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Are Adipokines Functional Biomarkers of Obesity?

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Glossary of Abbreviations
BMI: Body Mass Index
CRP: C-Reactive Protein
CVD: Cardiovascular Disease
DPP4-Inhibitors: Dipeptidyl Peptidase-4-Inhibitors
FFAs: Free Fatty Acids
GLP-1: Glucagon-Like Peptide-1
HMW: High-Molecular Weight
HOMA-IR: Homeostatic Model of Insulin Resistance
MI: Myocardial Infarction
NAFLD: Non-Alcoholic Fatty Liver Disease
NT-proBNP: N-Terminal Pro-B-Type Natriuretic Peptide
PPAR-γ: Peroxisome Proliferator-Activated Receptor-γ
T2D: Type 2 Diabetes

Abstract
A wealth of investigations, ranging from human and companion animal clinical studies, animal models and *in vitro* analyses, have generated great interest in the alterations in adipokine levels demonstrated in obese individuals under a variety of clinical conditions. It is difficult to define causality, however, between adipokine dysregulation and the development of concurrent, obesity-associated diseases in patients. Enhanced awareness of these relationships is expected to provide greater understanding of the pathophysiology of obesity, generate new therapeutic targets and endpoints for management of obesity and its co-morbidities, and enhance the use of individualized risk-factor profiling in preventive medicine. Despite much intriguing current information about adipokine biomarkers, none appear ready for clinical use. Additional study is needed to determine how these biomarkers will fit into diagnostic, therapeutic and preventive algorithms for patient management.

Introduction
Over the past two decades, our view of white adipose tissue has undergone a dramatic change from an inert energy storage tissue to an active endocrine organ. There is substantial epidemiological evidence that obesity is associated with increased risk for the development of vascular diseases, type 2 diabetes (T2D), malignancies and premature death in human patients.[1] Adipose tissue communicates with other central and peripheral organs by synthesis and secretion of a host of molecules (over 100) known as adipokines. Adipokines play a central role in energy and vascular homeostasis, as well as in immunity, coagulation, reproductive status and inflammatory processes.

Abnormal production or regulation of adipokines occurs in obese individuals and has been implicated in the development of metabolic syndrome, T2D, hypertension, cardiovascular disease (CVD), cancer, and an ever-growing list of pathological changes in a number of organs. Much of the rapidly expanding body of literature about adipokines documents research into these relationships. Many adipokines are being examined as potential biomarkers for risk
assessment for development of complications related to obesity. At this juncture, it is interesting to examine information about these relationships to see how they can be used currently and in the future and to examine some of the complexities arising as a result of current studies. Although adipokine trends in obese companion animals appear to closely track those in human patients,[2] limited data are available linking adiipokine dysregulation to obesity co-morbidities in pets. This review, therefore, will focus on information available from human studies.

Biomarkers: Definition and Potential Uses

A National Institutes of Health (NIH) Biomarker Working Group consensus definition of a biomarker is "a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention."

A biomarker typically is a substance in the blood that can be objectively measured and serves as an index for health- and physiology-related assessments, such as disease risk, disease diagnosis, metabolic processes and epidemiologic studies.[3] Some biomarkers may serve for

drug development, safety and dosing. Biomarkers are indicators of molecular and cellular events in biological systems.[4] According to the NIH Biomarker Working Group, a diagnostic biomarker is defined as "a characteristic that is objectively measured and evaluated to identify a disease process in patients."[5] Prognostic biomarkers serve as tools to classify and evaluate the extent of disease progression. Efficacy biomarkers are used to monitor clinical responses and beneficial effects of new therapeutic intervention strategies.[6] The large number and range of adipokines that are altered in obesity generates a huge potential for some of these compounds to be used as biomarkers. Table 1 outlines some potential medical uses for adipokines as biomarkers.

Table 1. Potential Functions for Adipokines as Biomarkers

Diagnosis
Obesity (not an anticipated use)
Metabolic syndrome
Obesity-related co-morbidities
Risk management
Obesity prevention
Identifying individuals at risk for obesity co-morbidities
Response to therapy (individualized therapy)
Identifying new therapeutic targets (enhanced understanding of pathophysiology)
Pharmacological
Nutritional (nutragenomics)
Lifestyle interventions
Response to therapy (surrogate endpoints)
Drug development
Treatment of individual patients

Since the diagnosis of obesity in humans and animals is usually straightforward, there would seem to be little need to develop biomarkers as diagnostic tests. Although obesity is associated with an increased risk for a wide variety of diseases, not all obese individuals develop these co-morbidities. A useful role for biomarkers in obesity would therefore be identifying subsets of obese individuals at greatest risk for developing specific co-morbidities such as T2D, CVD and cancer. Biomarkers of susceptibility must consider host, environmental and lifestyle factors, and, particularly, genetic predisposition (personal behavior or lifestyle, environmental exposure, or inborn or inherited characteristics). These processes can be envisioned as a continuum that links exposure, dose and effect. Biomarkers useful for disease prevention and lifestyle/nutritional/therapeutic intervention may appear anywhere along the pathway. Earlier markers have the greatest potential to avert disease; later markers are most closely related to the disease.[4] For optimal usefulness, levels of a biomarker would be minimally influenced by factors extraneous to the system of interest. For example, N-terminal pro-B-type natriuretic peptide (NT-proBNP) increases with stress, stretch or strain on cardiomyocytes due to occult or overt heart disease but is also increased in pulmonary hypertension, renal disease and severe systemic disease.[7]

Cardiovascular Disease

An important distinction to be made is between adipokines (and other biomarkers) as risk factors versus risk markers.[8] For example, a recent study reported that low adiponectin was independently associated with high brachial-ankle pulse-wave velocity in the general population.[9] The authors concluded that low adiponectin may directly contribute to arterial stiffening. However, that interpretation has been questioned due to conflicting data, such as that showing plasma adiponectin levels are reproducibly increased in chronic kidney disease patients despite this group having markedly stiffer large arteries than the nonrenal population.[10] Similarly, heart transplant recipients exhibit high-plasma adiponectin levels and yet frequently have arterial stiffness.[11] Further complicating evaluation of such results is the knowledge that adiponectin circulates as three distinct protein complexes of which the high-molecular weight (HMW) form is the most biologically active.[12] Assessment of only total adiponectin, therefore, may confound rather than clarify.

In other intriguing connections between adipokines and CVD, low levels of adiponectin are associated with hypertension, hyperlipidemia and coronary artery disease, but patients who have heart failure have higher levels of adiponectin.[13] Moreover, higher adiponectin levels are associated with an adverse prognosis in heart failure.[13] Such apparently conflicting observations have highlighted the mechanistic complexity and temporal nature of CVD. The so-called "obesity paradox" of CVD remains unexplained: Obese individuals are more likely to suffer myocardial infarction (MI), yet these patients often have improved post-MI survival.[14] Studies have centered on the contribution of leptin to cardiac remodeling and whether the effects of leptin on metabolism, apoptosis, extracellular matrix remodeling and hypertrophy could explain the obesity paradox.[14] While contributing greatly to our understanding of the complexity of the role of leptin in obesity, such studies to date have failed to yield clear mechanistic answers.

Insulin Resistance and Type 2 Diabetes Mellitus

Although progression to T2D occurs more frequently in obese humans compared with lean individuals, this association is highly dependent on genetic background

and/or environmental factors.[15] Recent study has shown that patients with high body mass index (BMI) values undergoing gastric bypass surgery segregate into high- and low-insulin sensitivity, based on homeostatic model of insulin resistance (HOMA-IR) calculations.[16] Surprisingly, a significant population of these patients exhibit HOMA-IR values that are within the range of insulin-sensitive lean patients. This study is only one of many showing that BMI does not necessarily correlate with IR, suggesting that adipose mass alone cannot explain IR and triggering a wider search for biomarkers that may link obesity and IR.

It generally is accepted that two features are particularly critical for obesity to elicit T2D. First, impaired responsiveness of skeletal muscle to insulin is a primary condition in obesity and a precondition for the onset of T2D. The relationship between obesity and skeletal muscle IR is thought to be causal, as studies in humans, companion animals and animal models indicate that weight loss and gain correlate with increasing and decreasing insulin sensitivity, respectively. In insulin-resistant individuals who are not diabetic, glycemic control can be maintained by compensatory increases in insulin secretion by pancreatic β-cells. Thus, a second defect required for progression from IR to T2D is the failure of β-cells to secrete the required levels of insulin that maintain normal fasting blood glucose levels. The key question that has been difficult to solve is which factor or factors actually mediate IR in skeletal muscle, but at least one such important mediator has been identified — free fatty acids (FFAs).[15] The hypothesis that FFAs mediate IR, at least in part, is consistent with data that show a strong association of obesity and IR with high-circulating FFA levels.[17] Inflammation also could cause IR by a direct action of TNFα on muscle insulin signaling, but the evidence for this is less compelling.[15]

Metabolic Syndrome

The term "metabolic syndrome" has developed to describe those individuals at increased risk of T2D and CVD due to the metabolic dysfunction commonly seen in individuals with IR. A consensus statement on the definition of the metabolic syndrome, representing the views of six major organizations and societies, recently was published.[18]

The metabolic syndrome illustrates the difficulty using phenotypic changes and/or biological markers to predict disease risk. For example, 3% of metabolic syndrome patients are not obese and are defined by IR.[19] Meta-analysis of the studies examining risk for incident diabetes showed that the metabolic syndrome conferred a relative risk between 3.1 and 5.1. A similar meta-analysis focused on CVD showed that the metabolic syndrome is a comparatively poor predictor of CVD (estimated relative risk of 1.7 to 1.9) as well as all-cause mortality (estimated relative risk of 1.2 to 1.4).[20] Clinical definitions of the metabolic syndrome, while obviously important, have been criticized for being suboptimal in their ability to predict the development of T2D and CVD. The mission statement for clinical definitions of metabolic syndrome, however, is to identify those at high lifetime risk of both T2D and CVD, and evidence is accumulating that it is highly useful for that purpose. Much of the diabetes risk associated with the metabolic syndrome is due to the

Table 2. Consensus criteria for clinical diagnosis of the metabolic syndrome[18]

Measure	Categorical Cut-Points
Elevated waist circumference (using guidelines for different ethnic groups)	Population and country-specific definitions
Elevated triglycerides (drug treatment for elevated triglycerides is an alternate indicator†)	> or = 150 mg/dl (1.7 mmol/L)
Reduced HDL-C (drug treatment for reduced HDL-C is an alternate indicator†)	<40 mg/dL (1.0 mmol/L) in males and <50 mg/dL (1.3 mmol/L) in females
Elevated blood pressure (antihypertensive drug treatment in a patient with a history of hypertension in an alternate indicator)	Systolic > or = 130 and/or diastolic > or = 85 mm Hg
Elevated fasting glucose‡ (drug treatment of elevated glucose is an alternate indicator)	> or = 100 mg/dL

HDL-C indicates high-density lipoprotein cholesterol.
† The most commonly used drugs for elevated triglycerides and reduced HDL-C are fibrates and nicotinic acid. A patient taking one of these drugs can be presumed to have high triglycerides and low HDL-C. High-dose omega-3 fatty acids presumes high triglycerides.
‡ Most patients with type 2 diabetes mellitus will have the metabolic syndrome by the proposed criteria.

presence of "prediabetic" fasting glucose in the definition. It is not at all surprising that a large proportion of those with already elevated glucose levels go on to develop frank diabetes. Research has shown, however, that in fact simple measurement of fasting or 2-hour post-load glucose may be at least as predictive of the development of diabetes as use of the metabolic syndrome as a whole.[21,22]

The metabolic syndrome differentiates from short-term risk calculators in that it does not include age and can therefore indicate high risk at any age. Obesity is central to the concept of the metabolic syndrome but is considered a cause rather than a symptom. Because obesity is important years before the development of the other abnormalities that together constitute the syndrome, it is important to recognize that obesity in isolation is the important risk factor for future metabolic deterioration. Clinical and public health interventions may be more effective in those who have not yet developed the full metabolic syndrome but are obese.[19] In patients with Type 2 diabetes, adiponectin concentrations are closely related to IR and the components of the metabolic syndrome. Adiponectin concentration may be a useful addition to the criteria used for identifying obese subjects with the metabolic syndrome.[23]

Type 2 Diabetes Mellitus

Obesity and T2D are linked with a low-grade inflammatory state in which the role of endoplasmic reticulum stress and unfolded protein response are increasingly being recognized.[24] Chronic energy imbalance produces adipocyte hypertrophy and hyperplasia, endoplasmic reticulum stress and mitochondrial dysfunction. These processes lead to increased intracellular and systemic release of adipokines, FFAs and inflammatory mediators that cause adipocyte dysfunction and induce adverse effects in the liver, pancreatic β-cells, skeletal muscle, heart and vascular beds.[25] Important inflammatory mediators in obesity include TNF-α, serum retinol binding protein and C-reactive protein (CRP).[25,26] Studies suggest that apelin, a newly described adipokine, is associated with hyperinsulinemia and inflammation. Low-grade inflammation also is important in the development of other obesity-related pathologies such as non-alcoholic fatty liver disease (NAFLD) and CVD. Chemerin and vaspin are newly described adipokines that may modulate inflammatory response and insulin sensitivity in NAFLD, one of the most common forms of chronic liver disease and one that is closely associated with obesity and IR. The sheer number of adipokines involved and their complex relationships, while ultimately enhancing our understanding of pathophysiology, serve as reminders that insights related to the release of these adipokines and the connection to adipose tissue remodeling during obesity are still rudimentary.[27]

Classification of patients with T2D by means of the classical clinical and laboratory markers (Hemoglobin A1c [HbA1c], glucose, lipids, BMI and blood pressure) is a classification by symptoms and provides little insight into the underlying pathophysiological disorders, IR, β -cell dysfunction and adipogenesis. The assessment of β -cell dysfunction has become of special interest as more drugs have been developed that are supposed to protect these cells or preserve their functional capacity, such as glucagon-like peptide-1 (GLP-1) analogs or dipeptidyl peptidase-4-(DPP4)inhibitors. Next to conventional means of β-cell function assessment, HOMA-score and meal-related functional parameters, the determination of fasting intact proinsulin or the proinsulin/insulin ratio have used to describe the impact of new drugs on the insulin-secreting cells. They have been investigated and validated in multiple cross-sectional and interventional-controlled clinical studies. Routine assessment of these biomarkers, in addition to adiponectin and high-sensitivity CRP (hsCRP) might allow for a better understanding of the underlying disease conditions and optimization of anti-diabetic and anti-atherosclerotic therapy.[28]

A prominent role for the HMW form of adiponectin has been highlighted in the context of studies with peroxisome proliferator-activated receptor-γ (PPAR-γ) agonists. Almost invariably, treatment of patients with various PPAR-γ agonists results not only in an improvement in insulin sensitivity but also in a robust increase in circulating adiponectin levels.[12] This increase is primarily due to the induction of the HMW form of adiponectin. Individuals with T2D tend to have lower levels of the HMW form compared with insulin-sensitive individuals, and the development of T2D in an individual is associated with a progressive decrease of the HMW form.[29]

Obesity Biomarkers in Drug Development

Biomarkers linked to patient outcomes (safety and efficacy) have an increasingly important role in drug development. Consequently, validation and qualification of such biomarkers are essential (Table 3), often requiring large data sets from well-controlled randomized clinical trials. Biomarkers present an opportunity in understanding target engagement and disease impact while accelerating drug development. For effective integration in drug development, it is essential for biomarkers to aid in the elucidation of mechanisms of action and disease progression. Biomarkers play a central role in target validation for novel mechanisms. They also play a central role in the learning/confirming paradigm, particularly when utilized in concert with pharmacokinetic/pharma-

Table 3. Fit-for-purpose qualification for disease-related biomarkers[30]

Biomarker	Description	Drug Development Use	Example
Exploration	Biomarkers are research and development tools accompanied by in vitro and/or pre-clinical evidence, but there is no consistent information linking the biomarker to clinical outcomes in humans	Hypothesis generation	Gene expression
Demonstration	Biomarkers are associated with adequate preclinical sensitivity and specificity and linked with clinical outcomes, but have not been reproducibly demonstrated in clinical studies. This category corresponds to "probable valid biomarkers" in nomenclature suggested in draft guidance from FDA	Decision-making, supporting evidence with primary clinical evidence	Adiponectin
Characterization	Biomarkers associated with adequate pre-clinical sensitivity and specificity and reproducibly linked clinical outcomes in more than one prospective clinical study in humans. This category corresponds to "known valid biomarkers" in nomenclature suggested in guidance by FDA*	Decision-making, dose finding, secondary/ tertiary claims	Fasting plasma glucose
Surrogacy	A holistic evaluation of the available data demonstrates that the biomarker can substitute for a clinical endpoint. The designation of "surrogate end point" requires agreement with regulatory authorities	Registration	Hemoglobin A1C

*Food and Drug Administration

codynamic modeling. Clearly, these attributes make biomarker integration attractive for scientific and regulatory applications to new drug development.

The area of biomarker development is the process of establishing the link between a biomarker and the clinical/therapeutic endpoints that the biomarker is intended to relate. A fit-for-purpose biomarker qualification is a graded evidentiary process linking a biomarker with biologic and clinical endpoints and is dependent on the intended application (Table 3).

Although biomarkers have been used for many years in decision making, clinical practice, drug development and regulatory evaluation of new drugs, there currently is an increased focus on them as a means to facilitate and expedite regulatory decision making. The reasons for this heightened interest include greatly expanded technical possibilities to probe pharmacological, physiological and pathological processes using new technologies, such as microarrays and imaging. When the purpose of a biomarker is to predict clinical outcomes, it can be useful to think of four general phases of qualification, as shown in Table 3.

Summary

Biomarkers are indicators of molecular and cellular events in biological systems and help epidemiologists and clinicians better understand relationships between specific variables, such as obesity and health effects. Virtually all known adipokines are markedly dysregulated in obesity, T2D and/or metabolic syndrome.[31] Adipokines may link obesity to its co-morbidities, however, given the complex interplay among disease, host, environmental factors and inter-individual variability, more research is needed before adipokines enter common clinical use as diagnostic or prognostic biomarkers for care of individual patients. Efficacy biomarkers are likely to enter clinical use more rapidly, aided by active and coordinated efforts to utilize adipokine biomarkers in drug discovery. Targeting changes in the cellular composition of adipose tissue, the molecular mechanisms leading to these changes and the altered production of adipokines (or their receptors/actions) might have great therapeutic potential for obesity-related diseases particularly T2D and metabolic syndrome.

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