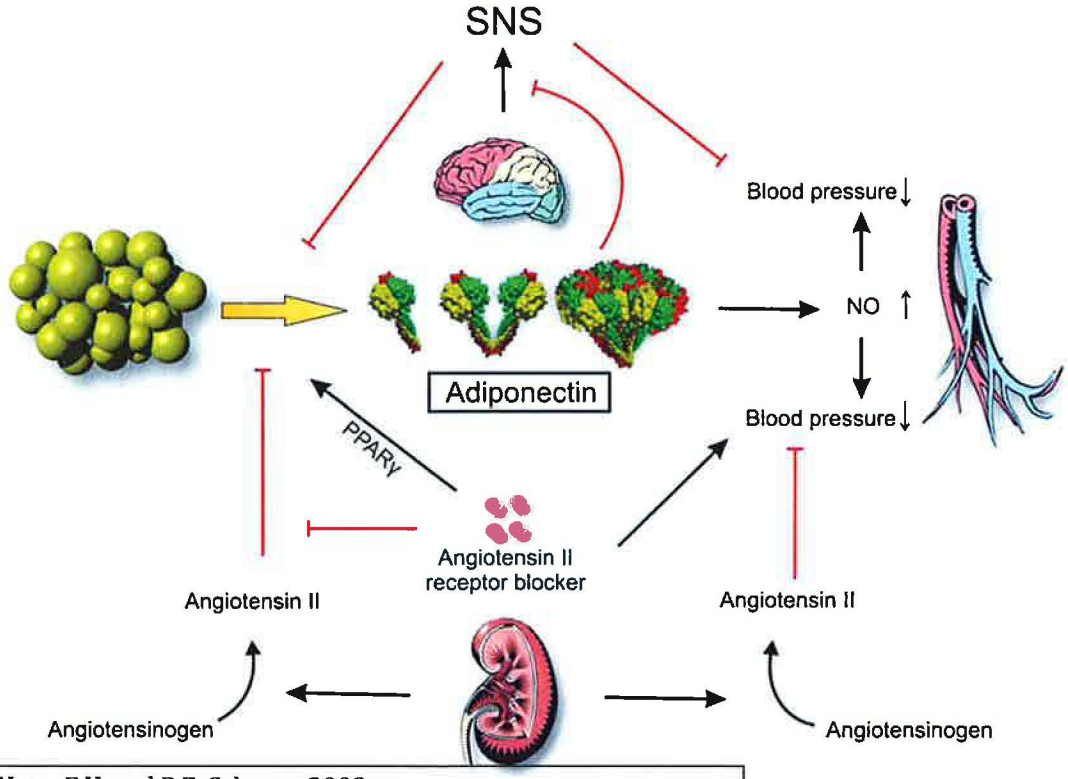
Iwashima, Y. et al. Hypertension 2004;43:1318-1323

Adiponectin and endothelial dysfunction

The endothelium is not only the inert interface between circulating blood and the vessel wall but also a major paracrine organ which plays critical roles in controlling vascular tone, inflammation and smooth muscle cell proliferation. Nitric oxide (NO) is considered to be the mediator for vasoconstriction and vasodilation, adhesion molecule expression and leukocyte transmigration, smooth muscle cell growth control under physiological conditions. The endothelial nitric oxide synthase

(eNOS) activity and NO production can be controlled by availability of substrates and cofactors, transcription of eNOS, mRNA stability of eNOS, subcellular localization of eNOS protein, enzymatic uncoupling and posttranslational modifications (Dudzinski, 2006).

Recently, a growing body of evidence shows that hypoadiponectinemia is associated with endothelial dysfunction. By measuring forearm blood flow in response to reactive hyperemia, Ouchi et al found that plasma adiponectin levels are correlated with an endothelial vasodilation response (Ouchi, 2003). These results were confirmed in animal studies by showing that adiponectin deficient mice display impaired endothelium-dependent vasodilation and NO production (Ouchi, 2003). Similarly, Tan et al showed that hypoadiponectinemia is associated with a lower vasodilation response in diabetic patients (Tan, 2004). The same group found that adiponectin administration increase NO production in human aortic endothelial cells. By analyzing nitrate/nitrite as NO metabolites, Ohashi et al showed that adiponectin knockout mice had decreased nitrate/nitrite levels compared to wild type littermates after high salt feeding (Ohashi, 2006 #5510). The obesity-associated metabolic syndrome causes a decrease in the expression and secretion of adiponectin. The dysregulated production of adiponectin may be one of the critical factors mediating obesity-associated NO decrease, endothelial dysfunction and cardiovascular disease.



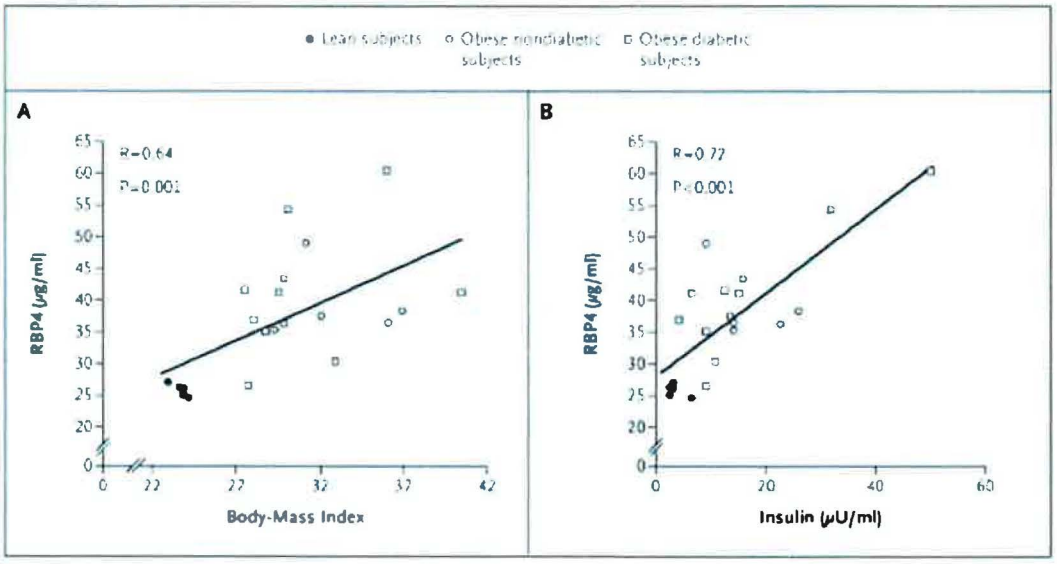
Wang, Z.V. and P.E. Scherer. 2008.

ADIPONECTIN, CARDIOVASCULAR FUNCTION AND HYPERTENSION.

Hypertension, 51(1):8-14

Additional adipokines

In the race to identify and characterize new adipokines many find the task a daunting one. Advancements in proteomics and microarray technologies have proved useful screening tools that provide a multitude of potential new adipokine molecules. However, characterization of putative adipokines is a slow and arduous process often taking 3-5 years of focused effort. A very exciting recent example of adipokine discovery is the production of retinol binding protein 4 (Rbp4) by adipocytes (Graham, 2006).



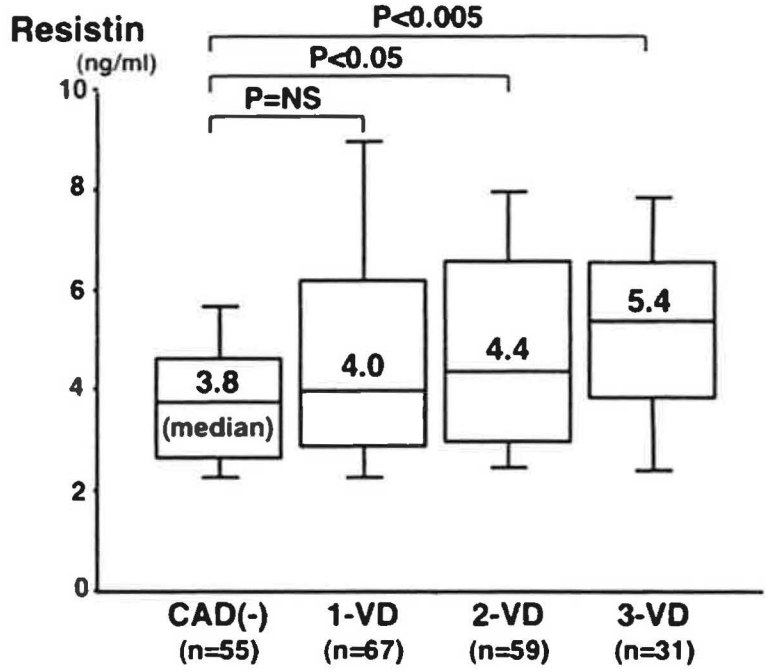
N Engl J Med. 2006 ;354(24):2552-63.

Resistin

Resistin (Steppan, 2001; Rajala, 2002), is member of the resistin-like molecule ("RELM") hormone family. Initial findings demonstrated that resistin expression is reduced by TZD treatment and increased in obesity. The physiological role of resistin has proven to be more challenging to figure out than originally anticipated. mRNA levels and protein levels do not correlate very well, and in fact in many instances show a inverse relationship. Other issues include the differential expression of resistin between rodents (that express resistin predominantly in adipocytes) and humans (where the primary sources are the stromal vascular cells in adipose tissue); causing some to question whether resistin is truly an adipokine

or merely a cytokine produced by immune cells within the SVF. While the direct clinical relevance of resistin is still under investigation, it represents a fascinating cytokine at every possible level.

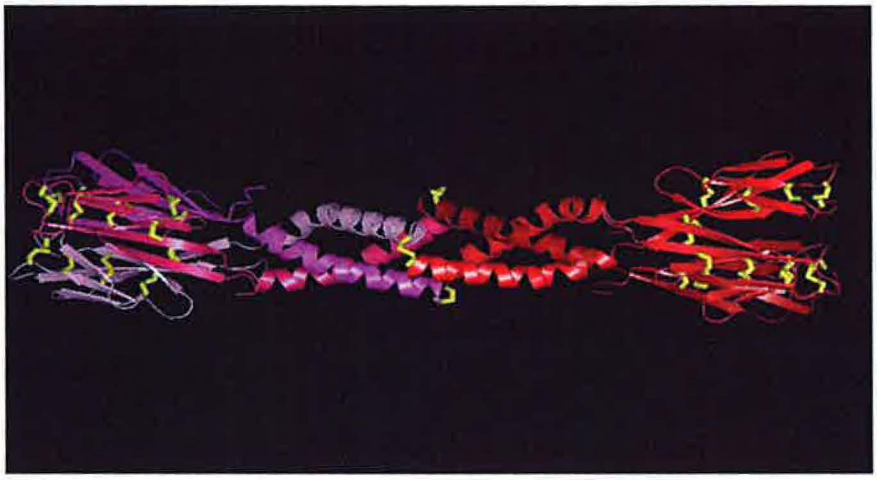
Serum resistin levels in patients with coronary artery disease (CAD) and age- and gender-matched patients without CAD



Ohmori, R. et al. J Am Coll Cardiol 2005;46:379-380

Structure

Similar to adiponectin, resistin may be secreted in two distinguishable multimeric forms. Each form is composed of two domains, a carboxy-terminal disulfide-rich ß-sandwich domain and an amino-terminal a-helical segment. These protomers combine to form trimers via three-stranded coiled coils within the a-helical segments. Two trimers likely bind via tail-to-tail interchain disulfide bonds to form hexamers. Whether there is indeed a measurable pool of reduced resistin lacking intact disulfide bonds at the trimer interface in serum remains to be shown. Current efforts in our laboratory are directed toward addressing this issue genetically. A close relative of resistin, RELMb, which is expressed primarily in the colon, shows a very similar overall structure as well and may in fact have partially overlapping functions.



High Resolution Structure of Resistin

From: Patel, S.D., Rajala, M.W., Rossetti, L., Scherer, P.E., and Shapiro, L. (2004). Disulfide-dependent multimeric assembly of resistin family hormones. Science 304, 1154-1158.

Function

Several studies using mouse models have determined that resistin antagonizes insulin action in the liver. During a hyperinsulinemic-euglycemic clamp, administration of mammalian produced resistin results in severe hepatic but not peripheral insulin resistance demonstrating acute resistin treatment blunts hepatic insulin action. In addition, comparable experiments performed with recombinant RELMb resulted in insulin resistance in hepatocytes. Similar to acute affects of resistin administration, transgenic overexpression of resistin results in increased fasting glucose and decreased glucose tolerance suggesting insulin resistance. The reciprocal of these findings were found in resistin knockout mice such that there were decreased fasting glucose levels, hepatic glucose production, and gluconeogenic enzymes in the liver}. Together these studies suggest resistin may counterbalance the insulin sensitizing affects of adiponectin in the liver. Whether these effects are a result of direct interaction of resistin with cell surface receptors on hepatocytes is not yet known, since the resistin and RELMβ receptors have not yet been identified.

Concluding remarks

The role of adipose tissue as an important source of local mediators in the stroma of a host of organs as well as its role as an endocrine gland is now widely appreciated. As a whole, adipose tissue can make up a significant proportion of total body weight. So by sheer mass action, it is difficult to ignore the contribution it makes to plasma protein. In addition, since fat pads are interspersed in many different places systemically, it is important to note that these pads constitute different "miniorgans" with unique characteristics depending on their location and a differential proteomics fingerprint. We have attempted here to provide a comprehensive overview of protein factors that have been described in the

literature. We have devoted much of the discussion on aspects of the adipocyte secretome that usually get less attention. It is not clear in all instances to what extent it is the adipocyte that serves as the primary site of production or whether another adipose tissue cell type in concert with the adipocyte is the major production site. In either case, if we have to treat the whole tissue as an entity. Thus much remains to be understood, both about the cross talk between the different cell types within the adipose tissue, as well as the secretory pathway.

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