Relationship of Adiponectin with Insulin Sensitivity in Humans, Independent of Lipid Availability

Stuart M. Furler, Seng Khee Gan, Ann M. Poynten, Donald J. Chisholm, Lesley V. Campbell, and Adamandia D. Kriketos

Abstract

FURLER, STUART M. SENG KHEE GAN, ANN M. POYNTEN, DONALD J. CHISHOLM, LESLEY V. CAMPBELL, AND ADAMANDIA D. KRIKETOS. Relationship of adiponectin with insulin sensitivity in humans, independent of lipid availability. *Obesity.* 2006;14: 228–234.

Objective: To test in humans the hypothesis that part of the association of adiponectin with insulin sensitivity is independent of lipid availability.

Research Methods and Procedures: We studied relationships among plasma adiponectin, insulin sensitivity (by hyperinsulinemic-euglycemic clamp), total adiposity (by DXA), visceral adiposity (VAT; by magnetic resonance imaging), and indices of lipid available to muscle, including circulating and intramyocellular lipid (IMCL; by ^{1}H-magnetic resonance spectroscopy). Our cohort included normal weight to obese men ($n = 36$).

Results: Plasma adiponectin was directly associated with insulin sensitivity and high-density lipoprotein-cholesterol and inversely with plasma triglycerides but not IMCL. These findings are consistent with adiponectin promoting lipid uptake and subsequent oxidation in muscle and inhibiting TG synthesis in the liver. In multiple regression models that also included visceral and total fat, free fatty acids, TGs, and IMCL, either alone or in combination, adiponectin independently predicted insulin sensitivity, consistent with some of its insulin-sensitizing effects being mediated through mechanisms other than modulation of lipid metabolism. Because VAT directly correlated with total fat and all three indices of local lipid availability, free fatty acids, and IMCL, an efficient regression model of insulin sensitivity ($R^2 = 0.69$, $p < 0.0001$) contained only VAT (part $R^2 = 0.12$, $p < 0.002$) and adiponectin (part $R^2 = 0.41$, $p < 0.0001$) as independent variables.

Discussion: Given the broad range of total adiposity and body fat distribution in our cohort, we suggest that insulin sensitivity is robustly associated with adiponectin and VAT.

Key words: adipokine, body composition, intramyocellular lipid, magnetic resonance spectroscopy

Introduction

Disturbances in lipid metabolism are thought to contribute to insulin resistance and other aspects of the metabolic syndrome. Skeletal muscle is the primary site for insulin-stimulated glucose disposal, and many studies in animals and humans have associated excess accumulation of triglycerides (TGs)[1] (and/or other lipid moieties) within muscle with insulin resistance. Although TGs per se are unlikely to directly affect insulin action, excess TG content indicates increased availability of intracellular fatty acids and their derivatives, which may impede glucose metabolism through substrate competition, inhibition of insulin signaling pathways, or modulation of gene transcription (1).

In previous studies (2,3), we have shown that direct indices of local lipid availability, such as muscle lipid content and circulating lipids, are related to whole body

Received for review December 28, 2004.
Accepted in final form November 30, 2005.
The costs of publication of this article were defrayed, in part, by the payment of page charges. This article must, therefore, be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Diabetes and Obesity Research Program, Garvan Institute of Medical Research, St. Vincent's Hospital, Sydney, Australia.
Address correspondence to Stuart Furler, PhD, Garvan Institute of Medical Research, St. Vincent's Hospital, 384 Victoria Street, Sydney NSW 2010, Australia.
E-mail: s.furler@garvan.org.au

[1] Nonstandard abbreviations: TG, triglyceride; FA, fatty acid; AMPK, adenosine monophosphate-activated kinase; MRS magnetic resonance spectroscopy; MRI, magnetic resonance imaging; IMCL, intramyocellular lipid; VAT, visceral adiposity; GIR, glucose infusion rate; *I*, prevailing plasma insulin concentration; FFM, fat-free mass; HDL, high-density lipoprotein; IL, interleukin; CRP, C-reactive protein.

insulin sensitivity independently of the size of various fat depots. These results strongly suggest that remote fat depots can influence skeletal muscle insulin sensitivity by secretion of circulating factor(s) other than fatty acids (FAs). Adipose tissue produces cytokines (adipokines), many of which are putative modulators of insulin sensitivity. An abundant circulating adipokine, adiponectin (4), has been associated with protection against cardiovascular disease, obesity, and type 2 diabetes (5–8). Produced predominantly in adipocytes, circulating adiponectin levels in humans are paradoxically inversely related to adiposity, but directly with insulin sensitivity (6).

Results from in vitro and animal studies suggest that adiponectin improves insulin sensitivity in skeletal muscle by promoting FA oxidation leading to reduced intracellular TG and circulating lipids (9), primarily by activation of adenosine monophosphate-activated kinase (AMPK) (10), but activation of peroxisome proliferator-activated receptor α may also be involved (11). Adiponectin may have additional effects on glucose metabolism that are independent of modified lipid metabolism, and our group has recently shown that acute AMPK activation (in addition to promoting FA uptake) stimulates muscle glucose uptake (12). Moreover, adiponectin may have anti-inflammatory properties through modulating nuclear factor-κB signaling (13). These actions could potentially affect insulin sensitivity independently of modulation of FA metabolism. Importantly, the actions of adiponectin have been studied predominantly in cellular or rodent models.

Using state-of-the-art techniques, we previously studied the relationships in humans between insulin sensitivity and various aspects of FA metabolism. Here we have measured adiponectin levels, in baseline samples from two previously published studies (14,15) that used the same protocols (together with some unpublished data), to determine whether adiponectin is related to insulin sensitivity independently of indices of FA availability and adiposity in humans.

Research Methods and Procedures

Subjects

Thirty-six men, ranging from normal weight to obese, were studied. All subjects undertook <2 h/wk of strenuous exercise. Physical activity was assessed from questionnaire response detailing time spent each week on specific activities. Total activity was calculated by multiplying time spent on activities by estimates of their intensity expressed in metabolic equivalents. (One metabolic equivalent is basal energy expenditure.) Subjects were excluded if they had a history of any major illness or use of systemic steroids or lipid-lowering agents within the preceding year. All subjects underwent an oral glucose tolerance test; frank diabetes, but not impaired fasting glucose or impaired glucose tolerance, was an exclusion criterion.

The studies were approved by the St. Vincent's Hospital Human Research Ethics Committee (conducted according to the Declaration of Helsinki). All subjects gave written informed consent before participation.

Overview

The following procedures were performed (in the order described) in a single morning, after an overnight fast. ^{1}H-magnetic resonance spectroscopy (^{1}H-MRS) and magnetic resonance imaging (MRI) were used to determine intramyocellular lipid content (IMCL) and abdominal fat content, respectively. DXA was used to assess total body fat. Fasting blood was sampled for assay of circulating factors. A euglycemic-hyperinsulinemic clamp was performed to estimate whole body insulin sensitivity.

Intramyocellular Lipid Content by ^{1}H-MRS

As previously described (16), a 1.5-T medical magnetic resonance scanner and extremity coil (General Electric, Milwaukee, WI) were used to acquire spectra from a right soleus voxel (2.0 × 2.0 × 2.0 cm; PRESS sequence; echo time, 135 ms; repetition time, 1500 ms). Proton resonance quantitation with AMARES (MRUI version 99.2, European Union, available at http://sermn02.uab.es/mrui/) used constraints comparable to recent approaches (17). IMCL was expressed as intramyocellular CH_2 peak area as a percentage of water peak area.

Anthropometry

BMI was calculated as weight (kilograms)/height (meters) squared. Overall body composition was estimated by DXA (Lunar DPXL, software version 1.35 y; Madison, WI). Visceral fat volume was determined by MRI (General Electric) as previously described (16). Briefly, 12 T1-weighted axial scans (5 mm thickness, 5-mm intervals) were performed between the levels of L4/5 and L1/2 intervertebral discs. For each scan, the area of visceral adipose tissue within the inner margin of abdominal wall (VAT) was determined by planimetric analysis (NIH Image 1.62; NIH, Bethesda, MD). Total VAT volume was calculated by interpolating between slices (18).

Euglycemic-hyperinsulinemic Clamp

A 150-minute clamp was performed as previously described (19). Insulin (Actrapid HM; Novo Industries, Copenhagen, Denmark) was infused at a rate of 50 mU/m^2 per minute to achieve serum insulin ~100 mU/liter. Plasma glucose was monitored every 10 minutes (YSI 2300; Stat-Plus, Yellow Springs Instruments, Yellow Springs, OH) and maintained at ~5.0 mM by a variable infusion of 25% dextrose. The steady-state glucose infusion rate (GIR) and prevailing plasma insulin concentration (I) were determined over the last 40 minutes of the clamp. Indices of whole body insulin sensitivity were calculated as M = GIR/FFM, where

FFM is the fat-free mass determined by DXA, and *M/I* to correct for any variation in clamp plasma insulin.

Basal fat oxidation rate was estimated using indirect calorimetry (GE Medical Systems, Rydalmere, Australia). Measurements were taken 30 minutes before commencement of the clamp.

Biochemical Analysis

Radioimmunoassays were performed for insulin and adiponectin (Linco Research, St. Charles, MO). Serum total and high-density lipoprotein (HDL)-cholesterol, TGs, and free FAs were measured by enzymatic colorimetry (Roche Diagnostics Corp., Indianapolis, IN, and Wako, Osaka, Japan). Interleukin 6 (IL-6) concentrations were determined using a high sensitivity IL-6 ELISA system (Amersham Pharmacia Biotech, Piscataway, NJ). C-reactive protein (CRP) was measured by a highly sensitive particle-enhanced turbidimetric immunoassay technique (Dade Behring Diagnostics, Sydney, Australia) as previously described (18). Inter- and intra-assay coefficients of variation were $<10\%$ for all assays.

Statistical Analyses

Unless stated otherwise, values are presented as mean ± SE.

Associations among continuous variables were studied using simple or multiple regression analyses. The unique contribution of an independent variable to a multiple regression model was assessed using the standard *F*-test based on the incremental sum of squares when the variable was added last to the regression equation (20). Analyses were performed using general-purpose statistical software (JMP; SAS institute, Cary, NC). $p < 0.05$ was regarded as significant.

The distributions of plasma TGs, CRP, and IL-6 were highly skewed. In all analyses, log-transformed values of these variables were used.

Results

Subjects

Subject characteristics are shown in Table 1.

Table 1. Subject characteristics ($n = 36$)

Characteristics	Mean ± SD
Age (years)	35.2 ± 8.0
BMI (kg/m^2)	28.6 ± 4.2
FFM (kg)	59.2 ± 6.7
Total body fat (kg/kg FFM)	0.441 ± 0.163
VAT (mL/kg FFM)	21.51 ± 10.33
VAT/total fat (mL/kg)	48.0 ± 14.1
IMCL (soleus)	1.80 ± 0.59
Plasma adiponectin (μg/mL)	8.16 ± 4.67
Plasma CRP (μg/mL)	3.10 ± 0.30
Plasma IL-6 (pg/mL)	1.09 ± 0.26
Plasma glucose (mM)	5.55 ± 0.47
Plasma insulin (mU/liter)	14.0 ± 6.7
Plasma cholesterol (mM)	4.43 ± 0.94
Plasma HDL-cholesterol (mM)	0.866 ± 0.278
Plasma TGs (mM)	1.86 ± 1.87
Plasma FFAs (mM)	0.397 ± 0.133
Fat oxidation (g/day/kg FFM)	1.10 ± 0.07
Physical activity (MET/h/wk)	3.41 ± 0.26
Glucose infusion rate (μmol/min/kg FFM)	40.7 ± 15.1
Clamp plasma insulin (mU/liter)	109 ± 21.0
M/I	0.398 ± 0.190

FFM, fat-free mass; VAT, visceral adiposity; IMCL, intramyocellular lipid; CRP, C-reactive protein; IL, interleukin; HDL, high-density lipoprotein; TG, triglyceride; FFA, free fatty acid; MET, metabolic equivalent.

All plasma concentrations are fasting values. *M/I* (μmol/min/kg FFM)/(mU/liter) is the glucose infusion rate normalized by the clamp plasma insulin. IMCL was determined in the soleus muscle by ^{1}H-MRS. Values shown are the intramyocellular CH_2 peak area expressed as a percentage of water peak area. Values for plasma TG, CRP, and IL-6 values were log-transformed before the analysis. One MET is the energy expenditure of an individual at rest.

Bivariate Relationships

The set of simple correlation coefficients for insulin sensitivity, adiponectin, and indices of lipid availability and inflammation is shown in Table 2. This includes a direct correlation ($R^2 = 0.27$, $p = 0.0003$) between insulin sensitivity and fasting plasma adiponectin consistent with numerous previous reports (21). Also, as expected, total fat was associated inversely with insulin sensitivity ($R^2 = 0.50$, $p < 0.0001$) and adiponectin levels ($R^2 = 0.12$, $p = 0.04$).

In addition to the correlations involving lipid substrates (Table 2), relationships were also detected among circulating cholesterol, adiponectin, and insulin sensitivity. There were direct relationships between HDL-cholesterol and adiponectin ($R^2 = 0.28$, $p = 0.0008$) and insulin sensitivity, *M/I* ($R^2 = 0.46$, $p < 0.0001$). An inverse ($R^2 = 0.27$, $p = 0.001$) correlation between total cholesterol and *M/I* was also observed.

In this group of men, there were no associations between estimated physical activity and insulin sensitivity, adiponectin, or any of the indices of lipid availability considered in Table 2. However, the range of activity was small because subjects were recruited for this study only if they were

Table 2. Correlation matrix of insulin sensitivity, indices of adiposity and inflammation, and adiponectin

	M/I	Total fat	VAT	IMCL	Plasma free FAs	Plasma TGs	CRP	IL-6	Adiponectin
M/I	1								
Total fat	**−0.71**	1							
VAT	**−0.76**	**0.71**	1						
IMCL	**−0.34**	**0.34**	**0.38**	1					
Plasma free FAs	**−0.49**	**0.43**	**0.41**	0.24	1				
Plasma TGs	**−0.58**	0.30	**0.52**	0.01	0.19	1			
CRP	**−0.49**	**0.44**	**0.43**	0.24	0.23	0.17	1		
IL-6	−0.19	0.07	0.05	−0.04	**0.34**	0.09	0.32	1	
Adiponectin	**0.55**	**−0.36**	−0.30	0.11	−0.28	**−0.50**	−0.23	−0.24	1

VAT, visceral adiposity; IMCL, intramyocellular lipid; FFA, free fatty acid; TG, triglyceride; CRP, C-reactive protein; IL, interleukin. Numbers shown are Pearson product-moment correlation coefficients (R). Values shown in bold are significant ($p < 0.05$). Critical R value = 0.33. Indices of insulin sensitivity (M), total body fat mass, and visceral adiposity have been normalized by FFM. Values for plasma TG, CRP, and IL-6 values were log-transformed before the analysis.

sedentary (<2 h/wk of strenuous exercise), specifically to avoid the confounding effects of exercise.

Despite the putative action of adiponectin to promote lipid oxidation, there was no association between fasting adiponectin levels and absolute basal fat oxidation ($R^2 = 0.0002$, $p = 0.93$). However, there was a strong positive association between basal fat oxidation and circulating free FAs ($R^2 = 0.35$, $p = 0.0001$). Moreover, when rates of fat oxidation were normalized by circulating lipid substrate levels, direct correlations (free FAs: $R^2 = 0.14$, $p = 0.03$; TGs: $R^2 = 0.12$, $p = 0.04$) with adiponectin concentration were observed.

Somewhat unexpectedly, there was also no simple relationship between IMCL and adiponectin. However, in a previous study (22), we showed that many of the relationships observed between indices of fatty acid availability and insulin action in lean individuals are absent or reversed in obese subjects. To determine whether a similar phenomenon was occurring here, IMCL was modeled as a fully factored function of adiponectin and total fat. A strong relationship was evident ($R^2 = 0.48$, $p < 0.0001$), with all independent variables, including the interaction term, being highly significant ($p < 0.0005$). The model predicted an inverse relationship between adiponectin and IMCL in lean subjects but a direct association in obese individuals.

Multivariate Relationships

The degree to which adiponectin independently predicted insulin sensitivity (*M/I*) was studied by the series of multiple regression models shown in Table 3. These results indicate that adiponectin is a significant predictor of insulin sensitivity independently of each of the major indices of lipid supply (total fat, VAT, IMCL, plasma free FAs, and plasma TGs). Moreover, when adiponectin was included as an independent variable together with all five of the above indices, it alone was significant ($p < 0.04$). This result is of physiological importance because it shows that adiponectin also predicts insulin sensitivity independently of the above indices of lipid supply in combination. However, this model has little statistical value because it is clearly overparameterized. To identify a parsimonious model of insulin sensitivity, the best of the simpler models ($R^2 = 0.69$), which included adiponectin and VAT as independent variables, was assessed more closely.

The superiority of VAT over other indices of adiposity in the models considered in Table 3 is at least partly caused by the significant correlations between central fat and each of total fat, IMCL, plasma TG, and plasma free FA levels, which themselves also correlate with insulin sensitivity (Table 2). VAT, therefore, acts as a single efficient surrogate for multiple predictors of insulin sensitivity. Indeed, the two-parameter model is only marginally inferior ($R^2 = 0.69$) to an overparameterized model that also included total fat, IMCL, plasma free FAs, and plasma TGs as independent variables ($R^2 = 0.71$). We also studied whether it was possible to add a third independent variable to the two-parameter model, but none of the adiposity indices considered above, physical activity, or the inflammatory markers IL-6 and CRP contributed significantly.

The degree to which the regression model containing only VAT and adiponectin predicts insulin sensitivity in our cohort is shown in Figure 1.

Table 3. Binary regression models with insulin sensitivity *M/I* as the dependent variable and adiponectin and a single index of adiposity as the two independent variables

Dependent variable: *M/I*								
Independent variable 1	**Standardized coefficient**	**Part R^2**	***p***	**Independent variable 2**	**Standardized coefficient**	**Part R^2**	***p***	**Model R^2**
Adiponectin	0.31 ± 0.19	0.09	0.0123	Total fat	−0.60 ± 0.19	0.32	<0.0001	0.59
Adiponectin	0.35 ± 0.10	0.12	0.0016	VAT	−0.65 ± 0.10	0.41	<0.0001	0.69
Adiponectin	0.57 ± 0.13	0.32	0.0001	IMCL	−0.41 ± 0.13	0.17	0.0033	0.44
Adiponectin	0.42 ± 0.14	0.16	0.0052	Plasma free FAs	−0.37 ± 0.14	0.13	0.0128	0.40
Adiponectin	0.33 ± 0.16	0.08	0.0442	Log_{10} (plasma TGs)	−0.39 ± 0.16	0.11	0.0202	0.39

Model R^2 is the fractional variance explained by the complete model. For each independent variable, part R^2 is the increase in the model R^2 when it is added last to the regression equation that already contains the other term. It represents that proportion of the model R^2 that can be assigned uniquely to a given variable and hence quantifies the extent to which each variable independently contributes to the prediction of insulin sensitivity (20). The standardized regression coefficients result from analyses where all variables have been scaled to have a mean of 0 and SD of 1.

Discussion

This study confirmed previously reported associations (5,6,21) among plasma adiponectin concentration, insulin sensitivity, and indices of adiposity and lipid availability in a diverse cohort, consisting of normal weight to obese sedentary men. Such correlations, by themselves, cannot show causation. Hence, we did not seek to deduce mechanisms but to study whether the observed relationships in our human population were consistent with mechanisms previously shown in animal and cellular systems.

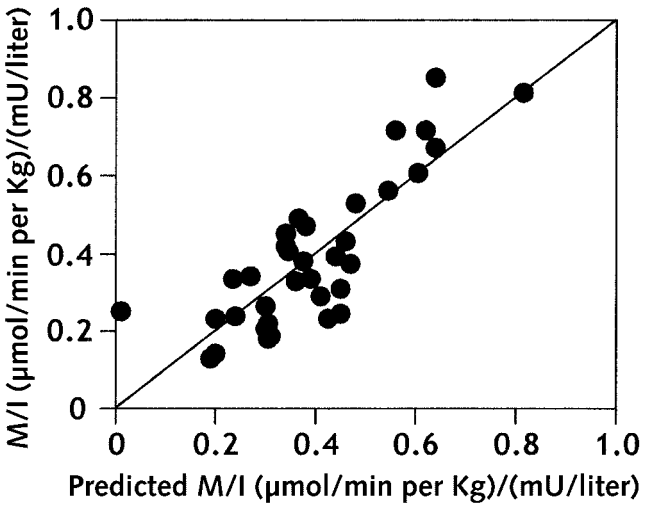

Figure 1: The relationship (R^2 = 0.69, $p < 0.0001$) between measured whole body insulin sensitivity and that predicted by plasma adiponectin and VAT (Table 3, model 2).

Adiponectin is thought to act predominantly by promoting lipid oxidation (9), thereby reducing local lipid availability in insulin-sensitive tissues. However, as previously reported (23), no association was detected between basal lipid oxidation and adiponectin levels in our data. However, lipid oxidation is also limited by substrate supply (24). When rates of fat oxidation were normalized by plasma free FA or TG levels, direct correlations with adiponectin concentration were apparent, most likely reflecting the action of adiponectin to increase the efficiency with which lipids are extracted from the circulation and subsequently oxidized.

The inverse relationship observed (Table 2) between plasma adiponectin and circulating TGs supports this view. It is of particular interest because it was accompanied by a direct association with HDL-cholesterol (R^2 = 0.28, p = 0.0008). Although very-low-density lipoprotein-cholesterol was not measured in this study, it is a relatively constant fraction (~1/5) of total circulating TGs (25). Thus, together, these results support the concept (26) that adiponectin, through activation of lipoprotein lipase, promotes the removal of TGs from the circulation, with a concomitant conversion of very-low-density lipoprotein particles to HDL.

Although our plasma data are consistent with the hypothesis that adiponectin promotes the removal of lipids from the circulation, overall there was no association between adiponectin and soleus IMCL. However, a reduction (or accumulation) of intracellular lipid requires an imbalance between influx and oxidation, and if adiponectin simultaneously increases both of these processes (26), changes in lipid stores need not occur. We previously showed (22) that obesity can distort the usual relationships between lipid

availability and insulin action. These results also indicate that the relationship between plasma adiponectin and IMCL is different in obese and lean subjects, possibly because of an altered balance between the dual actions of adiponectin to promote lipid influx and oxidation in the myocyte.

In animals, adiponectin has major effects on lipid metabolism of the liver, where it promotes FA oxidation (10) and, through AMPK activation, inhibits TG production (27). These actions would be expected to contribute substantially to the observed association between plasma TGs and adiponectin. In addition, by lowering overall lipid availability to muscle (despite unaltered IMCL), they could also contribute to the correlation between insulin sensitivity and adiponectin.

Other actions of adiponectin to affect insulin sensitivity have been proposed, including enhancement of glucose uptake through AMPK activation (12,28) and a reduction of subclinical inflammation (29,30). Although our results do not provide mechanistic detail, they are consistent with a mode of action of adiponectin in humans that is independent of altering lipid availability, because, in a regression model that included multiple indices of lipid availability, adiponectin was an independent predictor of insulin sensitivity.

The relationship between inflammatory markers and insulin resistance is the subject of much recent discussion in the literature. A previous study from our group (18) argued against a role for inflammation, suggesting instead that associations between insulin sensitivity and markers of inflammation such as CRP are largely secondary to the relationship between insulin sensitivity and measures of lipid availability. This argument is very consistent with the results presented here, where CRP was not an independent predictor of insulin sensitivity.

To explore the ability of adiponectin to predict insulin sensitivity independently of lipid availability and adiposity, we initially considered regression models that included multiple indices of these traits either alone or in combination. However, a parsimonious model including only VAT and adiponectin was selected for further study. As reported in numerous previous studies (31), there was a stronger (simple) correlation between visceral fat and insulin sensitivity than between total fat and insulin sensitivity. From a statistical point of view, this occurs, at least in part, because central fat is significantly correlated with total fat, IMCL, plasma TG, and plasma free FA levels, which themselves correlate with insulin sensitivity (Table 2). The associations between total fat and IMCL and plasma TGs are weaker. In a multiple regression model, VAT may, therefore, act as a single efficient surrogate for multiple predictors of insulin sensitivity.

The model may also have mechanistic implications. Although the action of lipid to modulate insulin sensitivity could occur within the myocyte by a variety of mechanisms (1), any response would ultimately depend on the lipid supply from local stores such as circulating lipid and IMCL. However, increased central fat could either be the cause of, or the response to, increased lipid availability to non-adipose tissues. Central fat is sensitive to adrenergic stimuli and is, therefore, more labile, releasing FAs more readily than remote fat beds (32). It is possible, therefore, that increased central adiposity would raise circulating lipid levels and increase tissue lipid deposition, resulting in the observed associations between central fat, and plasma lipids and IMCL. Alternatively, the labile central depot could be acting merely as a buffer, responding to excess body lipid availability; i.e., excess circulating lipid simultaneously promotes lipid deposition in central fat stores and muscle.

We showed (Figure 1) a close association between insulin sensitivity and the independent effects of adiponectin and visceral adiposity in a cohort of men with a large range of adiposity. We were unable to detect additional independent effects of other indices of adiposity or circulating factors, but this does not definitively preclude their existence. The number of subjects considered in this study is relatively small, and the power to detect subtle effects is, therefore, limited. It is likely that additional effects would be evident in a larger population. Other probable extensions to the model include the effect of lifestyle factors such as exercise and diet. In this study, we deliberately avoided the confounding effects of exercise by studying only sedentary individuals, and nutritional data were not available for all our subjects. However, irrespective of how the model might be further refined in the future, we established here the existence of a broadly applicable dependence of insulin sensitivity on both adiponectin and VAT.

The broader objective of this study was to investigate how adiponectin interrelates with local indices of lipid availability to predict insulin sensitivity. In all of the models considered, adiponectin was a strong independent predictor of insulin sensitivity, suggesting significant insulin-sensitizing actions of adiponectin through mechanisms other than modulation of lipid metabolism.

Acknowledgments

We acknowledge the assistance of the nursing staff of the Clinical Research Facility, laboratory technicians of the Diabetes Research Group, Dr. Judith Freund and technicians of the Nuclear Medicine and Radiology Departments, St. Vincent's Hospital Sydney, and the volunteers who participated in this study.

References

1. **Hegarty BD, Furler SM, Ye J, Cooney GJ, Kraegen EW.** The role of intramuscular lipid in insulin resistance. *Acta Physiol Scand.* 2003;178:373–83.
2. **Kriketos AD, Furler SM, Gan SK, Poynten AM, Chisholm DJ, Campbell LV.** Multiple indexes of lipid availability are independently related to whole body insulin action in healthy humans. *J Clin Endocrinol Metab.* 2003;88:793–8.

3. **Furler SM, Poynten AM, Kriketos AD, et al.** Independent influences of central fat and skeletal muscle lipids on insulin sensitivity. *Obes Res.* 2001;9:535–43.
4. **Scherer PE, Williams S, Fogliano M, Baldini G, Lodish HF.** A novel serum protein similar to C1q, produced exclusively in adipocytes. *J Biol Chem.* 1995;270:26746–9.
5. **Arita Y, Kihara S, Ouchi N, et al.** Paradoxical decrease of an adipose-specific protein, adiponectin, in obesity. *Biochem Biophys Res Commun.* 1999;257:79–83.
6. **Weyer C, Funahashi T, Tanaka S, et al.** Hypoadiponectinemia in obesity and type 2 diabetes: close association with insulin resistance and hyperinsulinemia. *J Clin Endocrinol Metab.* 2001;86:1930–5.
7. **Spranger J, Kroke A, Mohlig M, et al.** Adiponectin and protection against type 2 diabetes mellitus. *Lancet.* 2003;361:226–8.
8. **Lindsay RS, Funahashi T, Hanson RL, et al.** Adiponectin and development of type 2 diabetes in the Pima Indian population. *Lancet.* 2002;360:57–8.
9. **Fruebis J, Tsao TS, Javorschi S, et al.** Proteolytic cleavage product of 30-kDa adipocyte complement-related protein increases fatty acid oxidation in muscle and causes weight loss in mice. *Proc Natl Acad Sci USA.* 2001;98:2005–10.
10. **Yamauchi T, Kamon J, Minokoshi Y, et al.** Adiponectin stimulates glucose utilization and fatty-acid oxidation by activating AMP-activated protein kinase. *Nat Med.* 2002;8:1288–95.
11. **Yamauchi T, Kamon J, Waki H, et al.** Globular adiponectin protected ob/ob mice from diabetes and ApoE-deficient mice from atherosclerosis. *J Biol Chem.* 2003;278:2461–8.
12. **Iglesias MA, Furler SM, Cooney GJ, Kraegen EW, Ye JM.** AMP-activated protein kinase activation by AICAR increases both muscle fatty acid and glucose uptake in white muscle of insulin-resistant rats in vivo. *Diabetes.* 2004;53:1649–54.
13. **Ouchi N, Kihara S, Arita Y, et al.** Adiponectin, an adipocyte-derived plasma protein, inhibits endothelial NF-kappaB signaling through a cAMP-dependent pathway. *Circulation.* 2000;102:1296–301.
14. **Poynten AM, Gan SK, Kriketos AD, et al.** Nicotinic acid-induced insulin resistance is related to increased circulating fatty acids and fat oxidation but not muscle lipid content. *Metabolism.* 2003;52:699–704.
15. **Gan SK, Kriketos AD, Ellis BA, Thompson CH, Kraegen EW, Chisholm DJ.** Changes in aerobic capacity and visceral fat but not myocyte lipid levels predict increased insulin action after exercise in overweight and obese men. *Diabetes Care.* 2003;26:1706–13.
16. **Gan SK, Samaras K, Thompson CH, et al.** Altered myocellular and abdominal fat partitioning predict disturbance in insulin action in HIV protease inhibitor-related lipodystrophy. *Diabetes.* 2002;51:3163–9.
17. **Forouhi NG, Jenkinson G, Thomas EL, et al.** Relation of triglyceride stores in skeletal muscle cells to central obesity and insulin sensitivity in European and South Asian men. *Diabetologia.* 1999;42:932–5.
18. **Kriketos AD, Greenfield JR, Peake PW, et al.** Inflammation, insulin resistance, and adiposity; a study of first-degree relatives of type 2 diabetic subjects. *Diabetes Care.* 2004;27:2033–40.
19. **Furler SM, Zelenka GS, Kraegen EW.** Development and testing of a simple algorithm for a glucose clamp. *Med Biol Eng Comput.* 1986;24:365–70.
20. **Tabachnick BG, Fidell LS.** *Using Multivariate Statistics.* 4th ed. Boston, MA: Allyn and Bacon; 2001, pp. 140–4.
21. **Chandran M, Phillips SA, Ciaraldi T, Henry RR.** Adiponectin: more than just another fat cell hormone? *Diabetes Care.* 2003;26:2442–50.
22. **Gan SK, Kriketos AD, Poynten AM, et al.** Insulin action, regional fat, and myocyte lipid: altered relationships with increased adiposity. *Obes Res.* 2003;11:1295–305.
23. **Stefan N, Vozarova B, Funahashi T, et al.** Plasma adiponectin levels are not associated with fat oxidation in humans. *Obes Res.* 2002;10:1016–20.
24. **Groop LC, Bonadonna RC, Shank M, Petrides AS, DeFronzo RA.** Role of free fatty acids and insulin in determining free fatty acid and lipid oxidation in man. *J Clin Invest.* 1991;87:83–9.
25. **Friedewald WT, Levy RI, Fredrickson DS.** Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem.* 1972;18:499–502.
26. **Ng TW, Watts GF, Farvid MS, Chan DC, Barrett PH.** Adipocytokines and VLDL metabolism: independent regulatory effects of adiponectin, insulin resistance, and fat compartments on VLDL apolipoprotein B-100 kinetics? *Diabetes.* 2005;54:795–802.
27. **Muoio DM, Seefeld K, Witters LA, Coleman RA.** AMP-activated kinase reciprocally regulates triacylglycerol synthesis and fatty acid oxidation in liver and muscle: evidence that sn-glycerol-3-phosphate acyltransferase is a novel target. *Biochem J.* 1999;338:783–91.
28. **Kurth-Kraczek EJ, Hirshman MF, Goodyear LJ, Winder WW.** 5′ AMP-activated protein kinase activation causes GLUT4 translocation in skeletal muscle. *Diabetes.* 1999;48:1667–71.
29. **Haffner SM.** Insulin resistance, inflammation, and the prediabetic state. *Am J Cardiol.* 2003;92:18J–26J.
30. **Ouchi N, Kihara S, Funahashi T, et al.** Reciprocal association of C-reactive protein with adiponectin in blood stream and adipose tissue. *Circulation.* 2003;107:671–4.
31. **Samaras K, Campbell LV.** Increasing incidence of type 2 diabetes in the third millennium: is abdominal fat the central issue? *Diabetes Care.* 2000;23:441–2.
32. **Lafontan M, Dang-Tran L, Berlan M.** Alpha-adrenergic antilipolytic effect of adrenaline in human fat cells of the thigh: comparison with adrenaline responsiveness of different fat deposits. *Eur J Clin Invest.* 1979;9:261–6.